ABSTRACT

It is established that all British populations of wild cabbage, Brassica oleracea L. subsp. oleracea, show variation with respect to the Guignard picrate test. The picrate response in this species is due primarily to the volatile derivatives of sinigrin : especially allyl-nitrile which is released on damage to laminar tissue.

The picrate response varies during the year due to the effects of temperature, but individuals remain distinct.

Within a population, there is a relationship between picrate score and age, older plants being mostly of low picrate response. This is due to selection rather than age.

The Large White butterfly, Pieris brassicae L., can be a major predator of mature, sterile B. oleracea. Gravid female butterflies preferentially select high picrate response plants for oviposition (although the larvae are not selective with respect to picrate response). Larvae will cause considerable damage to host plants, although they are rarely directly responsible for plant death. However, plants which have been heavily predated by the larvae of P. brassicae are susceptible to further attack, e.g. by the aphid Brevicorvyne brassicae L., and are thus further weakened and may finally die. It is suggested that the numbers of high picrate response plants are thus reduced.

At the seedling stage the situation is reversed. High picrate response seedlings are at a selective advantage, the high levels of sinigrin derivatives protecting them from Molluscan and Fungal depredation. Thus selection by P. brassicae on the mature plants is balanced by reverse selection on seedlings.

A computer study of twelve morphological characters, demonstrates that high response plants are differentiated from lower response individuals.

A historical study strongly suggests that all the British populations are escapes from cultivation.

Studies on populations of the wild cabbage, *Brassica oleracea* L. subsp. *oleracea* by

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I certify that this thesis is my own work and has not been submitted for any degree other than that of

Doctor of Philosophy

in the University of Newcastle upon Tyne.

Neil Mitchell

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It is established that all known British populations of wild cabbage, Brassica oleracea L. subsp. oleracea, show variation with respect to the Guignard picrate test. The picrate response in this species is due primarily to the volatile derivatives of sinigrin : especially allyl-nitrile which is released on damage to laminar tissue.

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Within a population, there is a relationship between picrate score and age, older plants being mostly of low picrate response. This is due to selection rather than age.

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CONTENTS

1) INTRODUCTION

This study was based on a chance observation by Dr. A.J. Richards, that individual wild cabbages, Brassica oleracea L. subsp. oleracea at Kimmeridge, Dorset, vary markedly in the taste of their leaves. Some leaves were quite unpalatable, whereas others had a much milder taste. This led him to try Guignard's picrate test for cyanide on the leaves, in case there was some form of cyanogenic reaction producing the bitter taste. The picrate test proved positive for some plants, showing that there was variation within the population for some volatile, taste compounds.

In the cyanogenesis studies, particularly on Lotus corniculatus L., it is argued that preferential eating of acyanogenic plants by Molluscs, maintains a genetic polymorphism within populations (Jones, 1962, 1966). A similar argument has been proposed for cyanogenesis in Trifolium repens L. (Crawford - Sidebotham 1972; Angseesing 1974). In the light of this work, a similar hypothesis was proposed to attempt to explain the variability to the picrate test in B. oleracea.

It was suggested by Dr. Richards that there might be a polymorphism in B. oleracea, based on cyanogenic glucosides, the variation being maintained by selective feeding of an important predator. One such predator is Pieris brassicae L. It was initially suggested that the strong bitter taste might be a defence mechanism to predation by P. brassicae, the variation being maintained by the differential eating of the milder flavoured plants.

There were no previous reports of positive reactions to the picrate test in B. oleracea, neither were there reports of any potentially picrate reactive compounds being present in the wild species. Indeed, there was very little information available, of any kind, about B. oleracea subsp. oleracea, although there is a voluminous literature on commercial Brassica varieties.

Plate 1.1 A wild cabbage, Brassica oleraces subsp. oleracea, growing at Tenby, Pembrokeshire.

Initially the picrate test as applied to B. oleracea needed to be studied in some detail, to find out whether it was indeed reacting specifically to CN^- (as is generally reported) or to other volatile, picrate - reactive compounds. There was also a possibility, that if the reactive compound(s) proved to be of biological significance, the picrate test could be quantified.

It was also necessary to determine whether variation with respect to the picrate test, was common to all the wild populations of B. oleracea in the British Isles, and whether such variation showed a clinal trend. In the event, no such trend emerged, and comparable variation was found at all the sites. This suggested that common selective factors might be operating at every site. Subsequent investigations were able to allow suggestions to be made as to the nature of these factors.

During the course of the study, it became apparent that there was a certain amount of morphological variation within and between British populations of cabbages. This variation was studied in some detail, both with regard to co-variation with the picrate test, and to produce evidence concerning the taxonomic and historical status of 'wild' populations of B. oleracea in the British Isles.

2) VARIATION IN INDIVIDUAL RESPONSE TO THE PICRATE TEST.

2.1) Introduction

In the previous chapter the circumstances whereby the picrate test was first used to detect chemical differences between individuals of B. oleracea was described.

The picrate test is fundamental to many of the following studies and discussions, and consequently much analysis has been undertaken to accurately describe and if possible calibrate the test.

The test as first described by Guignard (1906) was carried out as follows: a strip of absorbant paper was soaked in a 1% solution of picric acid and dried. The strip was wetted with a 10% sodium carbonate solution immediately prior to use. The sample (originally 1-2g of Phaseolus lunatus seeds) was crushed and placed in a specimen tube, the moist picrate paper being suspended in the sealed tube from the stopper. The test was left to develop for one or two days at room temperature. If HCN is released the yellow picric acid is converted to red picramic acid. Since Guignard's original description of the picrate test, it has principally been used to determine the presence of HCN in laminar tissue rather than in seeds. Armstrong et al (1912, 1913) who were the first to analyse laminar tissue (of Lotus corniculatus) introduced a slight modification, in that the sample was macerated with a few drops of toluene during testing. The only other subsequent modification is to dip the paper strips in previously prepared alkaline sodium picrate.

The picrate test as used on Lotus corniculatus e.g. Jones (1962) Grant and Sidhu (1967) and Trifolium repens e.g. Pethybridge (1919), Rogers and Frykolm (1937), Corkill (1940, 1942, 1952), Daday (1954), Bishop and Korn (1969) and Angseesing (1974), has remained essentially qualitative although Melville et al (1940) and Jones (1966) have investigated

the quantitative basis of the picrate test, Melville et al (1940) finding a reasonably satisfactory correlation between the picrate test and extractable HCN; Jones (1966) compared the colours developed against British Colour Shades, having previously calibrated these against cyanide of known concentrations.

In the present study it became apparent at an early stage that other compounds in B. oleracea were causing the picrate reaction, free HCN being absent (Chapter 3). Experiments were set up to discover the causes of variation in the test and whether this variation was constant.

2.2) The Picrate Test

When the picrate test is used for B. oleracea a continuous series of colours develops (Plate 2.1) rather than the more discrete categories found in work on cyanogenesis. Therefore a method was developed to measure this colour series based on a standardised test (Mitchell 1974). A simple field technique was developed as follows: leaf discs of a standard area ($2.01cm^2$, size 12 cork borer), were cut from the edge of a lamina near the tip (Plate 2.2), avoiding any main veins. For comparative purposes, only mature leaves were sampled (see section 2.3).

The disc is lightly crushed between the fingers and placed in a specimen tube (24.5mm x 41mm) with 5 drops of toluene, equivalent to 123ul $\pm$ 1.4µl. A 1cm wide strip of Whatman No.1 paper soaked with one drop of 0.5% alkaline sodium picrate (Waller 1910) equivalent to 33.3µl $\pm$ 1.3µl, is immediately (still wet) suspended in the tube from the stopper. The strip was suspended such that it did not touch either the disc or the side of the tube. The test is developed for 72hours at room temperature.

The colour of the developed paper is measured using an Eel Reflectance Spectrophotometer. Maximum sensitivity is obtained using the 603 filter

Plate 2.1 The picrate test.

Plate 2.2 The positioning of a sample taken for picrate testing (to the left). Note the position on the lamina and its relation to 'leaf age'.

(against a standard orange background) with the attenuator open. A mask is used such that the aperture exactly fitted the test strips. Output leads from the light cell are connected to a digital millivoltmeter, rather than the spot galvanometer supplied (which was found difficult to use consistently at maximum sensitivity). Due to the small voltages involved, this system was found to be susceptible to small mains voltage fluctuations. Discrepancies arising from voltage fluctuations are overcome by regular (every 5 samples) checks on the "zero reading" (16mV) against a freshly scraped magnesium carbonate block. It was found easiest to use the spectrophotometer upside down, i.e. with the aperture uppermost.

Before this technique of measuring colour was evolved, the colours were scored subjectively on a scale 1 - 5 (1 lemon yellow, 5 deep red). Subsequently the scores were assigned millivolt values (Table 2.1).

Table 2.1 mV values assigned to picrate scores.

Picrate Score	mV Reflectance (Response)
1	>18
2	16 - 17.9
3	14 - 15.9
4	11 - 13.9
5	<10.9

2.3) The effect of leaf age on the picrate test

The picrate response of certain leaves from several plants at Tynemouth were determined. The plants were tested at hourly intervals during the day, the same leaves being used in each case (Table 2.2a).

It is clear from this data that the mature leaves of a plant maintain a consistent picrate response throughout the day. This suggests that each plant has a characteristic level of response for its mature leaves, despite the variation observed in the juvenile and senescent leaves. This study

Table 2.2) Variation of picrate response with leaf age.

a) Variation with time of day (values are as picrate scores).

Plant No.	Leaf position	Time of day 0945	1045	1145	1245	1345	1445
43	1	2	2	1	1	1	1
	2	1	2	2	2	2	2
	3	2	2	2	2	2	2
	4	2	2	2	2	2	2
	5	2	2	1	1	1	2
44	1	1	1	1	1	1	1
	2	1	1	1	1	1	1
	3	1	1	1	1	1	1
	4	1	1	1	1	1	1
	5	1	1	1	1	1	1
71	1	1	1	2	1	1	1
	2	3	3	2	3	3	3
	3	3	3	3	3	3	3
	4	3	3	3	3	3	3
	5	3	3	3	2	1	1
87	1	1	1	1	1	1	1
	2	1	1	1	1	1	1
	3	1	1	1	1	1	1
	4	1	1	1	1	1	1
	5	1	1	1	1	1	1

Key: leaf position 1 apical (juvenile) 2, 3, 4 intermediate (mature) 5 basal (senescent).

b) Variation between each leaf (values are mV reflectance)

Plant No.	Leaf position basal 1	2	3	4	5	6	7	8	9	10	basal 11
B10	14	14	15	14	12	11	11	12	11	12	21(s)
B22	21	21	20	20	19	20	20	21	22(s)		
B5	17	17	18	14	14	15	14	20(s)			
A14	20	20	20	18	18	18	19	18	21(s)		

(s) senescent leaf

also demonstrated that repeated sampling of the same leaf does not alter the level of picrate response, i.e. a wounding reaction does not appear to occur. To study this within-plant variation in some more detail, four further plants had every leaf tested simultaneously. (Table 2.2b).

These results confirm the previous study, in that a high degree of variation occurs within individual plants, but the mature leaves show a consistent level of response. The juvenile leaves appear to always have a lower picrate response than the mature leaves, as do senescent leaves. Consequently, for comparative purposes only mature leaves are used.

2.4) The relationship between weight of tissue and picrate test

Because leaf discs vary in thickness, they may also vary in weight; consequently the relationship between weight of tissue and picrate response was analysed. It was found that there was a linear relationship between square root reflectance and tissue weight $p < 0.001$ (Fig 2.1a). This relationship was found to be true for every plant tested, the only difference being in the slope of the regression. (Figs 2.1b, 2.1c) (Appendix A).

This relationship was studied in detail at Tynemouth, using 30 plants. Using the equation derived from the reflectance/weight study, it was possible to compare the 'crude' (i.e. constant area) reflectance and picrate value of each plant, with a 'corrected' value based on a standardised tissue weight of 0.1g (Table 2.3). (See Appendix C and additional data).

The data shows that for most plants there is no significant difference between reflectance or score values derived from discs of known or unknown weight i.e. under circumstances where it is not possible to weigh the discs, valid comparisons may still be made.

2.5) Temperature induced variation in the picrate test.

Thirteen plants were grown in a random array under standardised growth room conditions (light intensity at plant height 8280 lux from mercury vapour

Table 2.3 The comparison of 'crude' and 'corrected' reflectance and picrate responses for each plant at Tynemouth (Appendix C and additional data). The reflectance values were compared using the Wilcoxon signed-ranks test and the picrate scores by the Sign test.

Plant No.	Reflectance			Picrate Score		
	N[1]	T	p	N[1]	x	p
D1	14	50.0	N.S.	6	2	N.S.
P25	10	39.0	N.S.	5	2	N.S.
G18	12	16.5	N.S.	5	1	N.S.
A4	11	54.5	N.S.	3	1	N.S.
G8	11	27.5	N.S.	3	1	N.S.
B5	14	6.0	<0.001	5	1	N.S.
P2	14	39.6	N.S.	9	3	N.S.
G19	13	7.5	<0.001	6	1	N.S.
P24	12	17.5	N.S.	6	0	<0.02
A14	13	54.5	N.S.	8	2	N.S.
B22	9	35.0	N.S.	0	0	N.S.
G7	10	16.0	N.S.	1	1	N.S.
B19	10	19.5	N.S.	2	0	N.S.
P5	15	12.5	<0.001	4	0	N.S.
D3	13	45.0	N.S.	6	3	N.S.
B14	12	36.0	N.S.	1	1	N.S.
44	12	33.0	N.S.	5	2	N.S.
A6	14	42.0	N.S.	2	0	N.S.
43	14	21.0	<0.05	8	2	N.S.
P7	11	33.0	N.S.	6	2	N.S.
P12	13	57.5	N.S.	4	2	N.S.
G9	13	21.0	N.S.	4	0	N.S.
P17	12	38.5	N.S.	9	3	N.S.
P16	12	37.5	N.S.	2	1	N.S.
P11	9	48.0	N.S.	4	0	N.S.
P23	15	25.5	N.S.	2	1	N.S.
42	14	0.0	<0.001	8	0	<0.01
B10	11	54.0	N.S.	6	2	N.S.
A5	11	18.3	N.S.	2	0	N.S.
G16	11	14.0	N.S.	9	1	<0.05

Notes: 1 number of pairs of values which differed for that test.

T the smaller sum of like-signed ranks.

x the number of fewer signs.

N.S. probability not significant.

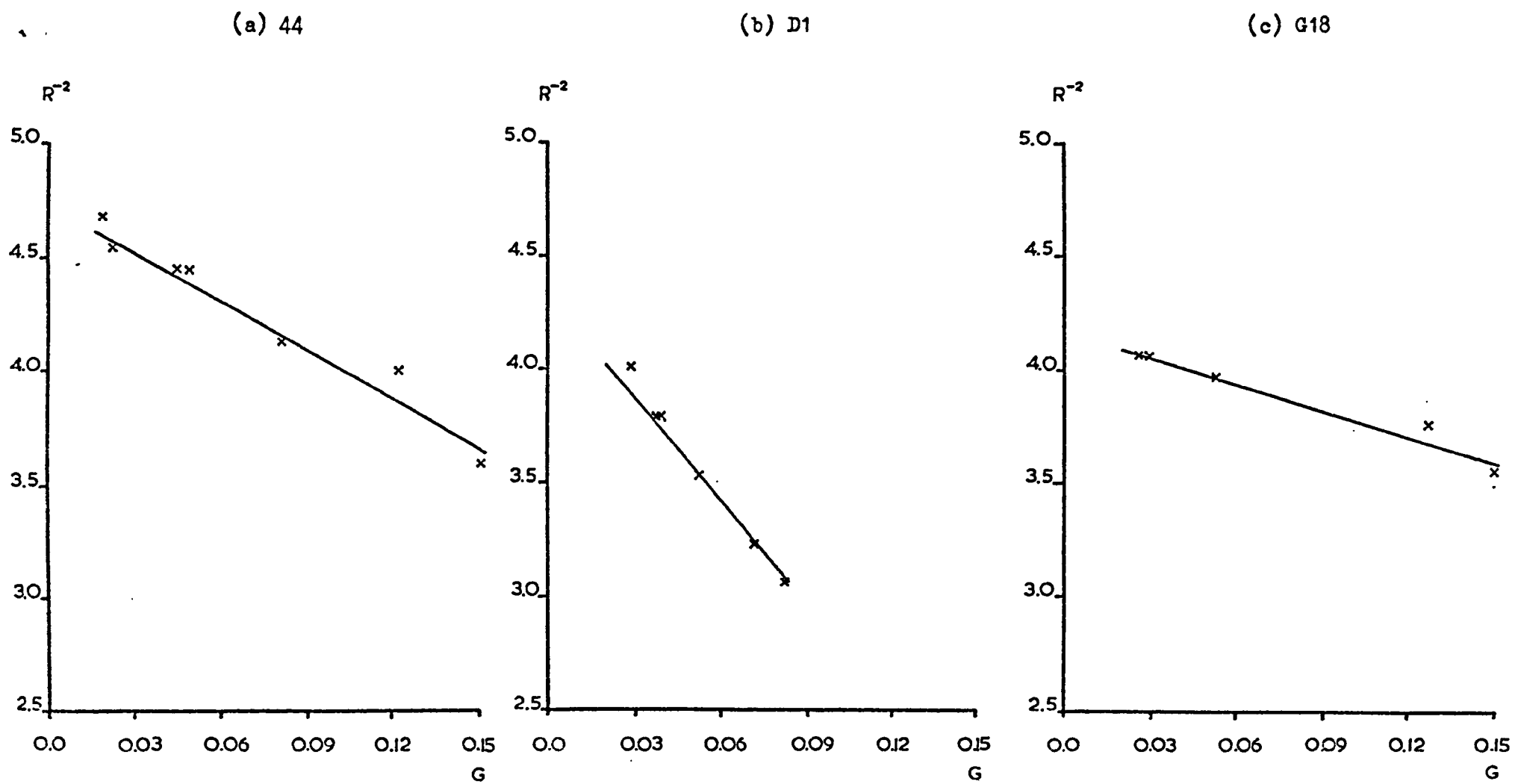


Figure 2.1 Examples (from plants at Tynemouth) to show the relationship between picrate reflectance (R) and the fresh weight of laminar tissue in grammes (G). (See Appendix A.).

lamps, plants watered once every two days with distilled water and grown in 25cm pots containing John Innes No.3 compost). The plants were maintained for four days at each of a series of temperatures, in the sequence: 25°C, 20°C, 15°C, 10°C, 5°C and 0°C. The picrate response of each plant was determined at the end of each four day period (Appendix B), the positions of the plants being changed to a new random array. Two features were analysed: (1) does each plant have a unique mean picrate response? (2) does temperature cause variation in the picrate response of a plant)? The data in Appendix B was analysed by means of a 2-way analysis of variance (Table 2.4).

Table 2.4 Anovar table of the 2-way analysis of variance.

Source	Sum Sq.	d.f.	Mean Sq.	F-ratio	p
Plants	233.89	12	19.49	9.38	$\ll 0.001$
Temperatures	71.86	5	14.37	6.91	$\ll 0.001$
Residual	124.75	60	2.08		
Total	430.50	77			

This result clearly demonstrates that there is a highly significant difference between at least some plants in mean picrate response under standardised laboratory conditions. It also demonstrates that there is a highly significant temperature effect. To further analyse these results, regression analyses were carried out, comparing the mean reflectance value of all the plants for each temperature against temperature. Linear regression suggested that this was not a simple response ($r=0.1550$, p not significant, d.f. 5). Consequently, a polynomial analysis was carried out using Hewlett-Packard computer program 'POLYFIT'. This fitted the curve and equation in Fig 2.2 to the data.

As figure 2.2 shows there was a correlation at $p < 0.01$ ($r=0.9169$, d.f. 5) between temperature and picrate response, suggesting that temperature is an important determinant of the picrate reaction of an individual plant. This curve shows that at low and high temperatures the picrate response is high, whereas at intermediate temperatures i.e. 10-20°C, the response is low. The curve shows a plateau between 10°C and 20°C, this implies that for individuals or populations to be compared, either the temperature at the time of sampling should fall between 10°C and 20°C, or the temperature (if outside this range) should be known.

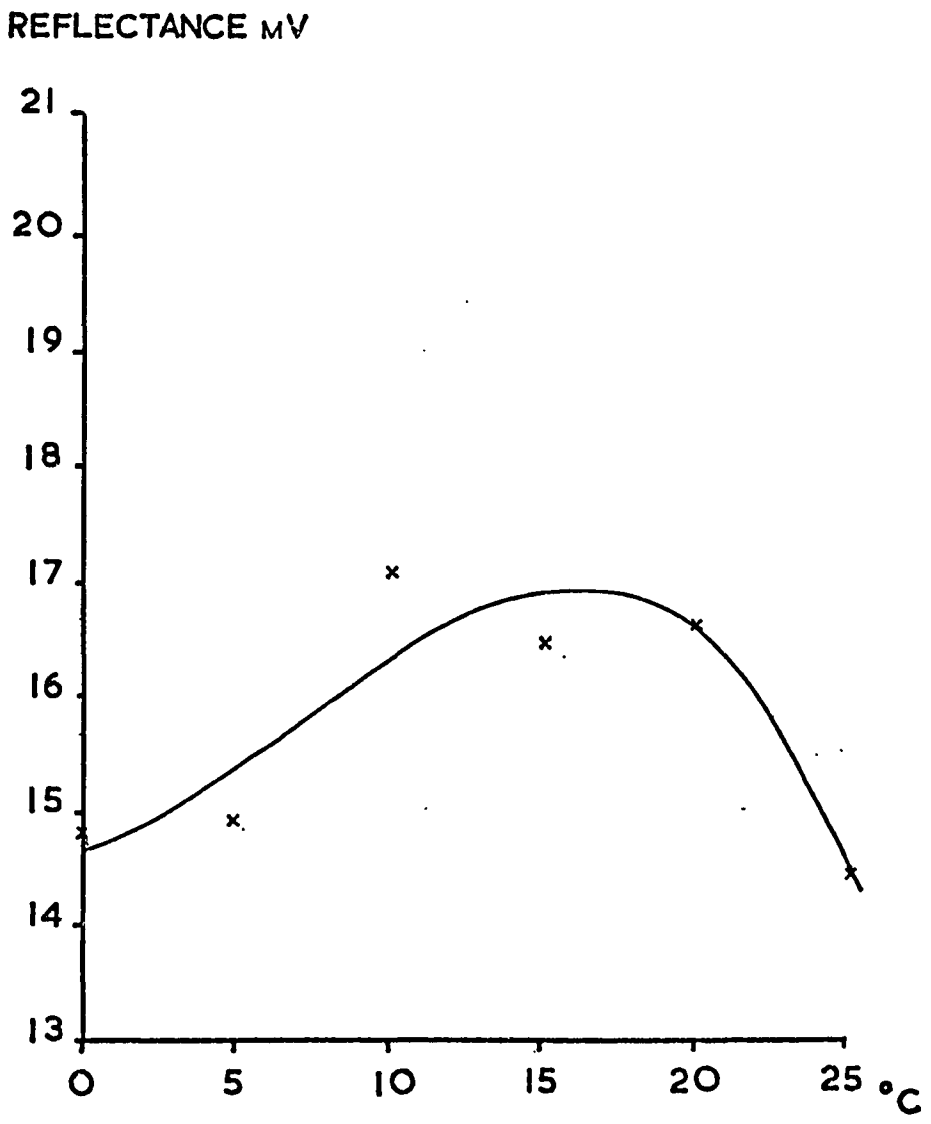


Figure 2.2 The relationship between mean picrate reflectance (R) under laboratory conditions and temperature.
$R = 14.66 + 0.075\ (^{\circ}C) + 0.0182\ (^{\circ}C)^2 - 0.00086\ (^{\circ}C)^3$, $r = 0.9169$ d.f. 5
$p < 0.01$ (See Appendix B.).

2.6) Variation in the picrate test with the time of year

Thirty plants at Tynemouth were labelled with jeweller's tags tied around the main stems (and were replaced whenever necessary). The 'corrected' picrate response (section 2.4) of each plant was determined by a series of monthly samples, the samples being taken between 9.30 and 11.30a.m. on about the same date each month (Appendix C). The mean reflectance values for each month (Fig 2.3) were subjected to a trend analysis ('running mean', Yeomans 1968; Fig 2.4). This shows that during May and June the picrate response reaches its lowest levels (high reflectance value = low picrate score = low response). The response steadily changes until it is at its strongest (low reflectance = high picrate = strong response) during September and October. There is then a decrease in response until December, after which it increases until February. The decrease to the May/June low then proceeds.

The population at Tynemouth grows around a Meteorological Office weather station. This proximity of weather station and population, allowed detailed studies to be made on the effect of climate on the picrate response. Those parameters concerning temperature and radiation were principally used (see also section 2.5 & Appendix D). Regression analysis using Pearson correlations showed that no simple correlations were present. Laboratory experiments (section 2.5) suggested that polynomial regressions were to be expected. Polynomial analysis by computer program (Polyfit) of the various parameters showed that only two were of high significance in their effect on the picrate response; the minimum temperature of the night prior to sampling (Fig 2.5) and the 30 year monthly mean (Fig 2.6) (the samples were taken at the beginning of the month, the mean for the previous month being used). Both these regressions were significant at $p = 0.01$.

These climatological relationships suggest that temperature is a

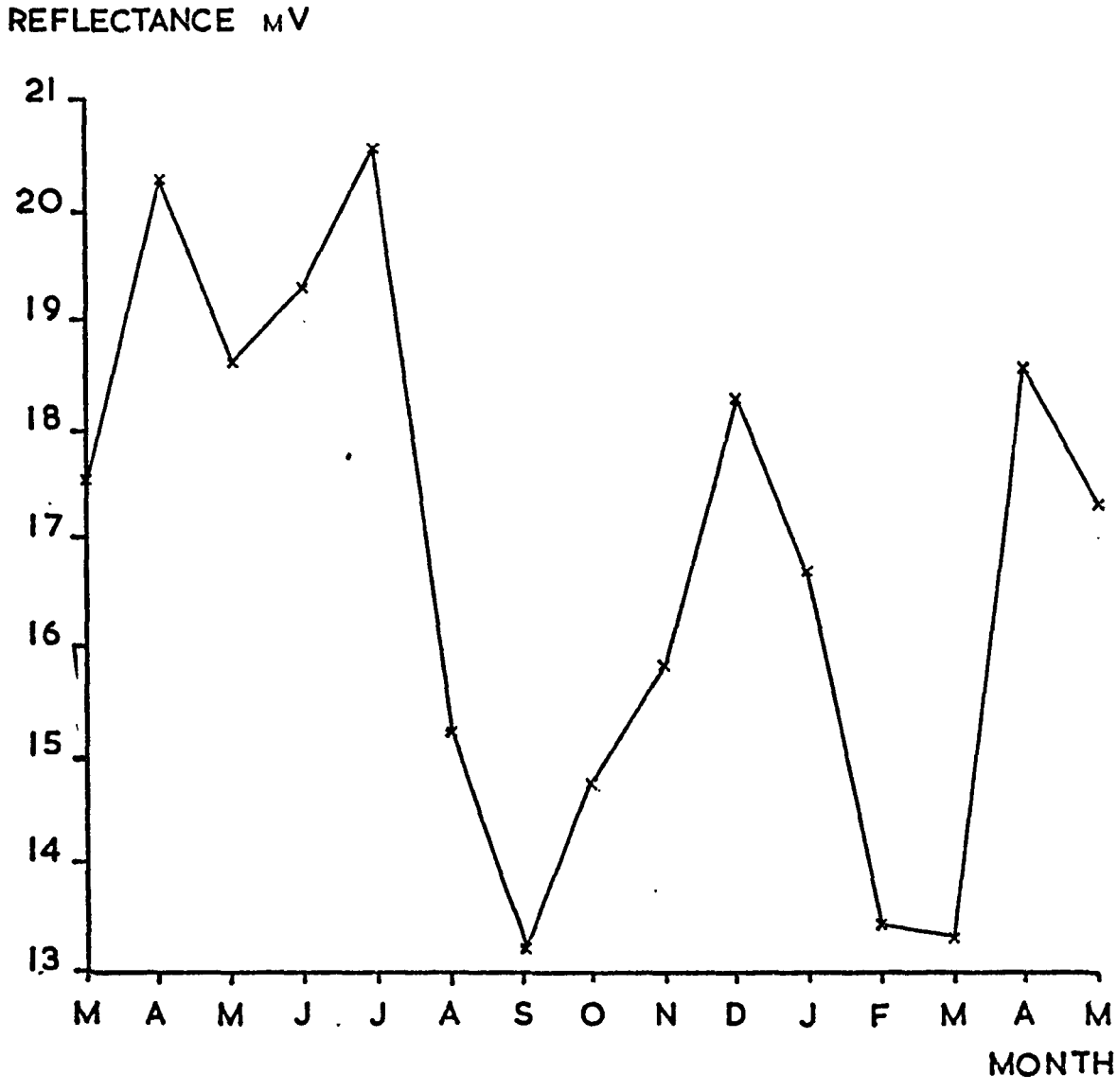


Figure 2.3 The mean monthly fluctuations in picrate reflectance at Tynemouth, from March 1974 to May 1975. (See Appendix C.).

Figure 2.4 A trend analysis (running mean) of the fluctuations in mean picrate reflectance at Tynemouth, to show the basic annual variation.

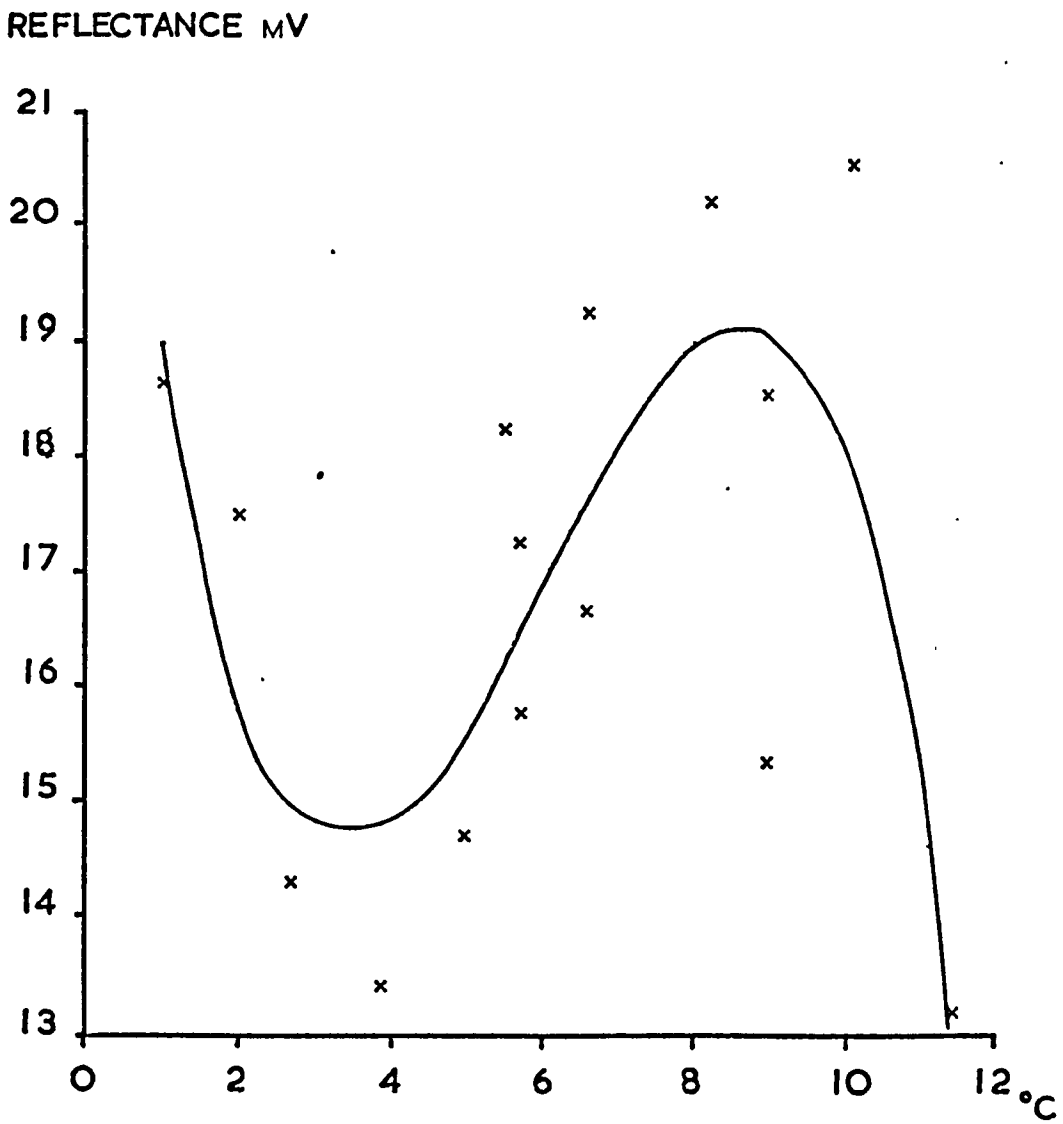


Figure 2.5 The relationship between mean monthly picrate reflectance ($\bar{R}$) at Tynemouth and the minimum temperature of the night prior to sampling.
$R = 23.82 - 6.050\ (^{\circ}C) + 1.2198\ (^{\circ}C)^2 - 0.06726\ (^{\circ}C)^3$, $r = 0.7275$, d.f. 1
$p < 0.01$ (See Appendices C and D).

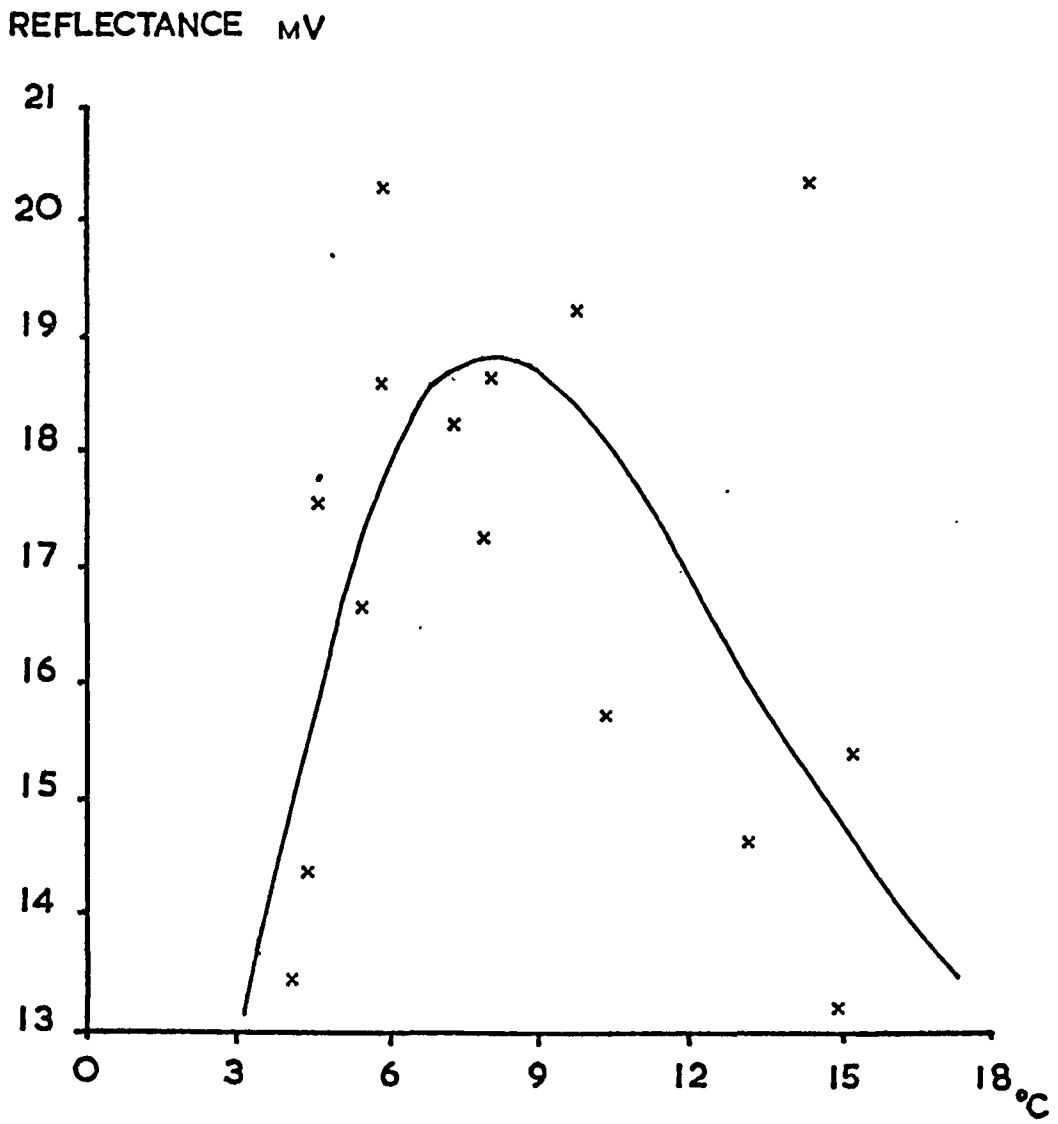


Figure 2.6 The relationship between mean monthly picrate reflectance (R) and the 30 year monthly mean temperature (of the previous month).

$R = 0.755 + 5.208\ (^{\circ}C) - 0.464\ (^{\circ}C)^2 + 0.0119\ (^{\circ}C)^3$, $r = 0.6356$, d.f. 14 $p < 0.01$ (See Appendices C and D).

major determinant of the picrate response of a plant in the field. The correlation with the minimum temperature of the night prior to sampling, implies that the day to day variation in the picrate test depends upon this night time temperature (N.B. in the laboratory experiments of section 2.5, the plants were maintained for 4 days at a constant temperature prior to sampling).

The relationship between the 30 year monthly mean and picrate response may be of considerable interest. There is the possibility that it is a spurious correlation since the 30 year mean and minimum night temperature are correlated ($r = 0.6858$, $p < 0.01$, d.f. 15) Partial correlation analysis did not clarify the matter (there are doubts as to whether it is valid to carry out such an analysis, involving linear and polynomial values). If this correlation is not spurious then it may indicate an underlying rhythm, the daily variation being superimposed upon this.

2.7) The variation between individual plants in response to the picrate test.

Although the study at Tynemouth suggests that individual plants follow similar trends in picrate response, it was necessary to demonstrate that individual plants in the field can have unique mean picrate responses. Thus the data in Appendix C was analysed by means of a 2-way analysis of variance (Table 2.5).

Table 2.5 Anovar table for the 2-way analysis of variance carried out on the data from Tynemouth (Appendix C).

Source	Sum Sq.	d.f.	Mean Sq.	F-ratio	p
Plants	603.95	25	24.16	2.35	$\ll 0.001$
Sample Interval	2128.62	14	152.05	14.79	$\ll 0.001$
Residual	3597.45	350	10.28		
Total	6330.03	389			

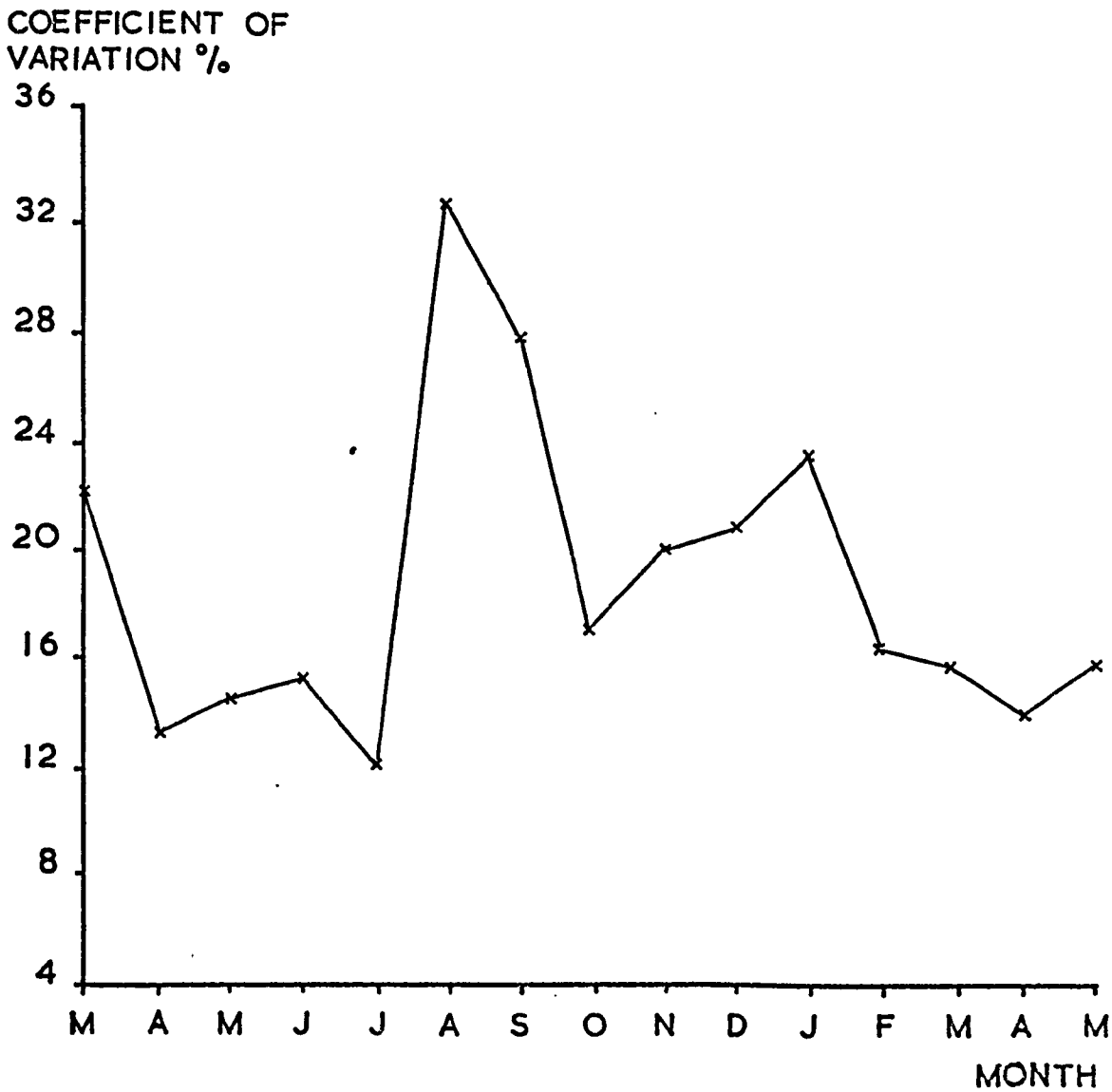


Figure 2.7 A measure of the monthly variation in picrate reflectance at Tynemouth.

These results clearly demonstrate that at least some plants have a highly significant difference in mean picrate response, i.e. some plants have unique mean picrate responses in the field. The results also demonstrate that there is a highly significant difference between the mean sample responses of at least some visits, suggesting that there are factors present in the field causing variation through the year. One such variate is minimum night temperature, investigated in section 2.6. Laboratory studies tend to support the hypothesis that this is among the most important parameters in determining picrate response.

The degree of overall variation in the population also varies (Fig 2.7), the variation reaching a mixiмum during August and September. The importance of the variation between individuals and within an individual are discussed later, with reference to selective mechanisms operating on populations.

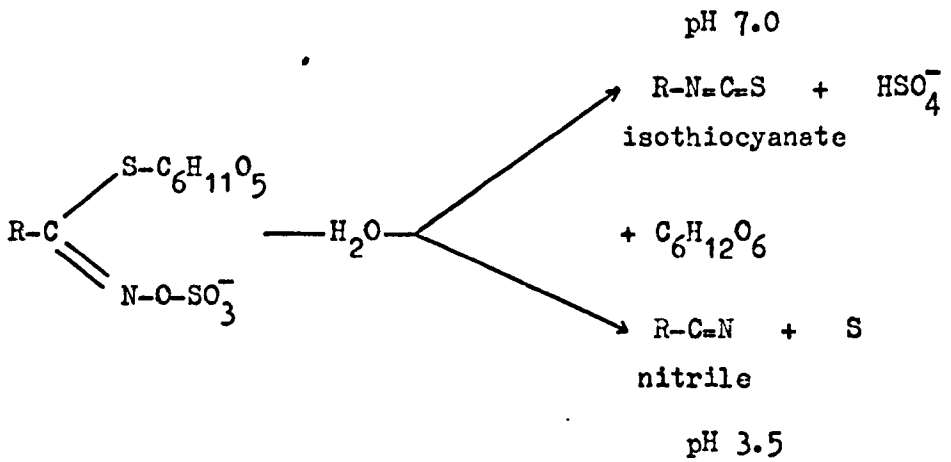
2.8) Summary

The picrate test shows several levels of variation. There appears to be a basic, genetically determined level of response, which varies between individuals. The basic response is varied by the effects of leaf age, sample weight and climatic effects. By standardising the age of a leaf sampled and the weight of sample, it has been possible to separate the effects of climate from variation between individuals. This has shown that variation between individuals is a major factor recorded by the picrate test.

3) THE CHEMICAL ANALYSIS OF THE PICRATE RESPONSE

3.1) Introduction

As mentioned in section 2.1, the picrate test has previously only been used to detect HCN release, HCN has never been detected in B. oleracea. However, there are quantities of glucosinolates (thioglucosides) found in species of the Cruciferae, which may hydrolyse to release nitriles (organic cyanides), although it is generally stated that the major derivatives are isothiocyanates (Kjaer 1960, 1963; Virtanen 1965). Glucosinolates are hydrolysed (on tissue damage) by the enzyme myrosinase, according to the reaction:



At pH 7.0 the major product is isothiocyanate and at pH 3.5 nitrile, although at intermediate pH's, both products are released (Virtanen 1965). Schwimmer (1960) states that nitrile release occurs only below pH 5.8.

Since the glucosinolates seemed a likely source of picrate reactive compounds, studies were undertaken to determine if there were any relationships between the picrate response and the glucosinolates present. A check was made for the presence of HCN.

A considerable body of work has been published on the analysis and characterisation of glucosinolates, and their derivatives, as reviewed by

Kjaer (1960). The work on the genus Brassica and its near relatives e.g. Sinapis, falls into several categories : (1) the analysis of glucosinolates in seed meals (Appelquist & Josefsson 1967; Bjorkman 1973; Ettlinger & Lunden 1956, 1957; Gmelin & Virtanen 1961, 1962; Jensen et al 1953; Josefsson 1968; Josefsson & Appelquist 1968; Kjaer et al 1953; Kjaer & Rubinstein 1954; McGregor & Downey 1975; Underhill & Wetter 1966; Vanetten et al 1974; Wetter 1955); (2) the analysis of laminar material for goitrogenic effects, due to the glucosinolate progoitrin (Altamura et al 1959; Astwood 1949; Bachelard & Trikojus 1960; Kreula & Kriesvaara 1959; Paxman & Hill 1974b; Wetter 1957); (3) general glucosinolate content (Ellestrom & Josefsson 1967; Gmelin & Virtanen 1960; Johnston & Jones 1966; Johnston & Gosden 1975; Josefsson 1967a, b; Paxman & Hill 1974a); (4) the volatile components of laminar material (Bailey et al 1961; Clapp et al 1959; McLeod & McLeod 1968, 1970a, b). Glucosinolates have also been used in taxonomic studies of B. juncea (Vaughan et al 1963, 1970; Vaughan & Waite 1966, 1967a, b; Vaughan & Denford 1968). There have been a few studies on myrosinase, which have principally concerned its preparation from seed meal (e.g. Björkman & Janson 1972; Howard & Gaines 1968; Schwimmer 1961). Some studies have been made of the enzymic action of myrosinase (e.g. Schwimmer 1960, 1961; Srivastava & Hill 1974).

Much of the work on Brassica glucosinolates concerns agricultural problems and little consideration has been given to the role of glucosinolates in the plant, nor of their adaptive significance.

3.2) Qualitative HCN analysis

Analyses were undertaken on buffered tissue macerates.

15g of laminar tissue was macerated in 30ml acetate buffer (pH 7.0) and incubated at 37°C for 30 minutes. The macerate was then acidified

with acetic acid and distilled; the distillate being collected in 10ml M NaOH (any HCN released should be collected by this method).

There are a number of tests for cyanide, three were employed here; the picrate test (Waller 1910), the copper/benzidine reaction (Kuhlberg 1937) and Aldridge's method (Aldridge 1944, 1945). Only the picrate test showed a convincing reaction, the copper/benzidine reaction and Aldridge's method showed barely detectable responses (both tests are capable of detecting 3µg CN^-). These results suggested that cyanide as CN^- was not the compound causing the picrate response.

A simple procedure was devised to analyse the volatiles released during the picrate test. Laminar tissue (25g) was lightly crushed and shaken in a 1l flask with 25ml toluene. The flask (incubated at 37°C) had a stream of "scrubbed" air passed through it. The air flow was divided to pass through five test solutions : (1) 2ml o.5% alkaline sodium picrate; (2) 2ml copper/benzidine reagent; (3) 2ml 5% ferric nitrate (Barker 1936); (4) 2ml iodine/sodium azide reagent (Feigl 1947); (5) 2ml 1% lead acetate (Vogel 1952). Between them these reagents will test for cyanides, isothiocyanates, thiocyanates and sulphides (Table 3.1), all of which may be produced by B. oleracea (Bailey et al 1961).

Table 3.1 The analysis of volatiles : compounds which should be detected by the reagents used (as suggested in the literature).

Reagent	Compounds detected			
	Cyanides	Isothiocyanates	Thiocyanates	Sulphides
Sodium picrate	*			*
Copper/benzidine	*		*	
Ferric nitrate			*	*
Iodine/Sodium azide		*	*	*
Lead acetate				*

Plants of all picrate types were analysed by this technique. In every case the sodium picrate and iodine/sodium azide reagents produced a response. This result suggests that the principal volatiles released during the picrate test were isothiocyanates. It also suggested that sodium picrate would react with isothiocyanates, although there was no previous report of this. Subsequent experiments in which allyl isothiocyanate (AITC) and sodium picrate were mixed, confirmed this view. The results also showed that simple sulphides were not an important component (no reaction with lead acetate), although the presence of organic sulphides was not ruled out.

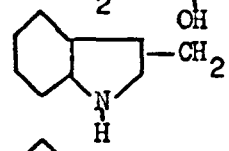
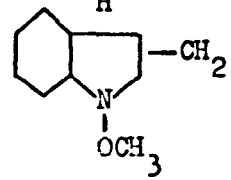
Nitriles have been detected in the volatiles from B. oleracea (McLeod & McLeod 1968, 1970a, b; Bailey et al 1961), there were though no simple tests for their presence. It was found that allyl nitrile (ACN), when mixed with sodium picrate would give the picrate response. Thus these compounds may have been detected in the above experiments.

These results suggested that volatile isothiocyanates (and possibly nitriles) were the major products detected by the picrate test in B. oleracea (this was later confirmed by gas chromatography, section 3.5) It thus seemed likely that the test measured the breakdown of glucosinolates.

3.3) Qualitative analysis of glucosinolates.

Two methods are available for the qualitative analysis of glucosinolates. One involves extracting the tissue with methanol and analysing the extract by paper chromatography (Josefsson 1967a; Schultz & Gmelin 1952). The second method is to convert the isothiocyanates released by myrosinase to thiourea derivatives and analyse the thioureas by paper chromatography (Kjaer & Rubinstein 1953; Jensen et al 1953). By means of this derivative analysis, Josefsson (1967a) has shown that up to 10 glucosinolates may be detected in B. oleracea plants (Table 3.2); the most commonly found being sinigrin, glucoiberin, glucobrassicin and neoglucobrassicin. Josefsson included

Table 3.2 Glucosinolates detected in B. oleracea (Josefsson 1967a)

Trivial Name	Chemical Name	Formula of R
Sinigrin	Allyl	$CH_2=CHCH_2$
Gluconapin	3-Butenyl	$CH_2=CH(CH_2)_2$
Glucoibervirin	3-Methylthiopropyl	$CH_3S(CH_2)_3$
Glucoerucin	4-Methylthiobutyl	$CH_3S(CH_2)_4$
Glucoiberin	3-Methylsulphinylpropyl	$CH_3SO(CH_2)_3$
Glucoraphanin	4-Methylsulphinylbutyl	$CH_3SO(CH_2)_4$
Gluconasturtiin	3-Phenylethyl	$C_6H_5(CH_2)_2$
Progoitrin	2-Hydroxy-3-butenyl	$CH_2=CH.CH(OH).CH_2$
Glucobrassicin	3-Indolylmethyl	
Neoglucobrassicin	N_1-methoxy-3-indolylmethyl	

a sample of 'wild kale' (B. oleracea var. sylvestris L.) from Italy, in his analysis of cultivated B. oleracea. This variety contained in its laminar tissue the glucosinolates gluconapin, glucoraphanin, progroitrin, glucobrassicin and neoglucobrassicin. The petiole and main vein additionally included sinigrin.

The methanol extraction technique is far simpler than the derivative analysis and much more suited to large scale screening (Kjaer & Hansen 1958), although it appears to be more limited in the glucosinolates detected. By this technique I detected up to three glucosinolates, although others (notably glucobrassicin, section 3.4.2) must have been present. The glucosinolates detected were of low molecular weight ; sinigrin, gluconapin and glucoiberin, the former two producing highly volatile derivatives. In these studies it was only those glucosinolates with volatile derivatives which were of interest, thus the methanol extraction technique was considered suitable. At its simplest, this procedure involves making a crude methanol extract of the tissue (Kjaer & Hansen 1958) followed by paper chromatographic analysis.

In this study large numbers of samples were analysed from a number of populations, thus a rapid and convenient procedure was devised. Two standard leaf discs (section 2.2) were taken from a plant : one disc was picrate tested and the other placed in 2ml of methanol. The disc was left in the methanol for 4-6 weeks. During this time satisfactory extraction of the glucosinolates occurred. The samples were then analysed by paper chromatography.

3 µl of extract were spotted on to Whatman No.1 paper and run by the descending method in a butanol - ethanol - water (4:1:4) solvent system. Sinigrin and glucoiberin were used as standards. At the end of the run (after 6 hours),the paper was air dried and sprayed with ammoniacal silver nitrate solution (400mg $AgNO_3$, 10ml 0.880 ammonia and acetone to 400ml).

The paper was dried at 120°C until light brown, then resprayed with the ammoniacal silver nitrate and dried at 120°C until dark brown. Finally the paper was sprayed with 0.4M HNO_3. The glucosinolates develop as purple spots on a yellow background (Josefsson 1967a). In this system sinigrin has an R_f of 0.12 and glucoiberin 0.06.

The frequency and combination of the glucosinolates is compared in terms of the populations (Tables 3.3, 3.4) and picrate score (Tables 3.3, 3.5, 3.6).

A number of features became apparent from this data. Each population has a unique frequency of the three glucosinolates and frequency of the combinations although glucoiberin becomes commoner on the east coast. This may reflect differing local selective pressures (chapter 4) or suggest an independent origin of each population (Appendix E).

The overall frequencies of the glucosinolates show that sinigrin is the commonest (82.5%), followed by gluconapin (65.3%), with glucoiberin being less common (38.4%) (Table 3.5). Overall, glucoiberin appears to be the "least important" glucosinolate, 6.4% of the plants contained it as their sole glucosinolate. The principal combination is sinigrin and gluconapin (37.0%), the remaining combinations being found at much lower frequencies (Table 3.6).

The results suggest that there is some relationship between picrate score and qualitative glucosinolate content. Presence of sinigrin increases with increasing picrate score (Kendall's tau = 0.80, $p = 0.04$ d.f. 4) whereas gluconapin frequency increases to score 2, then falls off to score 4, rising again in type 5 plants. Glucoiberin is present at a comparatively low frequency, rising to 49.2% in score 4 and falling off in type 5 plants. The relationship between picrate score and the various combinations is even more complex.

Table 3.3 The percentage of plants containing the glucosinolate combinations of glucoiberin (gi), sinigrin (s) and gluconapin (gn); detected by paper chromatography (data for each picrate type per population).

Population and Picrate Scores	Glucosinolate combinations							
	gi	s	gn	gi+s	gi+gn	gn+s	gi+s+gn	n
Great Orme								
1	3.3	3.3	30.0	-	3.3	36.7	6.7	
2	-	-	6.7	-	-	6.7	-	
4	-	-	3.3	-	-	-	-	
overall %	3.3	3.3	40.0	-	3.3	43.4	6.7	31
Tenby								
1	-	4.6	9.1	-	-	72.7	4.6	
2	-	-	-	-	-	4.6	-	
3	-	-	-	-	-	4.6	-	
overall %	-	4.6	9.1	-	-	81.9	4.6	22
Southerndown								
1	-	7.7	-	-	-	61.5	-	
2	-	-	-	-	-	23.1	-	
3	-	-	-	7.7	-	-	-	
overall %	-	7.7	-	7.7	-	84.6	-	13
St. Ives								
1	-	16.7	-	16.7	-	-	-	
2	-	16.7	-	-	-	-	-	
3	-	-	16.7	16.7	-	-	-	
5	-	-	-	-	-	16.7	-	
overall %	-	33.3	16.7	33.3	-	16.7	-	6
Torquay								
1	-	17.5	-	5.0	-	12.5	2.5	
2	-	2.5	-	2.5	-	5.0	10.0	
3	-	-	-	2.5	-	2.5	2.5	
4	-	7.5	-	5.0	-	12.5	2.5	
5	-	-	-	-	-	5.0	2.5	
overall %	-	27.5	-	15.0	-	37.5	20.0	40
Lulworth								
1	2.9	17.7	-	2.9	2.9	20.6	5.9	
2	-	5.9	-	2.9	-	14.7	-	
3	-	-	-	2.9	-	14.7	-	
4	-	-	-	-	-	5.9	-	
overall %	2.9	23.6	-	8.7	2.9	55.9	5.9	35

Table 3.3 (data continued)

Population and Picrate Scores	Glucosinolate combinations							
	gi	s	gn	gi+s	gi+gn	gn+s	gi+s+gn	n
St. Aldhelm's/ Winspit								
1	-	8.9	32.1	3.6	5.4	16.1	5.4	
2	-	1.8	8.9	1.8	1.8	7.1	1.8	
3	-	-	1.8	-	-	3.6	-	
overall %	-	10.7	42.8	5.4	7.2	26.8	7.2	57
Handfast Point								
1	-	-	-	-	-	25.0	-	
3	-	25.0	-	-	-	25.0	-	
4	-	-	-	-	-	-	25.0	
overall %	-	25.0	-	-	-	50.0	25.0	4
Freshwater I.O.W.								
1	-	8.7	4.4	-	-	8.7	8.7	
2	-	4.4	-	-	-	13.0	17.4	
3	-	-	-	4.4	-	8.7	-	
4	-	4.4	-	4.4	-	-	-	
5	-	-	-	-	-	-	4.4	
overall %	-	17.5	4.4	8.8	-	30.4	30.5	23
S. Foreland								
1	4.6	18.2	-	22.7	4.6	-	4.6	
2	-	4.6	-	4.6	-	-	4.6	
3	-	-	-	4.6	4.6	-	4.6	
4	-	4.6	-	4.6	-	-	4.6	
5	-	-	-	-	-	-	4.6	
overall %	4.6	27.4	-	36.5	9.2	-	23.0	22
Whitby								
1	4.0	8.0	-	8.0	-	8.0	4.0	
2	-	4.0	-	-	-	4.0	-	
3	-	4.0	-	-	4.0	-	-	
4	-	-	-	-	-	4.0	4.0	
5	-	12.0	-	-	-	16.0	16.0	
overall %	4.0	28.0	-	8.0	4.0	32.0	24.0	26
Staithes								
2	-	-	-	-	-	2.9	-	
3	2.9	2.9	-	-	-	-	2.9	
4	11.8	2.9	-	29.4	-	-	8.8	
5	-	2.9	-	14.7	-	2.9	14.7	
overall %	14.7	8.7	-	44.1	-	5.8	26.4	36

Table 3.3 (data continued)

Population and Picrate Scores	Glucosinolate combinations							
	gi	s	gn	gi+s	gi+gn	gn+s	gi+s+gn	n
Tynemouth								
1	-	3.5	-	-	-	-	-	
3	3.5	3.5	-	-	-	6.9	-	
4	3.5	10.3	-	3.5	3.5	17.2	6.9	
5	-	-	-	-	-	37.9	-	
overall %	7.0	17.3	-	3.5	3.5	62.0	6.9	29
Crail								
1	5.0	5.0	-	-	-	-	-	
2	-	-	-	10.0	-	-	-	
3	10.0	5.0	-	10.0	5.0	-	-	
4	10.0	15.0	-	5.0	5.0	10.0	-	
5	-	-	-	-	-	-	5.0	
overall %	25.0	25.0	-	25.0	10.0	10.0	5.0	20

Table 3.4 The percentage of plants in each population, containing the glucosinolates glucoiberin, sinigrin and gluconapin.

Population	Glucoiberin	Sinigrin	Gluconapin
Great Orme	13.3	53.4	90.4
Tenby	4.6	90.9	95.4
Southerndown	7.7	100.0	84.6
St. Ives	33.3	83.3	33.3
Torquay	35.0	100.0	57.5
Lulworth	20.6	94.1	64.7
St.Aldhelm's/ Winspit	19.6	49.6	84.0
Handfast Point	0.0	75.0	100.0
Freshwater Bay	42.9	95.2	61.9
S. Foreland	72.7	86.4	31.8
Whitby	72.0	92.0	60.0
Staithes	79.4	85.3	29.4
Tynemouth	20.9	89.7	71.7
Crail	65.0	65.0	25.0
mean	34.8	82.9	63.6
coefficient of variation %	78.7	19.6	40.5

Table 3.5 The percentage of plants of each picrate type containing the glucosinolates glucoiberin, sinigrin and gluconapin (based on the total data).

Picrate score	Glucoiberin	Sinigrin	Gluconapin	N
1	22.4	72.1	68.5	165
2	32.7	81.8	76.4	55
3	46.2	74.4	59.0	39
4	49.2	84.1	44.4	63
5	41.5	100.0	78.0	41
mean %	38.4	82.5	65.3	363

Table 3.6 The percentage of plants of each picrate type containing the glucosinolate combinations of glucoiberin (gi), sinigrin (s) and gluconapin(gn)

Picrate score	gi	s	gn	gn+s	gn+gi	s+gi	gn+s+gi
1	3.0	19.4	20.0	37.0	3.7	7.9	7.8
2	0.0	14.6	12.7	41.8	1.8	10.9	21.8
3	18.0	10.3	7.7	35.9	7.7	20.5	7.7
4	11.1	19.0	1.6	23.8	3.2	19.0	15.9
5	0.0	9.8	0.0	46.4	0.0	12.2	31.7
mean %	6.4	14.6	8.4	37.0	3.3	14.1	16.6

There appears to be little qualitative difference between the first four picrate scores, although there is a general increase in gluconapin + sinigrin frequency and a decrease in gluconapin alone (Kendall's tau = -1.0, d.f. 4 $p < 0.01$). However, the type 5 plants show several distinctions. They all contain sinigrin, implying that the breakdown products of sinigrin have to be present for the full type 5 response. Sinigrin is also the only glucosinolate found as the sole component. It may also be important for there to be a combination of glucosinolates (90.2% of these plants show this pattern). The combination of all three are much commoner in type 5 plants. Since the combinations found in type 5 plants, are also found in the other picrate types there must also be quantitative effects involved.

3.4) Quantitative variation in glucosinolate content.

3.4.1) Introduction

The quantitative analysis of glucosinolates has been undertaken on a number of Brassica spp. and their close relatives(e.g. Clapp et al 1959; Josefsson 1967a, b; Kjaer et al 1953). The analyses have principally been on seed meals although Josefsson (1967b) and Paxman & Hill (1974a) analysed B. oleracea laminar tissue. Josefsson (1967a) measured the content of all three kinds of glucosinolate found,i.e. the isothiocyanates,the oxazolidinethione,and the thiocyanate producing glucosinolates. Paxman & Hill (1974a) only measured the thiocyanate releasing glucosinolates. The latter glucosinolates i.e. glucobrassicin and neoglucobrassicin are easily determined, whereas the isothiocyanate and oxazolidinethione producers present some difficulty (sinigrin, gluconapin, glucoibervirin, glucoerucin, glucoiberin, glucoraphanin, gluconasturtiin, progoitrin).

In this study the main concern was with the isothiocyanate releasing glucosinolates, since only these have volatile derivatives. There are two

main methods used in quantitative analyses; direct extraction in methanol followed by purification and spectrophotometric measurement (e.g. Gmelin et al 1968) or crude extraction followed by hydrolysis with myrosinase and conversion of the products to thiourea derivatives, followed by spectrophotometric measurement (e.g. Appelquist & Josefsson 1967). Both methods were tried, but with no success. The main problem was in the spectrophotometric measurement, pure glucosinolates absorb at 225nm and thiourea derivatives at 243nm. At these wavelengths the products being measured have to be in a highly purified state. It was not found possible in these experiments, to produce a final extract of sufficient purity to allow spectrophotometric measurement. As an alternative, extraction of paper chromatograms was tried, but the same problem of purity was met with. Thus there is no data available on the quantitative variation in glucosinolates producing volatile derivatives. However, there is data on the volatile derivatives themselves (section 3.5).

As mentioned above, the thiocyanate releasing glucosinolates present no difficulty in determinations. However thiocyanates could not be of any direct influence on the picrate test, analyses were made in order to determine if there were any general relationships between picrate response and the content of other glucosinolates.

3.4.2) Thiocyanate determination of laminar tissue.

The technique was based principally on Paxman & Hill's (1974a) modification of Johnston & Jones (1966) technique.

50g of laminar tissue (excluding main vein) was homogenised in 100ml water until no obviously large pieces of tissue remained. It was further homogenised for 3 minutes in a high speed blender. Finally the homogenate was subjected to ultrasonic disintegration (at full power and an amplitude of 20 microns for 1 minute). Microscopic examination showed that all the

cells had been ruptured by this treatment. The homogenate was thoroughly shaken and left for 90 minutes at room temperature, with occasional shaking.

To 15g of homogenate, 0.5g of decolourising charcoal was added. This mixture was refluxed for 15 minutes, cooled, diluted to 100ml and filtered. 5ml of filtrate was pipetted into two test tubes and 5ml of 0.4M ferric nitrate (in M nitric acid) was added. To one tube, 1 drop of 5% mercuric chloride was added and mixed thoroughly. Both samples were measured against a reagent blank at 460nm (within 15 minutes). The thiocyanate content was determined from the difference in absorbance between the two samples (Table 3.7).

The results show that there is no simple relationship between picrate response and thiocyanate content. If the picrate test is a response to the volatile producing glucosinolates, this result was not unexpected, since Johnston & Gosden (1975) stated that they could not find any relationship between the thiocyanate and isothiocyanate producing glucosinolates.

3.5) Analysis of the volatiles released by B. oleracea.

A number of studies have been made on the volatiles released by B. oleracea, Bailey et al(1961) and MacLeod & MacLeod (1968, 1970a, b) analysed for the full range of substances released. These studies revealed that there occurred not only the derivatives of glucosinolates but also a range of organic sulphides, aldehydes, ketones and alcohols. A number of these compounds will react with sodium picrate solution, thus it became important to identify the major volatiles and to determine whether they had any effect on the picrate test.

The techniques and analyses were largely based on those of MacLeod & MacLeod (1968, 1970a, b) 30g fresh weight of laminar material (excluding main vein) were homogenised in 60ml iced water until there were no obviously

Table 3.7 The relationship between thiocyanate content and picrate reflectance.

Plant No.	Reflectance mV	Fresh wt. mg CNS^-/100g
T10	8.5	21.8
T2	8.5	14.3
T7	9.0	32.6
T3	9.5	13.0
GD	9.5	4.1
G5	10.5	4.1
T8	11.0	11.9
T1	11.5	9.2
T13	11.5	18.6
G2A	11.5	32.6
G2	12.0	14.0
T4	12.5	7.5
T11	13.0	14.2
G1	13.0	40.9
T5	13.5	13.2
T9	14.0	12.0
GH	15.5	1.5
T12	15.5	13.6
G11	16.5	14.0
GN	16.5	28.5
G4	20.5	16.7

Correlation between reflectance and CNS^- content $r = .019$ p not significant.

large pieces of tissue remaining. It was further homogenised in a high speed blender for 3 minutes. Finally the homogenate was subjected to ultrasonic disintegration for 1 minute (at full power and 20 microns amplitude).

After incubation at 37°C for 15 minutes (in a sealed vessel), the macerate was steam distilled for 10 minutes into a receiver cooled by liquid nitrogen. The distillate was carefully defrosted and 10ul immediately taken for GLC analysis. The sample was analysed using a Pye 104 gas chromatogram fitted with flame ionisation detectors. The stationary phase consisted of 20% polyethylene glycol 1500 on chromasorb, packed in a glass column of dimensions 150cm x o.4cm i.d. The column was held at 80°C for 26 minutes, then raised at a rate of 12°C per minute to 110°C and held there for 15 minutes; the nitrogen carrier flow rate was 25ml per minute. The detector oven was maintained at 150°C and the injector heater at 110°C. An amplifier attenuation of 2×10^2 was normally found suitable. The picrate response of the material analysed was also determined.

Up to 17 peaks were recorded (Fig 3.1, Table 3.8), the compounds detected including a number of aldehydes (peaks 1, 2, 4, 12 and 13), alcohols (peaks 5, 8?, 11?, 14? and 16), sulphides (peaks 3 and 7), isothiocyanates (peaks 15 and 17), nitriles (peaks 10 and 11?) and a ketone (peak 9). The majority of the peaks could be positively identified by the use of standards, although for three peaks (8, 11, 14) identification was tentative. Peak 6 could not be identified. The peaks were quantified by triangulation. An attempt was made to identify the unknown peaks by use of GLC coupled with mass spectrometry. It was tried using the systems in the Departments of Geochemistry at Newcastle and at Durham, but in both cases nothing could be resolved.

It is difficult to assess the sources of all these compounds. The

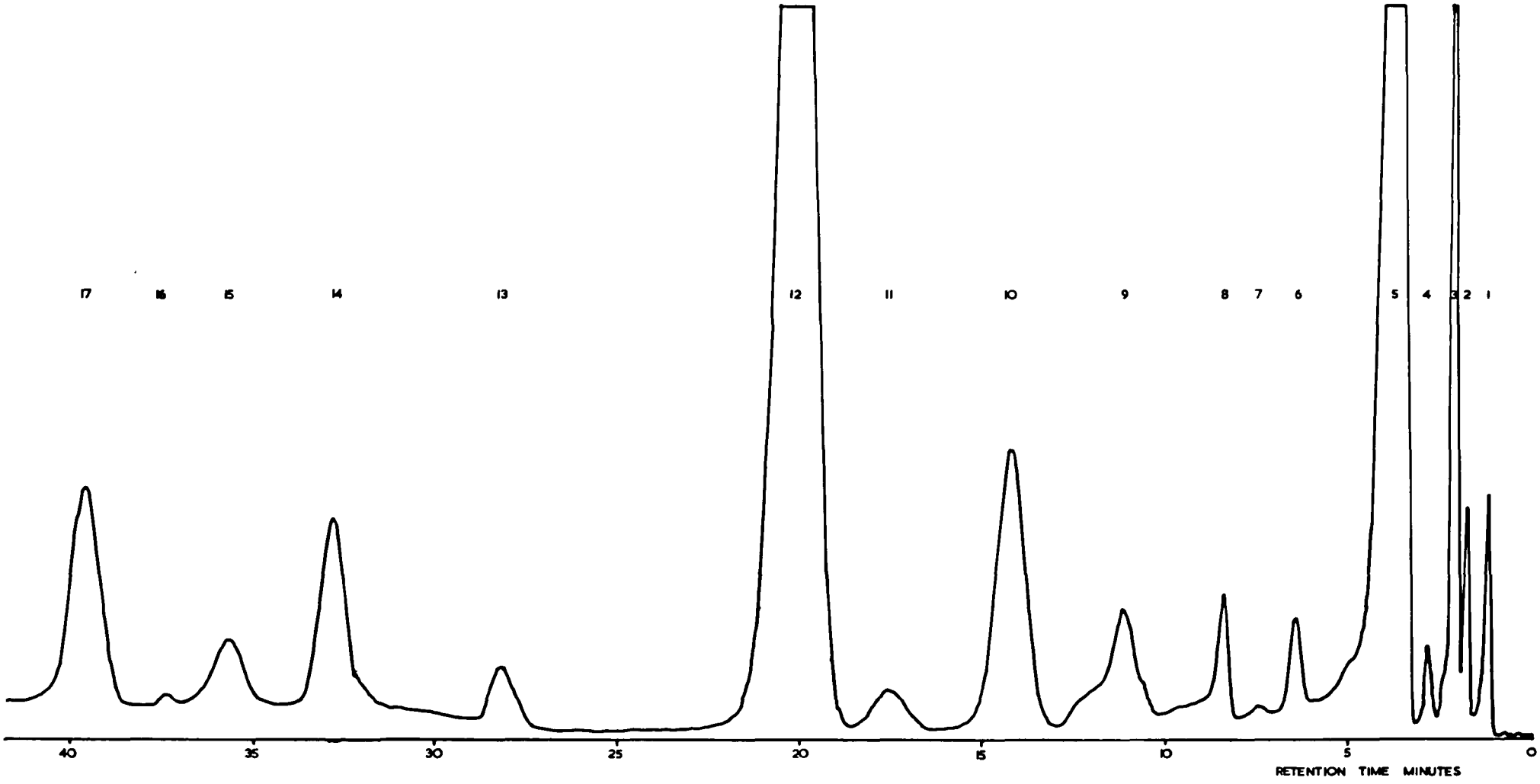


Fig. 3.1 The peaks recorded during the GLC analyses, showing the peak numbers and retention times.

Table 3.8 Compounds detected by the GLC analysis.

Peak No.	Retention time min.		Compound
1	1.2	*	Formaldehyde
2	1.8	*	Propionaldehyde
3	2.1		Dimethyl sulphide
4	2.9	*	N - Butyraldehyde
5	3.7		Methanol
6	6.4		?
7	7.5	*	Dimethyl disulphide
8	8.4	*	Allyl alcohol
9	10.2		Dipropyl ketone
10	14.2	*	Allyl nitrile
11	17.5		trans-But-2-en-1-ol or *Butyl nitrile
12	20.2	*	2-Hexen-1-al
13	28.1	*	Octanal
14	32.8		N - Hexane
15	35.7	*	Allyl isothiocyanate
16	37.4		3-Hexen-1-ol
17	39.5	*	Butyl isothiocyanate

* Compounds which will react with sodium picrate solution to produce a red colouration (as found by experimentation).

isothiocyanates and nitriles originate from glucosinolates. Macleod & Macleod (1968) believes that the sulphides and methanol are derived from the breakdown of pectin, and the aldehydes and ketones from amino acids, sugars and pectin.

A number of these compounds will react with sodium picrate to produce a red colouration (Table 3.8), thus it became essential to determine which of these compounds were major determinants of quantitative variation in the picrate test. No single compound showed a significant correlation with the picrate response. The data (i.e. peak areas, Table 3.9a) was then subjected to multiple regression analysis. The results (Tables 3.9, 3.10) clearly demonstrated that the allyl compounds accounted for the majority of the variation in the picrate response.

It was possible to produce an equation involving eight peaks, which accounted for 84.1% of the variation. The regression of this equation was highly significant at the $p<0.001$ level ($r=0.9172$, d.f. 15). Using this equation it was possible to predict reflectance values from the peak areas for each plant. These predicted reflectance values differed only slightly from the expected values ($X^2=2.31$ $p=1.0$, d.f. 15 i.e. the expected and calculated values were statistically indistinguishable). The regression analysis also demonstrated that allyl compounds were the prime determinants of the observed variation in picrate responses. They accounted for 63.1% of the total variation and 75% of the observed variation. Allyl nitrile in particular accounted for 58.1%

The lack of direct correlation between allyl nitrile and the picrate response, despite the high proportion of variation accounted for by the regression equation, required further investigation. By means of partial correlation analysis, it could be shown that if in the relationship between picrate response and allyl nitrile, the effects of formaldehyde were removed, then the correlation between allyl nitrile and picrate response rose from

Table 3.9a The GLC data (as peak areas) used to analyse the relationship between picrate response and volatiles released from B. oleracea laminar macerates.

Plant No.	R[1]	Peak reference number 1	4	7	8	10	11	12	15
G3	11.5	43.3	54.5	4.0	78.1	391.4	126.0	4465.0	2.5
GB	18.3	30.0	33.2	20.3	46.2	560.3	87.3	3386.0	3.8
G1	12.5	140.1	42.7	356.4	673.2	1426.0	99.2	4465.0	5.3
G11	12.5	43.3	55.4	40.8	736.4	701.2	108.9	4814.0	0.0
5	11.5	100.5	24.4	9.4	268.7	590.7	134.9	4305.0	17.0
6	12.5	191.4	44.0	24.6	401.9	1584.0	140.1	3920.0	0.0
TA6	12.5	68.6	35.2	14.6	123.3	668.5	99.2	2762.0	4.4
TA5	11.5	102.6	46.5	10.8	151.2	667.9	165.6	6095.0	30.0
TA4	11.0	122.4	39.4	15.5	91.4	763.1	85.5	1578.0	12.4
T13	12.5	86.6	43.8	7.5	190.9	1106.0	122.3	2088.0	19.3
TA3	10.0	88.8	37.2	12.5	115.1	669.2	119.3	3904.0	4.7
TA2	10.8	122.3	46.4	21.5	175.8	879.3	134.2	2318.0	9.5
TA1	11.5	54.6	32.8	7.9	81.9	518.5	78.0	2711.0	119.3
3	11.8	166.3	41.2	37.4	435.9	1255.0	189.0	5310.0	16.4
4	13.8	97.1	41.0	12.3	228.7	974.2	285.1	7700.0	0.0
GD	13.0	67.8	47.9	18.5	84.7	821.1	93.1	3451.0	64.7

1. Reflectance mV

Table 3.9b Multiple regression analysis of the GLC data relating picrate reflectance to peak areas.

	Peaks in the equation[2]	% variation accounted for	coefficient
Formaldehyde	1	9.6	-4.30×10^{-2}
N-Butyraldehyde	4	5.7	4.17×10^{-3}
Dimethyl disylphide	7	0.6	-4.91×10^{-3}
Allyl alcohol	8	0.6	1.80×10^{-3}
Allyl nitrile (ACN)	10	58.1	5.45×10^{-3}
?	11	0.1	-1.36×10^{-2}
2-Hexen-1-al	12	5.0	4.9×10^{-4}
Allyl isothiocyanate (AITC)	15	4.3	-5.8×10^{-3}
		constant	11.26

Total variation accounted for 84.1%

Multiple regression $r = 0.9172$ $p << 0.001$ d.f. 15

% variation accounted for by confirmed allyl compounds (P10 and P15)= 63.1%

2. These were the only peaks contributing significantly to the variation (as determined by a step-wise analysis).

Table 3.10 Comparison between observed picrate reflectance of individual plants and that calculated from the multiple regression equation.

plant no.	mean reflectance	calculated reflectance
G3	11.5	12.3
GB	18.3	13.6
G1	12.5	13.5
G11	12.5	14.5
5	11.5	11.0
6	12.5	12.4
TA6	12.5	12.2
TA5	11.5	11.6
TA4	11.0	10.0
T13	12.5	13.4
TA3	10.0	11.7
TA2	10.8	10.5
TA1	11.5	12.2
3	11.8	11.7
4	13.8	12.8
GD	13.0	13.5

$X^2 = 2.31$ (d.f. 15, $p = 1.0$) for the difference between expected mean reflectance and calculated reflectance.

$r = -0.0057$ (p not significant (N.S.)) to $r = 0.5585$ ($p < 0.05$, d.f. 14). i.e. picrate response is closely related to levels of allyl nitrile (ACN). By means of the same analysis, formaldehyde can be shown to have a negative correlation with picrate response ($r = -0.6344$, $p < 0.001$, d.f. 14), although ACN and formaldehyde levels are highly correlated ($r = 0.8254$, $p < 0.001$, d.f. 15). This strongly suggests that formaldehyde release interferes with the picrate response in some way, possibly by reacting with ACN to form a compound of lower picrate reactiveness. There is also the possibility that sodium picrate preferentially reacts with formaldehyde, with the effect that the reaction of ACN would be reduced. However, AITC is not correlated to reflectance, even after partial correlation analysis with formaldehyde ($r = -0.2342$, p = N.S.). Neither can AITC be shown to be correlated with ACN ($r = -0.2582$, p = N.S.).

These analyses clearly show that the quantitative variation in the volatiles (especially ACN) released from sinigrin, are the principal factors measured by the picrate response. However, AITC does not appear to be important in adult leaves. Due to the complex nature of the response, it was not possible to quantify the picrate test to measure sinigrin content. It was possible to determine the levels of ACN, which range from 1.75 to 0.43mg/100g fresh weight, and those of AITC, 0.56mg/100g to zero. It was also apparent that some unknown factors were involved (16% of the variation was unexplained).

It is still not known whether the picrate response is a measure of quantitative variation in glucosinolates or of glucosinolate breakdown (although glucosinolates are usually measured by their derivatives, Kjaer (1960)),and it is as yet unclear how important the levels of myrosinase are in the latter.

Greenhalgh (1976) using the identical technique of GLC analysis, but with seedling macerates, demonstrated that AITC is present in up to 10 times the concentration found in macerates of adult leaves. It is not known what causes this much higher release of AITC in seedlings, but it could be due

to a simple pH response (section 3.1). It is argued later that this high level of AITC release in seedlings may confer protection to grazing and infection. However AITC does not appear to be important in this respect in the leaves of adult plants. Thus although ACN apparently forms a major part of the picrate response in adult leaves, and AITC is unimportant in this reaction, AITC may well act with ACN in the picrate response of seedlings.

3.6) Enzyme assays.

Various studies have been reported on the extraction and assay of myrosinase from seed meals. Myrosinase may be extracted with the other B-glucosidases by ammonium sulphate precipitation (Schwimmer 1961, Howard & Gaines 1968). Björkman (1972) has characterised myrosinase after a series of complex purification stages; he demonstrated that it consists of three forms, each of molecular weight 151000. Each type shows some activity in glucosinolate breakdown.

No studies have been reported on myrosinase levels and extraction in laminar tissues.

As Björkman (1972) demonstrated, the purification of myrosinase is very complex, so no attempt was made to extract pure myrosinase. Instead the overall levels of B-glucosidases were assayed.

The plants were cultivated in a randomised array under standardised conditions of 8280 lux, 16 hour day at 20°C, in 25cm pots containing John Innes No.3. Under this regime any environmental effects should be mimimised.

The following assay is due to J.A. Lucas (pers.comm.) 10g of laminar tissue (excluding main vein) were cut up and ground in a mortar and acid washed sand, and 10ml of homogenising medium (0.1M Tris-HCl buffer, pH8.0 containing 17% sucrose, 1% cysteine and 1% ascorbic acid, at 4°C). The ground tissue was strained through double thickness muslin and the extract centrifuged at 2000g for 10 minutes. The supernatant was then recentrifuged at 37000g for 30 minutes (all at 4°C). 1ml of clear supernatant was

incubated with 2ml of 0.1M acetate buffer, pH5.5 (the mean pH of macerated tissue) containing 1mg/ml. p-nitrophenol-B-D-glucopyranoside, for 15 minutes at 37°C. The reaction was stopped using 5ml of 0.2M, NaOH. The nitrophenol released was measured immediately at 400nm (Table 3.11). A protein assay was also carried out on the supernatant, by the technique of Lovell, Illsey and Moore (1973) (Table 3.11).

Table 3.11 B-glucosidase and protein assay of laminar tissue and its relation to picrate reflectance (R).

Plant No.	mV R	mM activity/ g Fresh wt.	mM activity/ mg protein	mg protein/ g Fresh wt.
36	8.0	0.89	0.51	1.74
F	9.0	1.33	0.90	1.48
U	10.0	0.67	0.37	1.80
T5	10.0	0.48	0.30	1.64
A	10.5	0.86	0.61	1.40
T4	11.0	0.69	0.48	1.44
D	12.5	0.80	0.46	1.74
UB	12.5	0.37	0.26	1.43

Although this was only a small scale study, it was apparent that there was a wide range of B-glucosidase activity, which was not correlated with reflectance value, whether in terms of fresh weight ($r = -0.5394$, p = N.S.) or protein ($r = -0.4662$, p = N.S.). d.f 7 It should be noted that all the plants in this small sample were of high picrate response.

It is not known whether any inference may drawn as to myrosinase activity, although it is notable that protein levels vary very little.

3.7) Summary

The chemical analysis of the picrate response clearly shows that the variation in response is principally due to variation in volatile sinigrin derivatives. Whether this variation is due to quantitative variation in sinigrin and/or myrosinase activity is not known for certain. However results from later work (chapters 5 and 6) suggest it may be due to quantitative variation in sinigrin levels.

4) POPULATION VARIATION IN PICRATE SCORE

4.1) Introduction

It was demonstrated in chapter 2 that individuals vary in their degree of picrate response. Initially these studies were carried out at Tynemouth, subsequently populations from the rest of Britain were sampled.

There are approximately 25 populations of B. oleracea subsp. oleracea around the coasts of Britain. (Appendix E). In the course of this study I have visited 23 sites and obtained comparable data on the frequency of the different picrate types at 20 sites.

4.2) Methods

B. oleracea is a species found on sea cliffs and consequently presents a number of sampling difficulties. In most sites the majority of a population is inaccessible, so only those plants on the bottom few metres of cliff could be sampled. A further difficulty is the crumbling nature of the cliffs at many sites, which precluded the possibility of climbing without artificial aids.

The starting point for sampling was chosen randomly (by means of random number tables, counting individuals in a linear series along the cliff) and then from that point every fifth or tenth plant within reach was sampled. The sampling interval depended upon the size of the population and its linear extent. Each sample was picrate tested (section 2.2) and a number of other characters scored (Appendix F). At each site the ratio of flowering to sterile plants was calculated and the overall frequency of each picrate type was calculated.

The sampling was carried out at the same time of year each season, i.e. during the flowering period of late May/early June. It is to be noted that the picrate response is at its lowest during this period (section 2.5).

4.3) Results

The results were calculated as the percentage of a) Total (T), b) Flowering (F) and c) pre-flowering (S) plants in each category (Table 4.1, Fig 4.1).

Two analyses were carried out on the data in Table 4.1, firstly a clustering procedure (Ward's method, Wishart 1969) based on all the data in Table 4.1. The object of this analysis was to examine the relationships between the populations, on the basis of picrate score frequency, for geographic or other groupings. The second analysis was a non-parametric analysis of variance of the data, using Friedman's 2-way analysis of variance. For this analysis the data was split into 5% classes, i.e. 0-4.9%, 5.0-5.9% etc. This analysis demonstrates whether or not the populations are showing a common pattern of variation.

The cluster analysis (Table 4.2) demonstrates that although the data can be split into two main categories, these do not follow any clear-cut geographic distinctions, e.g. the similarity sequence of populations does not form an obvious cline, or a North/South or East/West split. Instead the populations are characterised by an absence or presence of picrate type 5 in the flowering plants, and a high or low frequency of pre-flowering and total type 1 plants. Possible causes of differences in picrate frequency are discussed later (Chapters 5 and 6), although the extent to which historical factors have a role is not known (Appendix E).

The Friedman analysis of variance (Table 4.3) demonstrates that at a high level of probability, the populations show a common pattern of variation with respect to picrate score frequency.

Table 4.3 Results from a Friedman analysis of variance of the data in Table 4.1

Frequency type	X^2_R	p	d.f.
total	12.71	0.85	19
flowering	3.83	1.0	19
pre-flowering	9.01	0.97	19

Table 4.1 Picrate structure of populations (May/June values), % relative frequencies.

Year	Population	Picrate score 1	2	3	4	5	N
1974	Great Orme						
	Total (T)	71.3	24.0	4.8	0.0	0.0	68
	Flowering (F)	100.0	0.0	0.0	0.0	0.0	23
	pre-flowering (S)	57.1	35.7	7.1	0.0	0.0	45
1974	Tenby						
	T	88.6	11.4	0.0	0.0	0.0	43
	F	87.5	12.5	0.0	0.0	0.0	16
	S	88.9	11.1	0.0	0.0	0.0	27
1974	Southerndown						
	T	89.6	7.8	2.6	0.0	0.0	33
	F	66.7	25.0	8.3	0.0	0.0	12
	S	100.0	0.0	0.0	0.0	0.0	26
1973	St. Ives						
	T	30.6	30.6	30.6	0.0	8.3	16
	F	25.0	25.0	25.0	0.0	25.0	4
	S	33.3	33.3	33.3	0.0	0.0	12
1975	Prussia Cove						
	T	38.2	17.1	14.5	30.4	0.0	30
	F	40.0	30.0	20.0	10.0	0.0	10
	S	37.5	12.5	12.5	37.5	0.0	20
1975	Polruan						
	T	32.5	27.0	15.2	20.5	4.8	82
	F	40.0	15.0	30.0	15.0	0.0	20
	S	29.1	32.4	8.5	23.0	7.0	62
1975	Looe						
	T	34.6	22.5	4.7	31.5	6.7	34
	F	64.3	7.1	14.3	14.3	0.0	14
	S	20.0	30.0	0.0	40.0	10.0	20
1973	Dartmouth						
	T	44.2	19.2	24.6	12.1	0.0	47
	F	78.6	14.3	0.0	7.1	0.0	14
	S	28.6	21.4	35.7	14.3	0.0	33
1973	Babbacombe						
	T	28.5	23.8	12.0	31.0	5.1	59
	F	47.4	21.1	5.3	21.1	5.3	19
	S	20.0	25.0	15.0	35.0	5.0	40
1973	Lulworth						
	T	35.9	31.0	22.6	10.6	0.0	48
	F	61.1	22.2	5.6	11.1	0.0	18
	S	10.0	40.0	40.0	10.0	0.0	30

Table 4.1 (Data continued)

Year	Population	Picrate score 1	2	3	4	5	N
1973	Kimmeridge Bay						
	T	45.6	28.8	10.6	15.0	0.0	32
	F	62.5	25.0	12.5	0.0	0.0	8
	S	40.0	30.0	10.0	20.0	0.0	24
1974	St.Aldhelm's/Winspit						
	T	78.8	18.6	2.6	0.0	0.0	97
	F	72.4	27.6	0.0	0.0	0.0	29
	S	81.5	14.8	3.7	0.0	0.0	68
1974	Handfast Point						
	T	20.8	10.4	49.4	19.5	0.0	10
	F	50.0	25.0	25.0	0.0	0.0	4
	S	0.0	0.0	66.6	33.3	0.0	6
1974	Freshwater Bay						
	T	29.3	33.3	22.6	9.9	4.9	36
	F	55.6	33.3	11.1	0.0	0.0	9
	S	20.0	30.0	26.7	13.3	6.7	27
1973	S. Foreland						
	T	40.4	23.5	14.9	14.9	6.3	65
	F	61.9	19.1	9.5	9.5	0.0	21
	S	33.3	25.0	16.7	16.7	8.3	44
1975	Whitby						
	T	29.2	26.8	14.2	21.1	3.5	42
	F	16.7	16.7	25.0	33.3	8.3	12
	S	34.9	31.4	9.3	15.6	9.8	30
1973	Staithes						
	T	70.3	16.9	6.8	6.1	0.0	124
	F	80.0	17.5	2.5	0.0	0.0	40
	S	66.7	16.7	8.3	8.3	0.0	84
1973	Tynemouth						
	T	60.7	27.0	11.0	1.4	0.0	66
	F	66.7	30.3	3.0	0.0	0.0	33
	S	56.1	24.3	17.1	2.4	0.0	33
1975	Crail						
	T	20.8	5.2	36.2	36.2	1.7	58
	F	55.6	16.7	5.6	16.7	5.6	18
	S	5.0	0.0	50.0	45.0	0.0	40
1975	Auchmithie						
	T	76.1	9.5	10.5	1.6	2.3	30
	F	87.5	12.5	0.0	0.0	0.0	8
	S	70.9	8.1	15.3	2.3	3.3	22
	mean values						
	T	48.8	20.7	15.5	13.1	2.2	1025
	F	61.0	19.8	10.1	6.9	2.2	332
	S	41.6	21.1	18.2	15.8	2.5	693

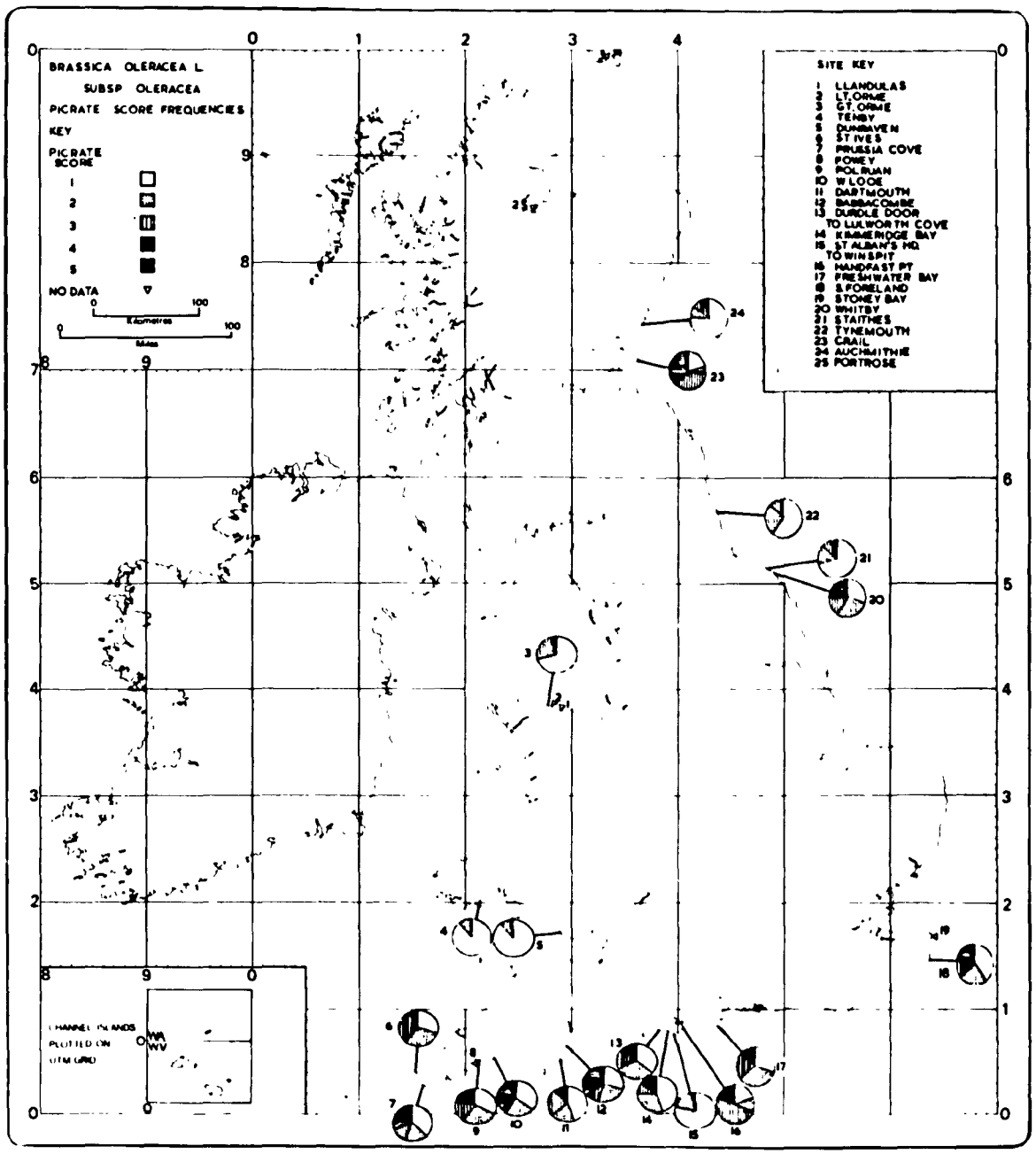


Figure 4.1 The frequency of each picrate type at the populations sampled.

Table 4.2 The result of a cluster analysis carried out on the data in Table 4.1, to whow the relationships between the populations and the characters which differentiate any groupings.

Cluster 1.

Great Orme, Staithes, Auchmithie, Tynemouth; Tenby, S. Aldhelm's/ Winspit, Southerndown;

Principal cluster diagnostics: absence of type 5 flowering plants; high frequency of type 1 pre-flowering plants (mean 74.4%) and total type 1 plants (mean 76.5%).

Cluster 2.

St. Ives, Dartmouth, Kimmeridge Bay, S. Foreland; Lulworth, Freshwater Bay; Prussia Cove, Looe, Babbacombe; Polruan, Whitby, Handfast Pt., Crail;

Principal cluster diagnostics: low frequency of total type 1 (mean 33.2%), pre-flowering type 1 (mean 24.0%) and pre-flowering type 2 (mean 23.0%)

Notes:

The sequency of populations starting with Great Orme and ending with Crail represents their relative similarity, thus Great Orme is more similar to Staithes than it is to Auchmithie; similarly Great Orme and Crail are the two most dissimilar populations. The ';' represent subdivisions within each cluster.

The results strongly suggest that there are common factors operating at each site, which determine the frequencies of each picrate type in the three categories. If the levels of probability (p) had shown significant differences instead of significant association, then the factors determining the picrate frequencies at each site would have been operating independently.

4.4) Summary of results in Table 4.1

Although the results appear to vary widely when the data is analysed, it becomes apparent that there are no overall distinctions between the picrate score structures of the populations either in geographic or absolute terms, since the structuring appears to be determined by common factors (see also Appendix E). In summary, type 1 plants are the commonest with a mean frequency of 48.8%, types 2, 3 and 4 have mean frequencies of 20.7%, 15.5% and 13.1% respectively with type 5 plants being the rarest with 2.2%. Despite local fluctuations, this distribution appears to be the basic structure and is presumably caused by some overall effect operating at all the sites.

4.5) The ageing of individual plants.

It is possible to age individual plants by counting the numbers of groups of closely spaced leaf scars (Plate 4.1). During the winter period when there is little stem elongation, the leaf scars are closely grouped; when stem elongation takes place in the growing season, the leaf scars from that period are spaced out. This leaves a marked distinction between the winter and growing seasons. The numbers of groups of closely grouped scars representing the age in years of the plant.

During the collection of the data in Table 4.1, each picrate tested plant was also aged (Table 4.5).

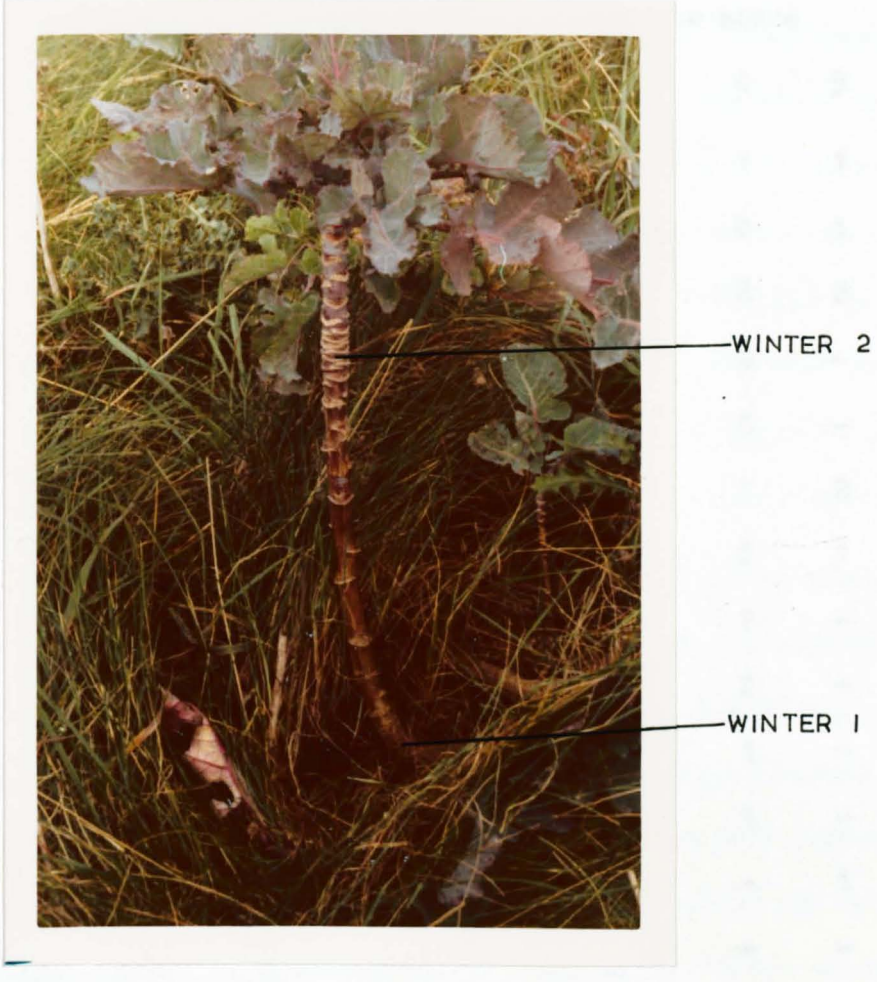


Plate 4.1 The technique of ageing individual plants.

Table 4.5 A breakdown of the numbers of sampled individuals of each picrate score in each age class.

Age yrs	Picrate score 1	2	3	4	5	totals
1	6	1	2	1	1	11
2	35	11	9	8	1	64
3	49	20	10	8	2	89
4	36	18	10	9	-	73
5	66	11	9	5	-	91
6	44	8	9	7	2	70
7	37	19	1	6	1	64
8	17	12	4	2	-	35
9	21	7	3	2	-	33
10	12	1	1	1	-	15
11	3	3	-	1	-	7
12	5	4	-	-	1	10
13	3	-	-	-	-	3
15	2	-	-	-	-	2
17	1	-	1	-	-	2
20	1	-	-	-	-	1
totals	338	115	59	50	8	570

Whichever way the data is summarised, the simple correlation between age and median picrate score is not very informative (due to the nature of the median statistic). The data had to be analysed in total to obtain a true idea of the overall relationships. The analysis undertaken was a Kendall correlations analysis (Table 4.6).

It is immediately apparent that there is a strong negative correlation between age and picrate score i.e. the younger the plant the higher picrate score tends to be. This holds at very high levels of correlation for the data when analysed in total and as analysed for flowering or pre-flowering / plants. (Table 4.6a). The negative correlation is particularly marked for flowering plants. Four of the larger population samples were also analysed individually and in each case the correlation was also negative (Table 4.6b) although in only two cases was the relationship significant.

The data suggests a number of features. Picrate score could be a function of age i.e. linked to the metabolic age of a plant, less glucosinolates and/or myrosinase being produced as the plant ages. The data tends to refute this, since although there are few type 5 plants, they are found throughout the age range up to 12 years (I have only recorded 8 older plants). Although no physiological studies have been carried out into this question of picrate score as a metabolic function of age there is no suggestion in the literature that this should be so. The alternative is that high picrate score plants are lost from the populations. There is a negative correlation between picrate score and frequency (see section 4.3). Thus some factor common to all the populations (section 4.2) is resulting in the loss particularly of the higher picrate scores from the populations.

4.6) Analysis of the picrate structure of seedlings.

It has been almost impossible to carry out any large scale seedling or

Table 4.6 Kendall correlation analysis of the total data in Table 4.5

a) Total data

	Kendall's tau	p	d.f.
overall	-0.1419	0.001	569
flowering	-0.0610	< 0.05	336
pre-flowering	-0.1498	< 0.001	233

b) The four largest population samples

	Kendall's tau	p	d.f.
Torquay	-0.0349	0.19	39
Winspit	-0.2295	0.006	56
Staithes	-0.2295	0.024	52
Tynemouth	-0.1119	0.079	75

genetic crosses since, for the past 3 years (at least) there has been an almost total seed loss due to attacks on the seeds and pods by Dasyneura brassica (Winn.) and Ceuthorrhynchus assiimilis (Payk.) These attacks have occurred at all the east coast sites (including our experimental gardens) as well as at some of the other sites in the south and west.

In 1974 a few plants at Tynemouth escaped complete seed loss, the fate of seedlings from six plants being followed. A sample (about 20% of the population) of emergent seedlings (at the cotyledon stage) was picrate tested during October and all the survivors tested the following May (first leaf stage) (Table 4.7). These latter seedlings were sampled just prior to the commencement of rapid growth.

Tabel 4.7) The distribution of picrate responses in the seedling patches.

		Reflectance category															Reflectance		
Plant		22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	mean	C.V.%	PR
D1	Oct	2	2	4	2	1	8	1									18.8	10.4	17.0
	May											3	10	5	5	1	12.7	13.9	
P2	Oct			1	1	1	2	1	3	5	4	1	1				14.9	15.7	14.0
	May										3	3	4	13	3	2	10.5	12.6	
P1A	Oct		1	1	4	4	3	2	1	1	1						17.8	11.8	15.5
	May								1		1	4	6		1	1	12.1	23.9	
P1	Oct							3	2	3	5	2	1	1	1	1	13.7	20.8	8.5
	May					1	1		1	4	6	9	6	1			12.7	13.7	
TS1	Oct	5	4	3	4	3	2	1	1								19.9	10.2	16.0
	May						1		1		1		3	2	3		11.6	22.9	
TS3	Oct	4	1	2	3	5	1	1	4		1						18.5	13.8	13.0
	May								3	1	3	5	5	2	1		12.2	14.3	

Notes: Reflectance categories are pooled to units, e.g. 17.0 and 17.5 in category 17. Means and C.V.% are based on the original, uncategorised data. PR female parent reflectance at time of October sampling.

It is apparent from the data that a major change in picrate response had occurred. In each case the mean picrate reflectance had fallen, in most instances by a large value. For several sets of seedlings there was little or no overlap between the October and May values. This difference in range of values could be due to a change in metabolism from cotyledon to first leaf, thus conclusions drawn from this data should be treated with caution. Despite this, the mean reflectance values of the surviving seedlings were very low. It might be expected that juvenile leaves would tend to have a high reflectance value (section 2.3) and that during May, leaf reflectance

values would be high (Fig 2.3). It should also be noted that seedlings with reflectance values of up to 20mV were present (thus the May seedlings are capable of high reflectance values).

These observations might suggest that seedlings tend to have low reflectance, perhaps adaptively so; alternatively, if the heavy seedling mortality (apparently up to 90%) is considered, then it might suggest that the observed low reflectance is a result of differential selection during the winter. If either argument is true, it would suggest that seedlings capable of releasing the higher levels of sinigrin derivatives, i.e. allyl-isothiocyanate and -nitrile, are at a selective advantage (it is later argued (Ch.6) that this may confer protection against predators.) In either case, the very limited data suggests that at the beginning of the second growing season, there will tend to be a predominance of high picrate score plants. Therefore, high picrate response plants must be lost from the populations after the first winter. The overall age structure of the pre-flowering plants illustrates this. (Table 4.8).

Table 4.8 The overall age structure of pre-flowering plants.

Picrate Type	1	2	3	4	5
mean age years	3.36	3.59	3.27	3.26	2.25
standard deviation	1.52	1.86	1.26	1.35	0.96
N	119	49	34	27	4
relative frequency	1	: 0.41	: 0.29	: 0.23	: 0.03

The mean age of the type 5 plants is significantly lower than that of all the other picrate scores (Table 4.9). This strongly suggests that during the post-seedling pre-flowering phase of B. oleracea life history, type 5 are selectively lost from the populations, although they are of selective advantage during their first (seedling) winter.

Table 4.9 t-test of the differences between the mean ages of the different pre-flowering plant picrate types (values are values for t and the probability).

	Picrate score 2	3	4	5
1	7.27	2.88	2.77	13.36
P	<0.001	<0.01	<0.01	<0.001
2		5.74	5.11	8.79
P		<0.001	<0.001	<0.001
3			0.16	7.31
P			N.S.	<0.001
4				6.13
P				<0.001

If a similar analysis is carried out on the flowering plants, then a different trend is observed. (Table 4.10)

Table 4.10 The overall age structure of the flowering plants.

Picrate type	1	2	3	4	5
mean age years	6.69	7.05	6.84	6.65	7.75
standard deviation	2.64	2.17	2.81	1.90	2.87
N	219	66	25	23	4
relative frequency	1 :	0.32 :	0.11 :	0.11 :	0.02

In this case there are no obvious trends, the mean ages being quite independent of each other and in most cases significantly different (Table 4.11).

Table 4.11 t- test of the differences between the mean ages of the different flowering plant picrate types (values are t- values and levels of probability).

	Picrate score 2	3	4	5
1	2.03	2.81	7.28	5.76
P	< 0.05	< 0.01	< 0.001	< 0.001
2		23.97	25.79	13.47
P		< 0.001	< 0.001	< 0.001
3			8.57	0.34
P			< 0.001	N.S.
4				11.12
P				< 0.001

These results suggest that once the flowering stage is reached (usually after 4 or 5 years) then whatever selective forces operate to reduce the numbers of pre-flowering / high picrate score plants, then they cease to operate at the flowering stage.

4.7) Conclusions based on the picrate score structures of the populations.

Wide differences in the absolute frequencies of each picrate type were

observed. Despite this, analyses suggest that there are common factors operating at all the sites, resulting in an underlying pattern of picrate frequencies. The basic structure of a population is that the majority of plants are of picrate score 1, then the higher the picrate score, the less frequently it is represented.

The picrate score of a plant is significantly correlated with its age, such that the higher the picrate score the younger the plant tends to be. Evidence is presented that during the pre-flowering stage in the species' life history, high picrate score plants are preferentially lost from the populations. This suggests that there are selective forces operating against the high picrate score plants. Possible selective agents are discussed in the following chapters.

It is to be noted that selection operating at the seedling stage appears to be the converse of that in older plants, with high picrate score seedlings being at a selective advantage. This indicates selection against low picrate score seedlings (this is particularly discussed in chapter 6).

4.8) Spatial distribution of individuals within a population.

4.8.1) Introduction

It was of some interest to investigate whether individuals of different picrate types were randomly distributed within a population, or whether there was any pattern to their distribution.

It was only physically possible to carry out such a pattern analysis at S. Foreland (Kent) where the population extends off the cliff face on to the cliff top, for approximately 80m inland. The various techniques used, were those described by Kershaw (1964).

4.8.2) Analysis of the total data.

A grid of dimensions 16m x 32m was laid out, with a quadrat size of $1m^2$

as the basic unit. The number of individuals in each quadrat was recorded, as was their picrate response. The expected Poisson distribution was then calculated, and compared with the observed distribution (Table 4.12).

Table 4.12 A comparison between the observed distribution of individuals with their expected Poisson (random) distribution.

	Individuals/quadrat							
	0	1	2	3	4	5	6	7
observed	374	92	25	10	4	3	3	1
expected	391.1	105.4	14.2	1.3	0.085	0.046	0.00021	0.000008

$$X^2 = 170050 \quad p \ll 0.001 \quad d.f. = 6$$

This analysis demonstrates that the individuals in a population are not randomly distributed. An analysis of the variance:mean ratio confirms this (variance:mean ratio = 2.09, t = 17.5, d.f. = 511, $p \ll 0.001$).

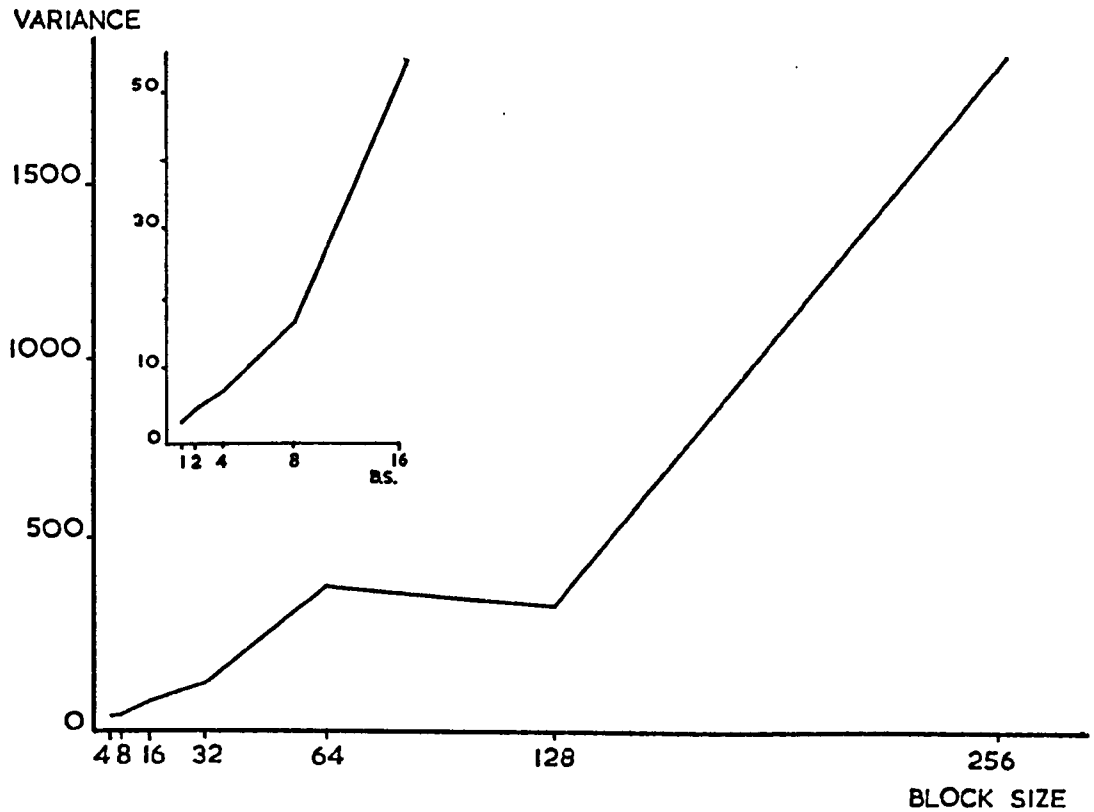
A pattern analysis was then carried out in order to determine whether the plants were 'clumped' at any particular block size (Fig 4.2a). Two peaks were discernable, a slight one at block size 16 and a more pronounced one at block size 64. The factors causing this pattern were not obvious, although the smaller peak might relate to the area of seed dispersal around an individual. Further microtopographic and covariance analysis with other species, would probably be necessary before any definite conclusions could be drawn.

4.8.3) Analysis of the distribution of picrate types.

A similar analysis was carried out for the distribution of each picrate type across the area studied (Table 4.13). This showed the very interesting result, that the higher picrate response plants are distributed randomly, whereas the low picrate response individuals are distributed non-randomly.

A pattern analysis was then carried out on the data (Fig 4.2b, c, d, e).

(a)



(b)

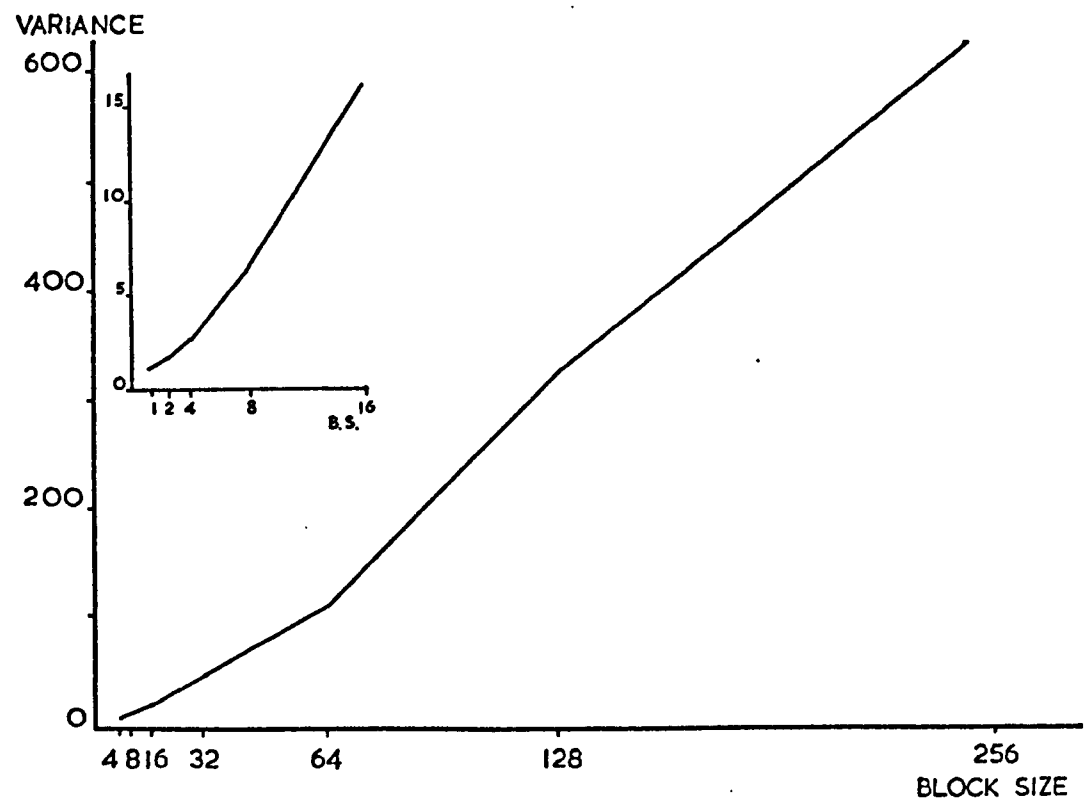
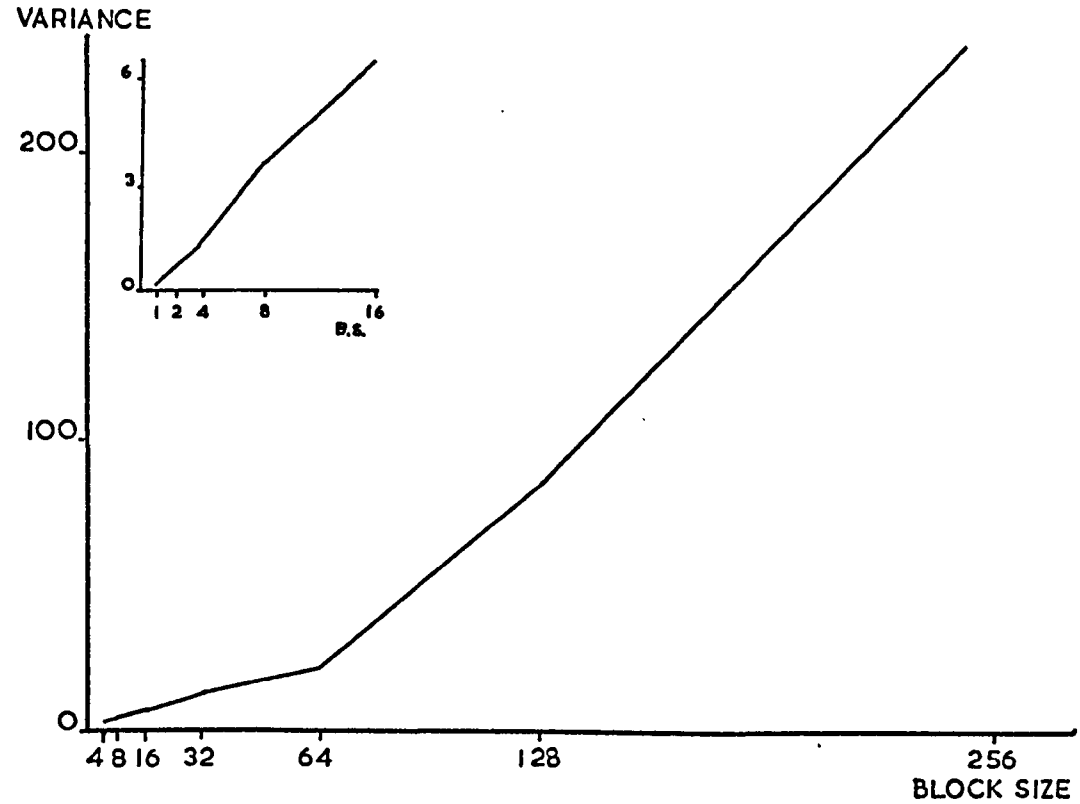


Fig. 4.2 The summary of the pattern analysis at S. Foreland, to show the relationship between the variance of the samples and the block size. (a) Total data. (b) Picrate type 1.

(c)



(d)

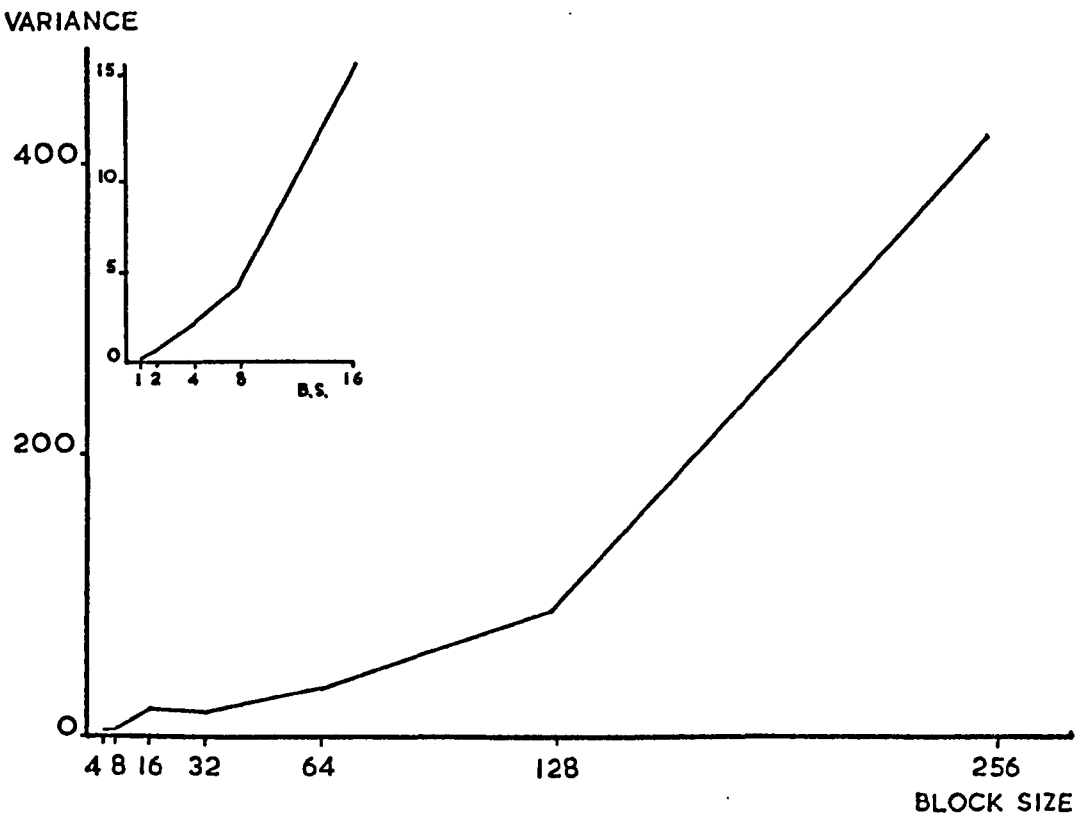


Fig. 4.2 (continued) (c) Picrate type 2. (d) Picrate type 3.

(e)

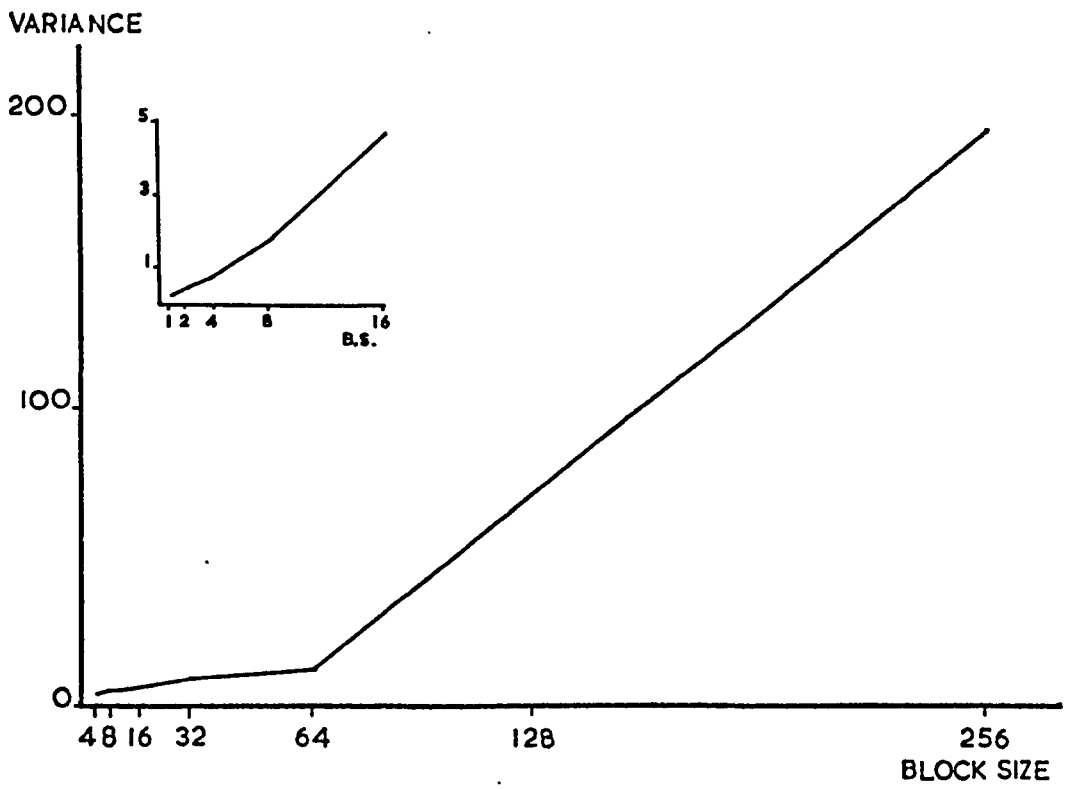


Fig. 4.2 (continued) (e) Picrate types 4 and 5.

Table 4.13 Analysis of the distribution of picrate types.

Picrate score		Individuals/quadrat 0	1	2	3	X^2	p	Variance:mean ratio	t	P
1	Observed (O)	442	58	11	1					
	Expected (E)	437.1	69.1	5.5	0.28	9.3	< 0.01	1.17	18.8	< 0.001
2	O	473	35	4	-					
	E	474.4	36.1	1.37	-	5.1	< 0.05	1.10	1.66	< 0.10
3	O	468	40	4	-					
	E	469.8	40.4	1.73	-	3.0	< 0.10	1.07	1.19	< 0.20
4	O	477	32	3	-					
	E	478.2	32.7	1.11	-	3.2	< 0.10	1.08	1.37	< 0.20
5	O	511	1	-	-					
	E	511.0	1.0	-	-	0.0001	1.00	1.00	-	1.00

Only picrate type 3 showed a noticeable pattern, at block size 16. If, as suggested above, block size 16 represents the principal area of seed dispersion, then it is suprising that the other picrate types do not show a similar peak.

The other picrate types all show slight peaks (1 at B.S. 128, 2 at B.S. 32 and 8, 4&5 at B.S. 32). Kershaw (1964) suggests that slight peaks may be due to a few major environmental factors operating, whereas sharp peaks probably represent some regular biotic pattern. He also warns, that results from species at a low density (as in this case) should be treated with caution. As has been pointed out previously, it is not obvious if any major environmental factors are operating to cause these patterns, although the peaks at a lower block size may represent the area of seed dispersal.

It was also of interest to determine if there was any association between individuals of differing picrate response. This was analysed by means of contingency tables, on the basis of the presence or absence of any particular type within each 1m quadrat (Table 4.14).

Table 4.14 The association between each picrate type. Values are as chi-squares with their positive (+) or negative (-) associations, and probability.

	Picrate score				
	1	2	3	4	5
1	+4.1	-5.5	-4.2	-4.9	0.9
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.75
2		3.6	0.1	3.3	-
P		< 0.1	< 0.75	< 0.1	-
3			3.3	0.1	-
P			< 0.1	< 0.75	-
4				1.0	-
P				< 0.40	-

It is noticeable that the higher picrate response plants show random association with each other. The association of high picrate plants with

type 1 plants are largely negative; type 1 plants showing positive association with each other.

4.8.4) Summary of pattern analysis study.

Overall, the individuals in the South Foreland population are not randomly distributed, there being a pronounced tendency for the plants to be clustered, probably due to the heavy seeds only being dispersed a short distance. However it should be noted, that this tendency is not sufficiently pronounced for a pattern analysis, at the scales used, to effectively demonstrate it.

The distribution of picrate types shows some interesting features. Type 1 plants are not randomly distributed (Table 4.13), neither are type 2 plants, whereas types 3, 4 and 5 are. Similar features hold for the association between picrate types (Table 4.14). Whether these distributions within a population are due to any selective pressures is not known. It could be argued that there is a selective pressure resulting in the higher picrate response plants showing a random distribution, whereas this pressure does not operate on the low picrate response plants.

5) STUDIES ON PREDATION BY PIERIS BRASSICAE L.

5.1) Introduction

Historically, P. brassicae has been a major predator of B. oleracea, but with the recent destruction of breeding grounds in the Baltic (where they fed on Lepidium latifolium L.), numbers of P. brassicae have since declined in W. Europe (Gardiner, no date). The widespread use of pesticides has probably speeded this decline. Nevertheless P. brassicae can still be an important predator of B. oleracea (Plates 5.1 and 5.2). The larvae of P. brassicae can completely strip a plant of its laminar tissue, although the plants appear able to survive this damage over one season. However it is likely that heavy predation in successive seasons would cause death of individual plants. Thus if P. brassicae shows a consistent choice of host plants, this might have strong selective effects on B. oleracea. Unpredated morphs would gain in numbers at the expense of those which have been predated, due to the gradual loss through secondary infections of damaged plants and subsequent death.

It has been demonstrated that populations of B. oleracea vary with respect to their picrate morphs (chapter 4). It is suggested that the principal variants are allyl derivatives of sinigrin, due to variation in sinigrin levels (chapter 3). Larvae of P. brassicae are known to be stimulated to feed by sinigrin (Verschaffelt 1910; David & Gardiner 1966a, b; Schoonhoven 1967) and the imagines stimulated to lay eggs (David & Gardiner 1962; Schoonhoven 1972). Ma & Schoonhoven (1973) demonstrated that the higher the glucosinolate concentration of laminar tissue, the stronger the electrophysiological stimulation of gravid females, although they did not report appropriate egg laying experiments. P. brassicae imagines do not seem to be stimulated by allyl isothiocyanate, although Ma & Schoonhoven do not rule out the possibility.

Plate 5.1 A 5th instar larva of *Pieris brassicae* feeding on *B. oleracea* at S. Foreland, Kent.

Plate 5.2 *B. oleracea* at St. Margaret's Bay, Kent, damaged by *P. brassicae* larvae.

This earlier work has suggested that no relationship exists between levels of sinigrin in host plants, and their attractiveness to predators. As long as the plants contain sinigrin, as the majority do (chapter 3), then they were thought to be suitable for oviposition. Consequently no relationship between the picrate test and predation by P. brassicae was to be expected. If such a relationship is found, those plants which are chosen as hosts might be expected to be represented at a lower frequency in the population.

5.2) Laboratory studies on larval food preference

Adult P. brassicae can be very difficult to rear under laboratory conditions, although various laboratory strains have been bred (Appendix G , for laboratory culture).

There are various methods of analysing the feeding preferences of insect larvae; one of the most favoured is the provision of a test diet for a fixed period of time,after which the numbers of frass pellets are counted (Nayar & Thorsteinson 1963). The greater the number of frass pellets, the more palatable the diet is thought to be.

5.2.1) Analysis of palatability by frass counts.

Six standard leaf discs were taken from plants of known picrate response (chapter 2) and placed in a 9cm crystallising dish, with four larvae of the final instar. The larvae had been starved for six hour prior to the experiments. The experiments were run two hours at room temperature and under even illumination (the larvae tend to be phototactic). At the end of the two hour period the number of frass pellets in the dish were counted. 40 such experiments were carried out and the results pooled (Table 5.1).

The results, based on mean values for the numbers of frass pellets, suggest that low picrate response plants are favoured by the larvae.

Table 5.1 Summary and analysis of the palatibility experiments based on frass counts.

Picrate score	1	2	3	4
mean no. frass	45.4	40.4	40.4	29.4
Coefficient of variation %	69.2	60.7	35.2	26.1

The coefficients of variation show that there is a considerable degree of variation around these means, reflecting either a wide individual or group variation in response. A t-test of the differences between the means, confirmed this wide variation, in that there was no significant difference between any of the means. (See additional data)

Nevertheless, mean values suggest a slight degree of preference for low picrate score plants. In the field even a slight degree of preference might have a selective effect over many years. Thus it was important to discover if larvae would show a preference for any type, when provided with a choice.

It is only at the fifth instar that choice would be important. In the first four instars the larvae remain on the plant the eggs were laid on, feeding and moving in unison. During the fifth instar the larvae lose their synchrony, dispersing to separate parts of the plant and sometimes to neighbouring plants. If the larvae found the plant they were on unpalatable, then this dispersion would probably be accentuated. To test whether the larvae preferred any one picrate type when offered a choice, a series of multiple choice experiments were set up.

5.2.2) Multiple choice analysis of larval palatability preferences.

The experimental technique was similar to that in section 5.2.1, except that three discs from each of two plants were provided, these being

arranged alternately. In total, 140 such experiments were made, not only between laminar tissue of different picrate score, but also between tissue of the same picrate score but of different reflectance values. The results were calculated in terms of the percentage of the total fresh weight eaten for each type (Table 5.2). Controls were run to take into account any loss in weight due to dessication. The simple total weight or total percentage weight of any one type was not used, because the total weights and percentages eaten varied from experiment to experiment. This seemed to depend upon the variation between individual larvae and to a limited extend upon the stage of development within the instar. Due to limited availability of picrate types three and five, not every combination of picrate types could be used.

The outstanding feature of these results is the overall lack of preference shown by the larvae. In nearly every case there is less than 10% difference between the means. The t-ratios show that in all but one case the differences are not significant, that is the variation in larval choice is so great that any differences between the means are negated. The coefficients of variation also illustrate this point. Not only is there no significant difference between the means, but there is no trend in the choice, i.e. the larvae do not tend to select, for example, the lower picrate type every time. If the results are analysed as high against low picrate response, then the mean percentage for the low picrate response is 49.7% and for the high response 49.9% ($t = 0.05$). This confirms the lack of preference shown by the larvae.

As a check on the reaction of larvae to the experimental conditions and to analyse their method of choosing a disc to feed on, a series of time lapse films were taken. These films clearly illustrate that the larvae have a search image. They can be seen moving round the discs, sampling each disc and then choosing one disc to start feeding on. This procedure may be repeated several times during the course of an experiment and a different

Table 5.2 Summary of the multiple choice experiments. The percentage values show the proportion of the total weight of each pair of picrate types eaten. (The right hand reading is of the plant with the lower reflectance value).

Picrate score		1	2	3	4
1	%	53.8 46.2			
	t	0.8			
2	%	54.4 45.6	47.1 52.9		
	t	0.7	0.4		
3	%	-	33.3 66.7	43.9 56.1	
	t	-	3.4*	-	
4	%	44.2 55.8	48.9 51.1	55.7 44.3	48.7 51.3
	t	1.1	0.2	1.5	0.3
5	%	51.2 48.8	-	-	54.9 45.1
	t	0.2	-	-	0.6

Key: % percentage of total weight eaten.
t t- ratio between means.
* probability 0.001

For original results see additional data.

disc may be chosen each time. The larvae did not necessarily feed as a group since each larva might choose a separate disc to start on, and then move independently to another disc. The picrate score of the disc was apparently immaterial.

5.2.3) Summary of laboratory studies on larval preference.

Under laboratory conditions and with the strain of P. brassicae used, the larvae do not show an overall preference for any one picrate type or types. Schoonhoven (1967) demonstrated that the larvae only require a very low concentration of sinigrin for feeding activity to be stimulated, higher concentrations of sinigrin failing to increase feeding activity. This suggests that even picrate type 1 plants may contain sufficient sinigrin to stimulate feeding activity. Further selection might depend upon other feeding stimulants, e.g. sugars (Schoonhoven 1967, 1972). However as Ma & Schoonhoven (1973) point out, some care should be taken when drawing conclusions based on laboratory strains.

5.3) Field studies on larval food preference

In section 5.2 it was demonstrated that under laboratory conditions, the larvae of P. brassicae show no overall food preference for different picrate types of B. oleracea. Field studies were undertaken to determine whether the larvae were to be found randomly distributed on all picrate morphs, as the laboratory experiments suggested they might be.

The study consisted of visiting various populations during August and early September (this is when P. brassicae predation is at its height) and carefully searching for larvae and/or eggs. When a plant was found to be predated a standard leaf disc was removed for picrate typing. A random sample of discs was also taken for picrate typing, these samples being used as the controls for comparative purposes. The results were calculated as

the relative percentage frequency of plants predated in each population, for each picrate type (Table 5.3). For each population a comparison was made between the observed picrate frequency of predated plants and the expected frequency, as based on the random sample of the population (Table 5.4).

In 11 out of the 16 studies, the distribution of larvae did not follow the expected random distribution. At nearly every site the larvae were found preferentially on the highest picrate score plants (as shown by the + sign in Table 5.4); at 10 sites this preference was shown to be significant. In the other cases, the trend although not significant, was present (doubtless due to the small size of the samples).

A Friedman non-parametric analysis of variance of the data in table 5.3 (the data was divided into 5% classes as in section 4.3) demonstrated that the distribution of P. brassicae larvae between the different picrate morphs, followed the same pattern at all the sites ($\chi^2_R = 21.4$ $p < 0.15$ d.f. 15).

These results strongly suggest that in the field, there is a selection of high picrate response plants as a food source for P. brassicae larvae. The results of section 5.2 show that it is unlikely that the larvae are selective in their food source. Larvae tend not to move very far from their site of egg emergence, particularly in the first four instars. Even in the fifth instar when the larvae tend to lose their synchrony and disperse, they rarely appear to leave their 'parent' plant (several fifth instar larvae are usually found on the same plant). This suggests that if the larvae are not selective as to host plant, then the gravid imagines must be. Radcliffe & Chapman (1966a) demonstrated that the preferences of gravid P. rapae L. was reflected by the distribution of their larvae on B. oleracea. If as seems likely, the same behavioural pattern is found in P. brassicae, then gravid imagines may seek high picrate score plants as their preferred site for oviposition.

Table 5.3 The relative frequency (%) of predated plants in each picrate category.

Site and Year	Picrate score 1	2	3	4	5	N[1]
Great Orme						
1974	0.0	0.0	0.0	25.0	75.0	8
S. Foreland						
1973	11.8	11.8	21.6	37.3	17.6	51
1975	5.3	0.0	2.6	36.8	55.3	38
St Margaret's Bay						
1973	6.3	14.6	14.6	33.3	64.6	48
1974	0.0	0.0	0.0	35.3	64.7	17
1975	0.0	0.0	3.8	23.1	73.1	26
Stoney Bay						
1974	0.0	0.0	7.7	53.8	38.5	13
1975	0.0	0.0	0.0	26.7	73.3	15
Whitby						
1974	0.0	14.3	14.3	28.6	42.9	7
Staithes						
1974	0.0	0.0	0.0	0.0	100.0	4
Tynemouth						
1973	14.3	14.3	14.3	57.1	-	14
1974	0.0	0.0	0.0	18.2	81.8	11
1975	0.0	0.0	0.0	0.0	100.0	20
Crail						
1974	0.0	0.0	8.3	50.0	41.7	12
1975	5.9	23.5	29.4	29.4	11.8	17
Auchmithie						
1974	13.3	6.7	13.3	33.3	33.3	15
mean	3.6	5.3	8.1	30.5	61.0	

1. number of plants predated in the population.

Table 5.4 Summary of the field survey of *P. brassicae* predation. The data is presented as chi-squared values and the probabilities associated with significant differences from random predation[1]. Comparisons are between plants of different picrate types within a population.

Site and Year	Picrate score 1	2	3	4	5	X^2 summary	N^2
Great Orme							
1974	0.3	0.4	0.7	0.9	2.9	5.2	8
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
	-	-	-	-	+		
S. Foreland[3]							
1973	0.6	7.7	0.9	2.1	14.2	25.5	51
p	N.S.	<0.01	N.S.	N.S.	<0.001	<0.001	
	-	-	+	+	+		
1975	2.0	0.0	1.9	3.9	2.2	10.0	38
p	N.S.	N.S.	N.S.	<0.05	N.S	<0.05	
	+	=	-	-	+		
St. Margaret's Bay							
1973	7.1	14.6	2.2	0.1	320.0	344.0	48
p	<0.01	<0.001	N.S.	N.S.	<0.001	<0.001	
	-	-	-	+	+		
1974	0.3	19.4	2.5	2.4	14.5	39.1	17
p	N.S.	<0.001	N.S.	N.S.	<0.001	<0.001	
	-	-	-	+	+		
1975	0.0	0.0	0.2	5.1	8.9	14.2	26
p	N.S.	N.S.	N.S.	<0.05	<0.01	<0.01	
	=	=	-	-	+		
Stoney Bay							
1974	1.0	0.5	0.2	0.1	0.9	2.7	13
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
	-	-	-	+	+		
1975	0.0	0.0	3.0	4.7	24.8	32.5	15
p	N.S.	N.S.	N.S.	<0.05	<0.001	<0.001	
	=	=	-	-	+		
Whitby[4]							
1974	0.1	7.0	0.7	0.6	0.2	8.6	7
p	N.S.	<0.01	N.S.	N.S.	N.S.	N.S.	
	-	+	-	-	+		
Staithes[4]							
1974	0.3	0.3	0.5	1.8	8.6	11.5	4
p	N.S.	N.S.	N.S.	N.S.	<0.01	<0.05	
	-	-	-	-	+		

Table 5.4 (Data continued)

Site and Year	Picrate score 1	2	3	4	5	X^2 summary	N^2
Tynemouth							
1973	3.8	0.9	0.0	68.8	*	73.5	14
p	N.S.	N.S.	N.S.	< 0.001		< 0.001	
	-	-	=	+			
1974	2.2	2.2	1.5	0.8	6.3	13.0	11
p	N.S.	N.S.	N.S.	N.S.	< 0.05	< 0.05	
	-	-	-	-	+		
1975	0.7	0.0	2.9	8.6	18.8	31.0	20
p	N.S.	N.S.	N.S.	< 0.01	< 0.001	< 0.001	
	-	=	-	-	+		
Crail							
1974	0.2	0.6	0.5	2.3	2.1	5.7	12
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
	-	-	-	+	+		
1975	0.1	1.6	0.4	2.5	2.5	7.1	17
p	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
	=	+	+	-	+		
Auchmithie[4]							
1974	2.1	1.2	0.4	1.1	6.4	11.4	15
p	N.S.	N.S.	N.S.	N.S.	< 0.05	< 0.05	
	-	-	-	-	+		

Key:
1. The +, - and = signs indicate whether the observed frequencies were greater, less than or equal to the expected frequencies.
2. N is the number of predated plants in the population.
3. No predation observed during 1974 visit.
4. No predation observed during 1975 visit.

* No type 5 plants recorded.

N.S. significance $p > 0.05$

5.4) Studies on the egg-laying preferences of P. brassicae.

To investigate the egg-laying (oviposition) preferences of gravid females, various laboratory studies were undertaken in which females were presented with a choice of plants of differing picrate response.

Wild P. brassicae imagines are almost impossible to use for experimental purposes under laboratory conditions; the laboratory strain used in section 5.2 were used wherever possible.

5.4.1) Cage experiments.

The initial experiments were carried out in cages of dimensions 45cm x 60cm x 75cm containing four potted B. oleracea of known picrate response. The cages were placed in a window (David & Gardiner,(1961a) considered daylight essential for copulation). Six females and six males were then released into the cages. The imagines fed on a 10% sucrose solution from artificial flowers (Appendix G). The numbers of eggs laid were recorded at the same time every day (Table 5.5), each batch being marked as it was counted. The position of the plants was changed every day, according to random number tables.

5.4.2) Controlled environment room experiments.

Subsequent to the cage experiments, a controlled environment room became available for use. The room was illuminated with mercury vapour and tungsten lamps (in a wattage ratio of 3:1) at an intensity of 8280 lux. The temperature was maintained at 20°C. Eight (or in one case six) plants of differing picrate responses were placed in the room, and periodically moved as before. The imagines were fed using artificial flowers (Appendix G) This combination of conditions promoted copulation. Under these conditions the imagines survived much longer than in the windows; consequently egg counts could be made every three days (Table 5.6). An indelible mark was made alongside each fresh batch of eggs. The potted B. oleracea were

Table 5.5 The number of eggs laid on caged plants.

(a)

Plant No.	mV R^1	Picrate score	days 1	2	3	4	5	6	7	8	9	sum
46	9.0	5	58	150	53	26	172	10	0	3	0	472
X	10.5	5	0	75	79	22	0	12	0	0	0	188
65	13.0	4	0	112	0	14	0	70	20	42	64	322
69	19.0	1	6	0	3	0	0	86	21	146	0	262

mean 311

(b)

Plant No.	mV R^1	Picrate score	days 1	2	3	4	5	6	sum
16	10.0	5	43	142	107	16	44	15	367
Z	11.5	4	93	0	0	0	16	82	191
28	12.0	4	0	0	0	158	33	68	259
22	13.0	4	0	12	43	25	0	5	85

mean 225.5

1. Reflectance values were used here in order to differentiate more accurately between the responses of individual plants.

Table 5.6 The numbers of eggs laid on plants in a controlled environment room.

(c)

Plant No.	mV R^1	Picrate score	days 3	6	9	12	15	18	sum
J	9.5	5	3	197	208	1322	55	0	1785
30	10.0	5	153	150	342	373	34	0	1052
A	10.0	5	13	36	43	78	54	0	224
T1	10.5	5	0	0	17	305	158	446	926
F	11.0	4	4	18	199	0	73	536	830
9	11.0	4	0	0	0	262	350	21	633
T2	11.0	4	0	60	64	116	229	34	503
B	12.5	4	200	125	421	0	370	270	1386

mean 921

(d)

Plant No.	mV R^1	Picrate score	days 3	6	9	12	15	18	21	sum
T6	9.0	5	4	94	171	614	279	50	33	1703
T4	10.0	5	0	0	0	18	0	79	541	638
36	10.0	5	0	166	146	22	0	0	0	334
D	10.5	5	110	66	286	416	165	0	0	1043
T5	11.0	4	33	19	33	25	121	286	53	570
E	12.5	4	76	63	173	71	0	12	0	395
T7	14.0	3	14	0	148	10	0	0	98	256
T3	20.0	1	0	0	0	269	0	218	0	487

mean 678

Table 5.6 (Data continued)

(f)

Plant No.	mV R^1	Picrate score	days 3	6	9	12	15	18	sum
T4	10.0	5	0	26	477	0	214	0	717
F	12.0	4	0	24	16	103	23	24	190
E5	14.0	3	0	13	24	316	103	17	473
D	14.0	3	0	0	0	116	0	6	122
T7	16.5	2	21	9	54	0	54	62	200
J	17.0	2	0	0	0	163	0	256	419
E4	18.0	1	85	0	0	7	212	91	368
36	20.0	1	0	12	40	40	0	16	108

mean 325

(e)

Plant No.	mV R^1	Picrate score	days 3	6	9	12	15	sum
A	11.0	4	35	55	301	119	76	586
E2	12.5	4	49	0	101	0	0	150
T5	14.0	3	14	107	0	36	0	157
E3	14.0	3	0	0	12	5	0	17
9	15.0	3	14	135	10	0	0	159
E1	17.5	2	14	0	77	14	0	105

mean 196

arranged according to a random array, the position of each plant being changed each day.

At the beginning of each experiment 40 females and 40 males were released into the room.

The results displayed in tables 5.5 and 5.6, clearly show that the plant with the stronger picrate response in an experiment, is preferred for egg-laying. When the data is analysed in terms of picrate score (Table 5.7), this preference is clearly shown.

Table 5.7 Analysis of the egg-laying preference experiments in terms of picrate scores. The data is as mean number of eggs/picrate group.

	Picrate score							
Experiment	1	2	3	4	5	X^2 summary	p	d.f.
a	262	-	-	322	330	9.1	<0.05	2
b	-	-	-	178	367	65.5	<0.001	1
c	-	-	-	838	997	13.8	<0.001	1
d	487	-	256	483	930	443.0	<0.001	3
e	-	105	111	368	-	232.0	<0.001	2
f	238	310	298	190	717	505.0	<0.001	4

When type 5 plants were present, then they were the preferred plants for egg-laying, or as in the case of experiment (e), type 4 plants, Chi-square analysis illustrates the degree of non-randomness in the egg-laying.

It was notable that the plants with the next highest totals of eggs, were not necessarily of the next highest picrate response e.g. experiment (c) (Table 5.6) (in which however all plants had rather similar and high picrate responses). This was also shown by the analysis of mean number of eggs/picrate group (Table 5.7 experiments d & f). This suggests that the egg-laying response may not be limited to the chemicals giving the picrate response. For instance, if the preferred plant has all the suitable sites for egg-laying occupied, another plant may be sought which may not necessa-

rily be that of the next highest picrate score. (The most favoured plant tended to have large numbers of eggs laid on it during the early stages of the experiment). Schoonhoven (1972) and Ma & Schoonhoven (1973) suggest that other chemical stimuli may be involved in the selection of host plants e.g. variation in appropriate levels of sucrose; these experiments may illustrate such variation. Individual imagines also vary in their degree of response to chemical stimuli (Ma & Schoonhoven 1973); again this might account for some of the observed variation.

5.5) Summary

The results in tables 5.5 and 5.6 agree with the field studies, in that the higher picrate response plants tend to be those selected by gravid P. brassicae for oviposition, although some eggs may be laid on plants of all picrate responses. The overall effect is for the higher picrate response plants to suffer greater damage from larvae, than will lower picrate response plants. It was very noticeable in the field that only pre-flowering plants were predated; this observation probably fits in with the work of Ilse (1937). She found that gravid P. brassicae are stimulated to egg-laying on green or green blue substrates; yellow substrates only elicit a feeding response. Predation is usually at a low level during the flowering season, but what predation there is would be confined to the pre-flowering plants (the flowering plants produce many spikes of yellow flowers). Later in the season when predation is at its peak, flowering plants have very few leaves, and as a result they probably have little 'visual impact', pre-flowering plants promoting a much greater visual response. On the few occasions when larvae have been observed on flowering plants, these plants have had leaves more typical of sterile plants.

As mentioned in the introduction (section 5.1), previous workers have suggested that there was no relationship between levels of sinigrin and

egg-laying preferences. The results in chapter 4 suggest that sinigrin will be present in most plants (every type 5 plant contains sinigrin), and that gravid P. brassicae are responding to variation in the levels of sinigrin derivatives (variation in the levels of glucosinolate derivatives are usually taken to indicate variation in glucosinolates, Kjaer 1960). My results suggest that the higher the levels of sinigrin in a plant, the greater its chance of being chosen for oviposition, and subsequent larval predation.

6) THE ROLE OF OTHER PREDATORS AND PATHOGENS ON B. OLERACEA

6.1) Introduction

It was demonstrated in chapter 5, that Pieris brassicae may be one of the major determinants of picrate score frequency in wild populations of B. oleracea. The importance of P. brassicae is confined to the mature, sterile phase of B. oleracea. It was noted in chapter 4 that strong selective forces also operate at the seedling stage, but in a reverse direction, low picrate score plants being preferred.

Deaths of mature plants were observed [illegible] ; stage,

Erratum.) picrate

For Helix aspera read Helix aspersa ıg the course

)ter 5,

P. brassicae appears to be most important as a debilatory factor rather than a cause of death. Observations in both the laboratory and field suggest other species may actually cause death. Also, at the seedling stage, no dead seedlings were observed, despite in some cases a 90% mortality being inferred (no doubt this is through the non-persistence of dead seedling tissue). It was apparent that at this stage, a number of predators and pathogens were important.

6.2) The role of Molluscs

Snails were commonly found on mature plants (both pre-flowering / and flowering), particularly Helix aspera (Müller) (Plate 6.1) and at some sites Cepea nemoralis L. H. aspera may cause extensive damage (Plate 6.2), although this is exceptional. There was no direct evidence of snails predating seedlings. Slugs were rarely found on mature plants, although both Arion ater L. and Agriolimax reticulatus (Müller) were recorded. There is some evidence that they are important as seedling predators.

Plate 6.2 B. oleracea extensively damaged by Molluscs at Tynemouth, Northumberland.

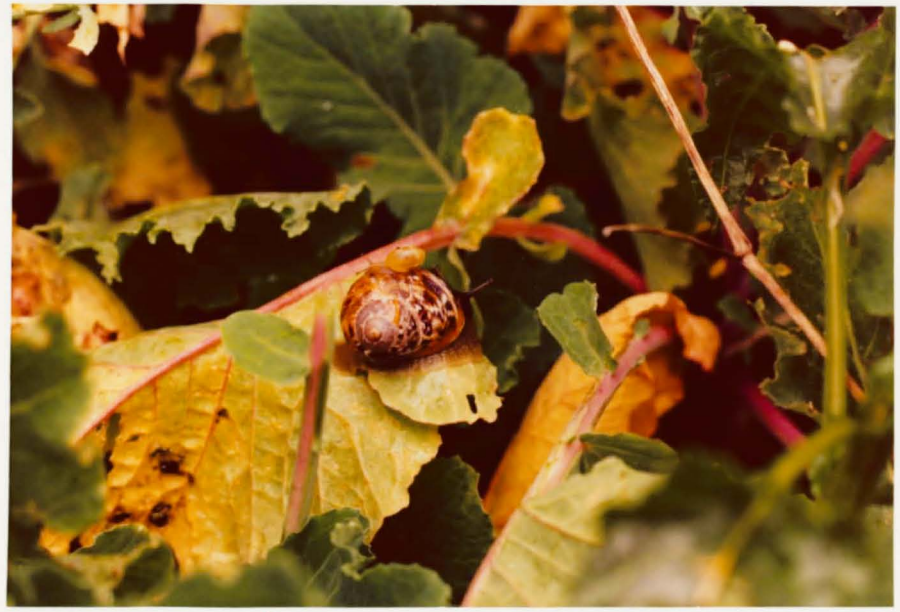


Plate 6.1 Helix aspera grazing B. oleracea at Looe, Cornwall.

6.3) Experiments on Molluscan food preferences.

Palatability experiments were performed in a similar manner to the P. brassicae studies. The major problem in studies of Molluscs, is the necessity of keeping the experimental chambers cool and moist. It was found throughout these studies, that Molluscs prefer to eat cellulose in the form of filter paper or cotton wool rather than B. oleracea leaves (a similar effect has been observed in cyanogenesis studies, see Jones 1972). Thus these materials could not be used to keep the experimental chambers moist. A comparatively satisfactory answer, was to line the dishes with saturated, fine sand. In some cases, particularly with slugs, this medium did not appear to be suitable (see later).

In all cases the animals were starved for 24 hours prior to the experiments. One animal was placed in each dish, containing two standard laminar discs (section 2.2) of different picrate response. The snail experiments were run for 4 hours and the slug for 6 - 8 hours. At the end of this period, the discs were lightly dried and re-weighed, the results being calculated as the percentage of the total weight eaten per discs, as against controls (Table 6.1).

In the case of H. aspera 106 such tests were carried out, although the shortage of picrate types 3 and 5 precluded the full range of comparisons being made. As the data suggests, (Table 6.1a) no overall preference was shown between the different picrate types. This bears out the field observations, that H. aspera (and C. nemoralis) are found on plants of all picrate types.

In the similar experiments carried out using the slugs A.ater and A.reticulatus, it was found almost impossible to successfully run experiments. Despite many attempts to provide suitable conditions, only one series of experiments using A. ater worked (involving 15 comparisons, Table 6.1b).

Table 6.1 Choice experiments using *H. aspera* and *A. ater*.

The results are as t- values with their associated probabilities.

(a) *H. aspera*

	Picrate score			
	1	2	3	4
1	2.7			
P	<0.1			
2	1.4	-9.6		
P	<0.2	<0.001		
3	-0.5	15.0	*	
P	N.S.	<0.001		
4	2.5	1.7	-0.2	-2.1
P	N.S.	N.S.	N.S.	<0.05

(b) *A. Ater*

	Picrate score	
	1	3
3	-1.2	
P	N.S.	
4	0.5	-0.3
P	N.S.	N.S.

Key: - the lower picrate response was favoured

N.S. probability > 0.2

* no data available

The main problem was that the slugs would not feed on the discs. Numerous deaths occurred, which were apparently not due to lack of moisture. It may have been due to starvation; alternatively toxic compounds released from the discs killed the slugs, or deterred the slugs from feeding.

If the few successful experiments are considered, then no preference was shown. As with H. aspera and C. nemoralis, A. ater has been found on mature plants of all picrate types. However some work by Withers (1974, unpublished data) on the selection of picrate morphs by Agriolimax agrestis is of note. She found that this species of slug when offered a choice, preferred low picrate response laminar tissue, particularly when the plants had been pretreated at 4°C as compared with 22°C.

A series of time-lapse films were made of the slug and snail palatability experiments. Various features emerged from these films. The animals all showed strong search-image patterns. Usually all the discs would be 'sampled', but it was not obvious why any one disc (which could be either of low or high picrate score) was eaten further. In several cases both slugs and snails appeared to be 'deterred' from eating more of the discs, after having 'sampled' only one or two discs.

The conclusion drawn from these studies, was that Molluscs probably do not show any preference as to different picrate types of mature laminar tissue (although Withers' work should be noted). The numerous slug deaths suggested that they either found, mature laminar tissue unpalatable or that it was toxic. In many slug experiments the tissue was not touched, which suggested that it was the volatile compounds in the tissue which were noxious. These reactions were not observed with H. aspera.

Mulluscs would not seem to be important as determinants of picrate frequency, by their grazing on mature plants. At the seedling stage this situation may be different.

As has been mentioned, there were few seeds set in the past few years and in only one season (1974-1975) could a few patches of seedlings be observed. These patches were visited each month, from October through to May. Despite the numbers of seedlings declining by up to 90% in one patch, few traces of Molluscan damage were observed and no 'slime trails' were found. From photographs taken during the visits, it is possible to make out damage due to slugs (Plate 6.3), which suggests that slugs may have been important predators.

A laboratory experiment using seedlings was attempted. If individuals of A.ater were left in a tray of seedlings, even for a few days, they would not eat the seedlings. Instead they buried into the soil or escaped through the drainage holes. This suggested that noxious, volatile compounds were being released from the seedlings. In a similar experiment using H. aspera, mechanical damage was the major cause of seedling death. An attempt was made to compare the reflectance values of the untouched and partially eaten seedlings. No significant difference could be found between the reflectance values of the two groups (untouched mean = 18.3, partially eaten mean = 18.8, $t = 0.68$, p not significant).

At the seedling stage, allyl isothiocyanate (AITC) is relatively more important as a volatile component than it is in mature leaves (Greenhalgh 1976), the ratio between AITC and allyl nitrile being more nearly equal. The quantity present in seedling cotyledons may be more than ten times that of mature laminar tissue. The maximum recorded from GLC analysis in B. oleracea subsp. oleracea mature tissue was 0.56mg/100g fresh weight, whilst in B. oleracea c v. January King seedlings, the mean level was 6.3mg/100g fresh weight (c v. January King is the cultivated cabbage with the most similar biochemistry to that of subsp.oleracea, sufficient seedlings of subsp. oleracea being unobtainable). It would be expected that higher levels than this may be found in seedlings of subsp. oleracea.

Plate 6.3 Damage to B. oleracea seedlings at Tynemouth due to Molluscs (M), Phyllotreta spp. (P) and Sitona spp. (S).

To test the effect of AITC on Molluscs, artificial media impregnated with AITC were prepared. Agar discs (2.01cm^2 in area) , consisting of 0.3g agar and 0.2g cellulose in 10ml water were used (Nayar & Thorsteinson 1963). As a food source these were found to be acceptable to both H. aspera and A. ater. The plates containing a known weight of agar were lightly dried when open, at 50°C for 20 mins. 0.5ml of a solution containing a known weight of AITC was then spread over the surface, the lid being replaced. By means of vegetable dyes which had no effect on the palatability, it was possible to follow the penetration of the AITC solution. Once the agar was fully penetrated, standard discs (section 2.2) were cut and used in palatability experiments. A range of concentrations of AITC were used, from 0.5mg/100g to 8mg/100g. Two discs of different concentrations were presented to the test animals, in petri dishes lined with saturated sand. After two hours the quantity of disc of each type eaten, was assessed by eye.

It was found that there was a threshold effect at 3mg/100g. At this concentration and below, no preference was shown for any one concentration. Above this concentration the discs were not touched. This relationship was quite clear for H. aspera. At concentrations above 3mg/100g the animals appeared to move as far away from the discs as possible. In the case of slugs, A. ater was used. A number of deaths occurred again (Withers 1974, (unpublished data) observed a similar effect), but where the animals did feed, the same threshold effect was observed. This suggested that AITC may be particularly toxic to slugs in enclosed situations, which could explain the problems met with in other experiments. The presumed lower susceptibility of snails to poisoning by gaseous AITC, may be because they can retreat into an impervious shell.

This result is important with regard to seedling damage. As mentioned in chapter 4, during the winter months the picrate frequency of seedling populations changes, and it is suggested that low picrate score seedlings tend to be lost;

a preponderence of high picrate score seedlings surviving. Work on cultivated B. oleracea seedlings shows the high levels of AITC reached. These levels (perhaps greater than 6 mg/100g) are probably well outside the palatability range of Molluscs. Thus it is likely that only those individuals with low levels of AITC would be eaten. This probably also explains the uniform distribution of Molluscs on mature plants of every picrate type, since these plants contain comparatively low levels of AITC.

It should be noted that there is evidence to suggest that many populations of B. oleracea are derived from cultivated plants (Mitchell 1976, Appendix E). The levels of AITC recorded in cv. January King are the maximum found in cultivated crops, the levels in other crops being much lower than this (Greenhalgh 1976). It might be expected that a wide range of AITC levels would be found in wild seedlings. There is at present no direct evidence linking the picrate test with levels of AITC. However it is shown (chapter 3) that the picrate test responds to allyl-nitrile and thus presumably to its progenitor sinigrin. As AITC is also a derivative of sinigrin it is reasonable to suppose that AITC may vary with the picrate test. The range of picrate types detected in seedling populations is equal to that in adults and thus high picrate score seedlings may be protected from Molluscan attack.

6.4) The role of fungal pathogens

Only one fungal pathogen was observed on mature plants, a powdery mildew of the genus Erisyphe (Plate 6.4). It was only observed at Tynemouth, where six plants became infected, all of picrate type 1. No deaths occurred due to the pathogen.

The other main pathogen is the downy mildew Peronospora parasitica (Pers ex Fr.) Fr. (Plate 6.5). Commercially this species is a very important pathogen of seedlings (Greenhalgh & Dickinson 1975). It is almost certainly found on wild populations (J.R. Greenhalgh, pers. comm.) where it could be important in determining the picrate frequency. Greenhalgh (1976) has shown that P. parasitica is very sensitive to AITC. The only cultivar

Plate 6.4 Powdery mildew, Erisyphe spp., on B. oleracea at Tynemouth, Northumberland.

Plate 6.5 Seedling B. oleracea infected by the downy mildew, Peronospora parasitica, during screening for resistance.

resistant to it is c v. January King, which produces large quantities of AITC as a seedling. A threshold effect operates such that at a concentration below 5mg/100g fresh weight, seedlings are susceptible to the pathogen. Greenhalgh and I screened several populations of B. oleracea for resistance to P. parasitica; the frequency of seedling resistance being compared to the overall frequency of picrates type 3, 4 and 5 in the populations (Table 6.2).

Table 6.2 A comparison of the overall frequency of picrate types 3, 4 and 5 in adult populations, against seedling resistance to P. parasitica.

	Picrate score			
Population	3	4	5	% Resistance[1]
S. Foreland	14.9	14.9	6.3	9.8
Crail	36.2	36.2	1.7	0.7
Tynemouth	11.0	1.4	0.0	0.1
Tenby	0.0	0.0	0.0	0.05
Great Orme	4.8	0.0	0.0	0.0

1. % of seedlings surviving infection under standardised incubation conditions, see Greenhalgh & Dickinson (1975).

The results in table 6.2 suggest that the percentage resistance to P. parasitica may be correlated with the frequency of adult picrate type 5 plants in the population. This further suggests that type 5 plants have preferentially survived from the seedling stage, in populations in which the infection of P. parasitica on seedlings may be important (although a survey of Molluscan population sizes would also need to be carried out, before any conclusions could be drawn, since they could play a similar role).

The role of P. parasitica in the field is otherwise unknown; no evidence of it was found in the patches of seedlings at Tynemouth. Infected seedlings will die and decompose very rapidly (in less than 10 days, J.R. Greenhalgh

pers. comm.), thus these may not have been detected.

6.5) The role of other species of potential importance at the seedling stage.

At the seedling stage two groups of insect commonly cause damage, the flea beetles (Phyllotreta spp.) and weevils of the genus Sitona. There is evidence that species of both genera are active in wild populations (Plate 6.3, damage identified by B.J. Selman), but their response to variation in the volatile compounds from B. oleracea is not known. The photographs suggest that the majority of seedlings are liable to be attacked.

6.6) Species occasionally found on mature sterile plants.

The larvae of another Lepidopteran, Pieris rapae are occasionally found. On the few occasions where it has been found, the larvae were on type 5 plants.

The cabbage aphid, Brevicoryne brassicae L. (Plate 6.6) is commonly found on mature plants (both pre-flowering and flowering), although it rarely causes severe damage on its own in the field. The main effect of this species is debilatory, particularly when a plant showed severe infestation (Plate 6.7) although except under laboratory conditions no deaths were recorded. In the laboratory B. brassicae infestations occurred after the conclusion of predation studies with P. brassicae larvae. The damaged plants would become infested with aphids, severe leaf curl resulting; the plants rarely recovered. Tertiary fungal and bacterial infections were also important at this stage. These observations suggest a means by which high picrate score plants may be lost from a population. After damage by P. brassicae larvae, the tissues will release AITC. Van Emden (1972) has demonstrated that B. brassicae responds positively to increased concentrations of AITC and sinigrin; thus they would tend to be attracted to damaged plants. The debilatory effect of B. brassicae perhaps prevents materials being stored in the tap

Plate 6.6 Brevicorvyne brassicae infesting a flowering spike of B. oleracea, at Durdle Door, Dorset.

Plate 6.7 B. oleracea severely infested with B. brassicae at Tynemouth, Northumberland.

root. This effect could cause over-wintering difficulties, or may hold the species back in the following growing season.

6.7) Predators of the flowering plants.

Few species are found feeding on flowering individuals. P. brassicae is rarely found on these plants; snails and B. brassicae are occasionally found, the latter may completely clothe the flowering spike (Plate 6.6).

Two species have become of importance since 1970, these are the cabbage seed weevil, Ceuthorrhynchus assiimilis (Payk.) and a gall midge Dasyneura brassicae (Winn.) The former species makes small holes in the seed pods through which it lays its eggs. The larvae usually eat all the seeds in the pod. The second species, D. brassicae lays its eggs through the holes made by C. assiimilis (Edwards & Heath 1964). These larvae eat the seeds and also cause premature ageing of the seed pods, with the subsequent complete loss of seed. I have also observed secondary fungal and bacterial infections of the damaged pods. Extensive studies at Tynemouth demonstrated that plants of all picrate scores were affected; in the seasons 1973-1975 there was almost 100% seed loss. All the populations on the N.E. coast were similarly affected. Lesser damage has been observed at S. Foreland and Tenby.(Plate 6.7)

6.8) Summary

Predators and pathogens were shown to be very important at the seedling stage, particularly P. parasitica and probably Molluscs; these might be responsible for the observed change in picrate response in seedlings. The high picrate response seedlings would apparently be resistant to these organisms, probably through high levels of allyl isothiocyanate.

At the mature, pre-flowering stage, B. brassicae in conjunction with P. brassicae is probably responsible for the loss of high picrate score

Plate 6.7 Damage due to Ceuthorrhynchus assiimilis and Dasyneura brassicae at Tynemouth. Note the damaged and infected seeds, as well as the premature ageing.

plants from the populations, counteracting the increased numbers of high picrate score plants entering the adult population.

Selection pressures from predators appear to become relaxed on the flowering individuals; instead there is damage to the seeds. The effect of seed predation can currently be seen in the wild populations, in which there are few plants of less than 3 years of age. (Table 4.5).

7) INHERITANCE OF THE PICRATE RESPONSE/GLUCOSINOLATE CONTENT

Very little research has been undertaken on the inheritance of glucosinolates. Principally it has been concerned with the glucosinolate content of various crop Brassica seed meals. Kondra (1967) showed that the levels of glucosinolates in the seeds of B. campestris were similar to that of the maternal parent after crossing. He also showed that the genes controlling the levels of gluconapin, glucobrassicanapin and progoitrin segregate independently in B. campestris. Kondra & Stefannson (1970) undertook similar analyses of B. napus L. seeds. They again found that gluconapin, glucobrassicanapin and progoitrin showed maternal effects on reciprocal crossing, except that in B. napus these three glucosinolates do not segregate independently. Kondra & Stefannson (1970) also obtained evidence on the numbers of alleles controlling low levels or absence of these three glucosinolates. Three recessive alleles control low gluconapin content, the control of high gluconapin content showing partial dominance. There are four or five alleles controlling absence of glucobrassicanapin, there being heterozygote superiority in the control of high production. For progoitrin, the control of high levels of production shows partial dominance, four recessive alleles controlling its absence.

There is a small quantity of work on the inheritence of the thiocyanate producing glucosinolates in laminar tissue, i.e. glucobrassicin and neo-glucobrassicin. Josefsson's (1967b) results indicated that the thiocyanate content of B. napus and B. oleracea were partly under genetic control. He found that the thiocyanate content of individual B. napus plants, was highly correlated with their selfed progeny. Johnston & Gosden (1975) working on forage kale (B. oleracea) have shown that there is a high degree of heritability between the thiocyanate content of F1 hybrids and their mid-parent values. They state that the volatile isothiocyanate content varies independently of the thiocyanate content. My results similarly show that the

picrate response and thus probably the sinigrin content varies independently from the thiocyanate content (section 3.4.2).

At present there are no published experiments on the inheritance of the glucosinolates producing volatile derivatives in B. oleracea. I attempted to analyse the inheritance of the picrate response both under laboratory and field conditions. However, I found it impossible to induce flowering in the laboratory by the usual techniques i.e. by varying day length and/or temperature. Under field conditions some crosses were made. However the total loss of seed resulting from Dasyneura brassicae and Ceuthorrhynchus assiimilis attack prevented any further analysis. Some data is available on the picrate response of naturally occurring seedlings at Tynemouth, arising from presumptive parents of known response (Table 7.1)

Table 7.1 A comparison of the maternal picrate response with the response of seedlings immediately after emergence in October.

Ref No.	Reflectance mV	
	Parent	Mean seedlings
P1	8.5	13.5
TS3	13.0	18.5
P2	14.0	14.9
TS2	15.0	18.0
P1A	15.5	17.8
D1	17.0	18.8
TS1	17.0	19.9

Analysis of this data suggests that the mean reflectance value of the seedling is correlated to that of the maternal parent ($r = 0.819$ $p < 0.02$ d.f. 6). As might be expected from this result, the X^2 of the difference between the seedling and parent reflectance values is not significant ($X^2 = 7.19$ d.f. 6 $p < 0.3$). This result suggests that for the inheritance of sinigrin, there may also be maternal effects. The generally lower mean picrate response (i.e. higher reflectance values) of seedlings compared with parents is not

understood, although it may reflect relatively low glucosinolate production in seedlings or pattern of volatile derivatives.

Work by Natti et al (1967) on the inheritance of resistance in cabbages to Peronospora parasitica provides indications of the allelic control of sinigrin levels. Greenhalgh (1976) has demonstrated that high AITC levels confer resistance to P. parasitica. Natti et al found that resistance to P. parasitica was governed by one or sometimes two independently segregating, dominant alleles. This suggests that high levels of sinigrin may be controlled by one or more dominant alleles.

Summary

There is no direct evidence as to the inheritance of glucosinolates producing volatile derivatives in B. oleracea. Work on other glucosinolates and species, suggests that there are maternal influences on the glucosinolate content of offspring. Indirect evidence for B. oleracea suggests a similar pattern of inheritance for sinigrin.

8) DISCUSSION

The role played by secondary plant substances such as glucosinolates, cyanogenic glucosides, alkaloids, phenols, tannins etc., in conferring protection against herbivores and pathogens has long been surmised, although little studied until comparatively recently. Fraenkel (1959) cites Stahl in 1888 as the first to consider that such secondary substances play a role in plant defences. Vershaffelt (1910) subsequently showed that they can also serve as attractants to insects. Brues (1920) extended this observation, in that he considered that insects were attracted to their host plants by a complex of chemical stimuli (not just secondary chemicals). He also suggested that the often exclusive association between Lepidopteran genera and various plant families, were based on specific chemical stimuli. This has led to the view that many animals and their host plants form a co-evolutionary relationship, which acts through the presence of secondary compounds in plants (Derthier 1954; Ehrlich & Raven 1964; Fraenkel 1959; Jones 1973).

A considerable body of work is now published on the stimulatory and inhibitory effects of secondary compounds in plants,on animal responses. A number of reviewers provide lists of compounds known to have such effects e.g. Fraenkel (1959), Levin (1971), Schoonhoven (1972). More general reviews on the effects of secondary compounds are provided by Thorsteinson (1960) on host selection and Beck (1965) on plant resistance.

The stimulatory effects of glucosinolates on insects is very well documented. Verschaffelt (1910) demonstrated that glucosinolates and their derivatives act as a feeding stimulant to larvae of Pieris brassicae and P. rapae. He demonstrated the acceptability of plants from the Cruciferae and from the closely related families Resedaceae, Tropaeolaceae and Capparidaceae to these predators; all these families possess glucosinolates (Kjaer 1960, 1974). However, Stepanova (1961) demonstrated that larvae prefer the species

of host plant on which they are normally found i.e. B. oleracea for P. brassicae.

It has been shown that sinigrin and AITC are the feeding stimulants for a number of species of insect including P. brassicae (Ma & Schoonhoven 1973), P. rapae (Hovanitz & Chang 1963),Plutella maculipennis Curt. (Thorsteinson 1953), Brevicorvyne brassicae (Wensler 1962; van Emden 1972) Athalia proxima (Klug) (Bogavat & Srivastava 1968) and Phaedon cochleariae (Fab.)(Tanton 1965). Other glucosinolates may act as feeding stimulants e.g. P. maculipennis shows greater stimulation from progoitrin than sinigrin (Nayar & Thorsteinson 1963).

AITC in particular also serves to attract the adults of a number of predator species of their host plant for egg laying, including Erioischia brassicae (Bouché) (Traynier 1965), Listroderes obliquus (Klug) (Matsumoto 1970), Phyllotreta cruciferae (Goeze) and P. striolata (F.) (Feeny et al 1970), Psylliodes chrysocephala L. (Queinnic 1967), Plutella maculipennis (Gupta & Thorsteinson 1960), Pieris rapae (Hovanitz et al 1963). Although P. rapae will respond to more than one isothiocyanate (allyl-, phenyl- and 2-phenylethyl- ITC; Hovanitz et al 1963),they suggest that most Pieridae are specifically attracted to the isothiocyanate group, hence their close association with the Cruciferae. P. brassicae is apparently not attracted to B. oleracea by AITC however (Ma & Schoonhoven 1973) (it is later argued that allyl nitrile may be the specific attractant in this case).

In a number of cases glucosinolates and their derivatives have been shown to have deterrent effects on insects e.g. Myzus persicae (Sulzer) (Wearing 1968, van Emden 1972) and Manduca sexta (Schoonhoven 1972).

Glucosinolates and their derivatives are also toxic to many organisms. Saarivirta in Virtanen (1962) has demonstrated antimicrobial activity of several naturally occurring isothiocyanates against Penicillium glaucum

and Staphylococcus aureus. Smissman et al (1961) have shown that indole-3-acetonitrile (a derivative of glucobrassicin) was toxic to Penicillium chrysogenum. However, Stahmann et al (1943) could find no relationship between levels of glucosinolate derivatives and resistance to clubroot (Plasmodiophora brassicae) in B. oleracea. The derivative of gluconasturtiin, 2-phenyl-ethyl-ITC, found in the roots of B. rapa has been shown by Lichtenstein et al (1964) to have insecticidal properties. Similar effects are reported for indole-3-acetonitrile (Smissman et al 1961).

The derivative of progoitrin, 5-vinyl-2-thiooxazolidethione, and the thiocyanate ion released on hydrolysis of glucobrassicin, can be very toxic to mammals. The principal effects are to cause iodine deficiency and haemolytic anaemia. Much plant breeding work has been carried out, to reduce the content of these compounds in seed meals of B. napus and B. campestris (Appelquist & Josefsson 1967; Josefsson & Appelquist 1968). The 3-butenyl-ITC present in these meals is also toxic, as is the p-hydroxybenzyl-ITC found in Sinapis alba L. seeds (Josefsson 1968). The use of B. oleracea as a fodder crop has caused similar problems (Johnston & Gosden 1975; Josefsson 1967b).

Depending upon the predator, glucosinolates and their derivatives have been shown to be feeding stimulants, attractants and deterrents (often toxically so). Sinigrin and AITC have been particularly studied with regard to insects, although some work implies that similar responses could be obtained from other glucosinolates and isothiocyanates. There is often a concentration effect : the higher the concentration, the greater the stimulus; although this is not universally so. For some species a threshold response operates as in P. brassicae (Ma & Schoonhoven 1973).

The role of nutritional factors is equivocal (for a review see Beck 1965). Fraenkel (1959, 1969) argues that secondary substances are the

primary factor in any attractant/deterrant response, nutritional factors being of little importance. Evans (1938) demonstrated that P. brassicae larvae reared on B. oleracea plants grown under a low light regime (and hence nutritionally poor), developed to the 5th instar more slowly than larvae on normally grown plants, but still reached their final development. Stepanova (1961) similarly found that larval development was independent of the nutritional status of the host plant. Adult members of the Pieridae do not necessarily respond to sugars (Ma & Schoonhoven 1973), although larvae of P. brassicae do possess amino acid receptors (Schoonhoven 1969). Benepal & Hall (1967) could find no relationship between the levels of protein in varieties of B. oleracea and their resistance to attack by P. rapae, although such a relationship may occur with respect to free amino acids. Using the larval stage of P. brassicae, Schoonhoven (1967) demonstrated that a greater feeding response was obtained on artificial media, when sucrose and sinigrin were combined. This will however occur subsequent to the selection of the host.

An alternative theory to that of Fraenkel, is the "dual-discrimination" theory of Kennedy (1958). In this theory, insects respond to a host-specific stimulus (probably a secondary compound) and a nutrient stimulus, which the insect 'assesses' for suitable levels of nutrients and/or deterrants. This theory does not seem to hold for the predation by Pieris spp. of B. oleracea, although it may be true for other interactions. Kennedy (1965) in support of his theory, makes the point that in agricultural systems, varietal differences are of importance. He believes that secondary substances do not vary sufficiently between varieties to account for differential varietal resistance. Instead he proposed that variation in nutritional factors accounts for these differences. The present work suggests that the converse is likely to be true.

Within the Brassicinae both qualitative and quantitative variations

have been observed in the levels of glucosinolates (Josefsson 1967a). Although much of this work has been concerned with seed meals, variation has also been observed in the levels of glucobrassicin in laminar tissue of B. napus, B. campestris and B. oleracea (Johnston & Gosden 1975; Josefsson 1967b). Greenhalgh (1976) has shown that there is a considerable range in the levels of AITC in laminar tissue of B. oleracea cultivars. Differential resistance between B. oleracea cultivars to predation by P. rapae and a number of other pests has been investigated (Radcliffe & Chapman 1966a, b) although they could only conclude that resistance or susceptibility was related to the colour of the variety, red cabbages apparently being less attractive (this has recently been confirmed by Dunn & Kempton (1976)). Size of plants did not appear to be important. Unfortunately however, glucosinolates were not investigated by Radcliffe & Chapman, although Dunn & Kempton concluded that volatiles did not have an important role in resistance or susceptibility. This colour response may be in accord with the observations of Ilse (1937), who found that gravid P. brassicae preferred to lay on green or green-blue substrates, whilst yellow substrates only elicited a feeding response.

It has frequently been reported that sinigrin serves as a short distance attractant for gravid, adult P. brassicae, but there is no previously published work which demonstrates that P. brassicae preferentially selects any particular variety or morph, with respect to glucosinolate content, as is shown here. It is notable that no work has shown how P. brassicae perceives B. oleracea from a distance. It is assumed here that volatile glucosinolate derivatives are involved.

In this study, the role played by glucosinolates as attractants to P. brassicae has been confirmed, but the attractants would appear to be one or several of the glucosinolate derivatives detected by the picrate test rather than sinigrin itself. The degree of perception that gravid

P. brassicae appear to possess to glucosinolate derivatives, has also not previously been demonstrated. It is clear that gravid females select certain picrate morphs, revealing a hitherto unrecorded level of discrimination. Sinigrin is probably present in most plants selected for oviposition (section 3.3). The picrate test is a measure of allyl nitrile release (for types 4 and 5 at least) which it is suggested follows the sinigrin content of leaves; however sinigrin itself is not volatile and thus P. brassicae would not detect it from a distance. It has been shown that AITC is not the attractive principal in B. oleracea (Ma & Schoonhoven 1973); thus allyl nitrile may fulfill this role, although this has not been investigated. Alternatively, P. brassicae might respond to one or more of the other volatiles released from B. oleracea e.g. various aldehydes, alcohols, ketones and sulphides. Sulphides in particular have been shown to be important in other insect/plant interactions (Matsumoto 1970), although not for the Lepidoptera.

It is not clear whether plant morphology can act as an attractant. Radcliffe & Chapman (1966a) could not detect any response in P. rapae to plant size; although my observation that, except on rare occasions, only sterile plants are predated (section 5.5) suggests that the plants have to present a certain visual stimulus prior to oviposition.

During the field collection of picrate data in the present work, a series of morphological characters were scored (Appendix F). Of these, it was found that picrate score was particularly correlated to leaf shape (Kendall's tau = -0.1304 $p < 0.001$, after partial correlation analysis). and plant width to height ratio (Kendall's tau = 0.1391 $p < 0.001$, after partial correlation analysis). It is not known whether these characters are of any significance to P. brassicae; previous work suggests they are not. However, an investigation was made to determine whether the high picrate score plants i.e. types 4 and 5, were morphologically distinct

from the lower picrate types. A number of morphological characters were compared using the t- test (Table 8.1); only characters of leaf and plant morphology were included, since Ilse's (1937) work suggested that yellow colouration (as in the flowers) does not stimulate oviposition. The data suggests that type 5 plants do differ morphologically from other picrate types; more so than type 4 plants (Table 8.1b) (although see Appendix F). Whether this is of any importance with reference to susceptibility to P. brassicae predation is not known. It could be argued that due to intense selection, type 5 plants have become differentiated from the other picrate types, becoming visually unattractive to P. brassicae. In this respect it is notable that type 1 plants show the greatest differentiation from type 5 plants. Any such morphological differentiation may be genetically linked to 'fitness characters', (such as low glucosinolate content) thus conferring an adaptive linkage. High glucosinolate content plants, due to their attractiveness are at a selective disadvantage with respect to P. brassicae. If the morphology of a plant has a role in attracting P. brassicae imagines, then plants which have an 'unattractive' morphology will be at a selective advantage. Thus there would be a selective pressure for plants which are attractive through their glucosinolate content, to be unattractive morphologically.

Alternatively, glucosinolate content and morphology may be correlated through epistatic or pleiotropic effects. Under these circumstances it could probably be concluded that morphology was not related to susceptibility to P. brassicae attack.

Within a population a range of picrate morphs are maintained, and it would seem that this occurs through selection by predators at both seedling and adult phases. It would seem likely that P. brassicae is the principal selective force operating to reduce the numbers of adult plants, with a high picrate response (chapter 5). The data suggests that gravid P. brassicae

Table 8.1 A comparison of several morphological characters on the basis of picrate score (see Appendix F).

(a) Mean values

character	Picrate score 1	2	3	4	5
lamina					
a) length/breadth	1.46	1.43	1.36	1.34	1.40
b) shape	1.10	1.09	1.12	1.18	1.15
c) no. basal lobes	3.13	3.25	3.71	3.50	3.38
d) edge[1]	3.00	3.00	2.73	2.25	2.17
plant					
e) width/height	1.84	2.34	2.54	2.18	1.56
f) width/stem diam.	29.01	29.99	33.61	36.26	33.38
no. observations.	338	115	59	50	8

Table 8.1 (Data continued)

(b) Comparison of the means. The values are as t-ratios with their associated probabilities.

Type 5 plants compared with picrate types

characters	1	2	3	4
(a)	4.86	2.70	2.68	3.85
p	<0.001	<0.01	<0.01	<0.001
(b)	11.86	9.75	3.36	4.52
p	<0.001	<0.001	<0.001	<0.001
(c)	4.09	1.19	2.29	0.80
p	<0.001	N.S.	<0.05	N.S.
$(d)^2$	3.16	2.94	2.96	2.5
p	<0.001	<0.002	<0.002	<0.006
(e)	8.29	11.01	6.52	6.63
p	<0.001	<0.001	<0.001	<0.001
(f)	10.03	5.32	0.25	2.91
p	<0.001	<0.001	N.S.	<0.01

Type 4 plants compared with picrate types

characters	1	2	3
(a)	4.15	3.16	0.47
p	<0.001	<0.01	N.S.
(b)	7.78	5.74	2.64
p	<0.001	<0.001	<0.01
(c)	3.06	1.18	0.71
p	<0.01	N.S.	N.S.
$(d)^2$	12.59	14.13	16.12
p	<0.001	<0.001	<0.001
(e)	3.44	0.79	0.92
p	<0.001	N.S.	N.S.
(f)	6.62	3.82	1.12
p	<0.001	<0.001	N.S.

Notes:

1 Median values

2 Z-statistic calculated from the Mann-Whitney U test

N.S. Probability >0.2

imagines respond to variation in levels of volatile glucosinolate products, the plants which release the largest quantities of allyl nitrile being the prime targets for egg-laying (chapter 5). Frequency dependent effects and/or variation in P. brassicae response, results in a certain proportion of lower picrate response plants also being predated. Type 5 plants show a certain degree of morphological differentiation from the other picrate types; whether this is of any adaptive significance with respect to predation is not known.

Although P. brassicae seems to be an important factor in determining which picrate morphs survive to flowering, a very important phase also occurs at the seedling stage. It has been suggested that genetic variation in glucosinolate content shows strong maternal effects (chapter 7). In the majority of populations, type 1 and 2 plants predominate in the flowering stage (chapter 4). Thus at the early seedling phase, these types also predominate (chapter 7). A change in glucosinolate types may occur during the winter period. At least two groups of organisms (Molluscs and Peronospora parasitica) may differentially select low picrate response seedlings which are thought to have low levels of AITC (chapter 6).

Molluscan responses to glucosinolates and their derivatives have not previously been reported, although similar effects have been previously reported in fungi (e.g. Saarivirta in Virtanen 1962).

By May, when rapid growth commences, the proportion of picrate morphs in seedlings has totally changed as a result of selection, high picrate response seedlings dominating. During subsequent years the high picrate response plants are gradually lost from the population.

Seasonal variation in the glucosinolate content of individuals (chapter 2) may have an important adaptive role in the protection of B. oleracea from predation. During the period of greatest P. brassicae predation (August/September), the mean picrate response of a population

is at its strongest, thus large numbers of plants might be expected to be attractive. Yet at this time of year, variation in picrate response is at its highest (section 2.7), resulting in a wide range of morphs being present. This results in there always being a certain proportion of unattractive morphs. There is also a strong picrate response during much of the winter period, when attack by Molluscs and Peronospora on seedlings would be of importance. The effect of this is to enhance any protective qualities of the glucosinolates. During the spring when protection against predation may not be of paramount importance, the mean picrate response is low (as is the variation), although this may reflect the metabolic mobilisation during rapid growth.

Thus it appears that at different stages in the life history of B. oleracea, sinigrin and its derivatives act as deterrents (allyl isothiocyanate), attractants (allyl cyanide) and feeding stimulants (sinigrin) to various organisms. Through a balance of their selective effects, these organisms help to maintain the variation of picrate morphs in populations.

APPENDIX A The regression equations derived for each plant samples at Tynemouth, relating the weight of tissue to the reflectance value (R).

Plant No.	equation	r	p	d.f.
D1	$R^{-2} = 4.32 - 14.40g$	-0.9903	< 0.001	5
P25	$R^{-2} = 4.66 - 7.46g$	-0.9633	< 0.001	5
G18	$R^{-2} = 4.25 - 3.79g$	-0.9746	< 0.001	5
A4	$R^{-2} = 4.76 - 3.22g$	-0.9974	< 0.001	5
G8	$R^{-2} = 4.28 - 4.63g$	-0.9913	< 0.001	5
B5	$R^{-2} = 4.58 - 13.65g$	-0.9871	< 0.001	5
P2	$R^{-2} = 4.29 - 7.79g$	-0.9675	< 0.001	5
G19	$R^{-2} = 4.57 - 7.09g$	-0.9946	< 0.001	5
P24	$R^{-2} = 4.74 - 6.71g$	-0.9944	< 0.001	5
A14	$R^{-2} = 4.79 - 6.48g$	-0.9976	< 0.001	5
B22	$R^{-2} = 4.59 - 8.22g$	-0.9513	< 0.001	5
G7	$R^{-2} = 4.19 - 4.84g$	-0.9704	< 0.001	5
B19	$R^{-2} = 3.99 - 4.46g$	-0.9669	< 0.001	6
P5	$R^{-2} = 4.33 - 14.62g$	-0.9763	< 0.001	5
D3	$R^{-2} = 3.90 - 8.36g$	-0.9881	< 0.001	5
B14	$R^{-2} = 4.33 - 10.22g$	-0.9646	< 0.001	5
44	$R^{-2} = 4.82 - 7.49g$	-0.9873	< 0.001	6
A6	$R^{-2} = 4.01 - 5.41g$	-0.9530	< 0.001	5
43	$R^{-2} = 4.26 - 9.36g$	-0.9933	< 0.001	5
P7	$R^{-2} = 4.50 - 4.74g$	-0.9956	< 0.001	5
P12	$R^{-2} = 4.93 - 9.51g$	-0.9952	< 0.001	5
G9	$R^{-2} = 4.57 - 6.30g$	-0.9670	< 0.001	5
P17	$R^{-2} = 3.92 - 6.83g$	-0.9812	< 0.001	5
P16	$R^{-2} = 4.64 - 10.28g$	-0.9863	< 0.001	5

APPENDIX A (Data continued)

Plant No.	equation	r	p	d.f.
P11	$R^{-2} = 4.79 - 4.14g$	-0.9693	<0.001	5
P23	$R^{-2} = 4.92 - 12.17g$	-0.9830	<0.001	5
42	$R^{-2} = 4.75 - 7.89g$	-0.9714	<0.001	5
B10	$R^{-2} = 4.19 - 10.17g$	-0.9201	<0.01	5
A5	$R^{-2} = 4.07 - 5.36g$	-0.9988	<0.001	5
G16	$R^{-2} = 4.82 - 5.31g$	-0.9952	<0.001	5

Appendix B. Data collected during the laboratory study of temperature induced variation in the picrate test. The results are as reflectance values.

Plant	Temperature °C					
ref. No.	0	5	10	15	20	25
9	10.9	12.5	14.2	13.3	14.2	14.5
16	13.2	12.5	13.6	14.5	17.1	11.9
18	11.1	12.5	14.5	13.1	12.8	12.8
22	15.0	14.8	18.3	15.1	15.1	12.8
36	14.8	13.9	17.7	15.9	15.4	14.2
46	13.8	15.4	17.7	15.1	13.9	11.6
53	16.5	15.5	17.7	18.8	16.2	15.1
65	16.5	16.8	18.5	15.9	14.5	12.8
68	14.4	19.4	18.8	20.3	20.3	15.4
69	14.4	13.5	16.8	18.3	17.7	16.2
X	18.3	14.2	18.3	18.5	16.8	15.9
Y	14.4	14.8	17.7	14.5	15.1	18.0
Z	18.9	18.5	18.3	20.8	20.3	17.4
Mean	14.8	14.9	17.1	16.5	16.7	14.5

$$\text{Reflectance} = 14.66 + 0.075\ (^{\circ}C) + 0.0182\ (^{\circ}C)^2 - 0.00086\ (^{\circ}C)^3$$

$r = 0.9169 \quad p < 0.01 \quad$ d.f. 12

APPENDIX C. The data collected at Tynemouth for analysis of the effects of climate.

The data are reflectance values for a sample corrected to 0.1g.

	PLANT REFERENCE NUMBER	D1	P25	G18	A4	G8	B5	P2	G19	P24	A14	B22	G7
1974	MARCH	22.1	11.2	18.8	16.6	17.3	21.6	8.8	19	24	21.1	20.7	16.5
	APRIL	22	17.9	19.9	21.8	20.4	26.6	14.3	15.3	20.4	22	19.6	21.3
	MAY	17.2	17.9	21	18.2	17.4	23.2	18.3	18.1	21.5	20.9	19.1	18.3
	JUNE	25.4	19.4	17.9	19.2	18.5	21.8	18.6	13.6	18.4	17.5	18.7	20
	JULY	23.8	20.2	18.3	21.8	18.6	27.7	17.2	18.2	20.7	17.2	21.0	20.6
	AUGUST	13	12.3	9.3	22.9	12	14.8	9.8	*	15.1	19.6	20.7	17.7
	SEPTEMBER	8.1	22.3	*	9.3	10.8	15.2	11	8.2	16.9	14.9	6.6	10.9
	OCTOBER	12.6	15.2	11.8	17.7	16.9	13.2	15.5	11.7	16.9	17.5	16.6	19.4
	NOVEMBER	21.3	14.5	14	22.4	15.4	16.9	17.9	10.3	17.2	14.8	19.3	17.1
	DECEMBER	17.3	16.2	15.5	23.7	10.3	15.4	21.7	16.2	18.4	19.4	17.1	19.9
1975	JANUARY	12.6	21.2	13.2	23.3	12.2	23.4	8.9	12.9	14.3	13.5	24.9	19.2
	FERRUARY	8.5	15	15.4	17.4	14.3	12.3	10.1	10.2	12.6	14	12.4	12.8
	MARCH	17.1	15.7	10.9	12.3	13.8	14.4	10.8	11.6	11.9	11.6	10.5	*
	APRIL	13.2	17.8	15.6	19	18.9	19.8	17.8	11.8	16.6	19.1	16.3	20.8
	MAY	13.1	18.6	16.9	19	14.7	15.8	17.4	18.8	15.3	13.9	22.9	17.6
	MEAN	16.5	17.0	-	19.0	15.4	18.8	14.5	-	17.4	17.1	17.8	-

APPENDIX C. (Data continued)

	PLANT REFERENCE NUMBER	B19	P5	D3	B14	44	A6	43	P7	P12	G9	P17	P16
1974	MARCH	21.8	11.9	18.7	16.7	13.4	20.4	15.8	11.1	18.1	19.5	18.2	15.4
	APRIL	16.5	23.1	19.3	22.7	20.3	21.6	13.9	19.4	21	21.2	21.1	19.7
	MAY	17	16.4	19.4	18.4	18.9	19.8	16.3	20.2	20.7	18.9	*	13.3
	JUNE	22.8	18.1	17.2	19.3	15.4	23.4	17.2	20.8	18.4	16.2	16.3	18.2
	JULY	20	17.3	17.4	26.9	18.9	23.5	20.2	21.5	16.2	19.8	20.7	20.7
	AUGUST	14.2	10.8	14.2	28.7	10.4	10.4	10.6	17.8	17.5	15.8	11.1	18.8
	SEPTEMBER	18	10.6	17.7	15.4	16	15	11.2	9	10.4	10.1	11.4	27.8
	OCTOBER	14.6	13.7	12.4	19.4	16.5	15.1	14.4	13.4	17	11.3	16.1	14.2
	NOVEMBER	12.7	13.5	13	17.1	16.8	10.3	12.6	16.5	21.4	12.3	18.2	13
	DECEMBER	22.2	27.5	19.1	21.8	20.5	13.2	11.7	18.7	20.3	20.4	*	15.4
1975	JANUARY	14	21.6	16.7	10.6	19.6	11.7	12.8	18	20.3	18.4	18.1	17.2
	FEBRUARY	11.6	11	12.4	11.1	17.7	13.5	12.7	11.8	18.4	14.8	12.1	12.5
	MARCH	16	16.2	13.2	16.7	16.8	17.9	11.9	13.9	16.2	12.3	14.8	17.0
	APRIL	17	18.9	18.4	21.1	21.2	18.8	17.6	15.8	21	20.2	22.1	16.6
	MAY	19.3	23.3	15.5	21.2	15.7	16.1	16.2	19.3	16.9	21.9	12	17.3
	MEAN	17.2	16.9	16.3	19.1	17.2	16.8	14.3	16.5	17.7	16.9	-	17.1

APPENDIX C. (Data continued)

	PLANT REFERENCE NUMBER	P11	P23	42	B10	A5	G16	MEAN	MINIMUM NIGHT TEMPERATURE °C	30 YR. MONTHLY MEAN °C
1974	MARCH	17.9	20.3	19	17.8	18.5	14.2	17.5	2	4.2
	APRIL	20.5	23.7	19.7	20.3	21.4	21.8	20.3	8.2	5.5
	MAY	18.1	26.9	20.6	10.9	19.3	18.6	18.6	9	7.5
	JUNE	19.5	23	19.8	13.7	20.7	21.4	19.3	6.6	9
	JULY	21.8	23.4	21.3	21.2	23.2	19.4	20.6	10.1	15
	AUGUST	17.6	24.3	13.3	11.2	19.3	11.2	15.4	9	14.5
	SEPTEMBER	13.7	8.7	17.2	13.3	11.3	13	13.2	11.4	14
	OCTOBER	13.7	11.3	16.9	12	13	10.9	14.7	5	13
	NOVEMBER	20	14.9	14	12.4	17.1	12.1	15.8	5.7	10
	DECEMBER	20.4	22.3	16	12.7	21.7	17.7	18.3	5.5	7.5
1975	JANUARY	15.7	21.2	13.7	15.1	16.4	17.4	16.7	6.6	5.3
	FEBRUARY	15.6	9.8	17.1	15.4	13.7	13.1	13.4	3.9	4
	MARCH	13.2	11.9	16.4	14.9	15.7	13.7	14.3	2.7	4.2
	APRIL	19.3	17.7	22.3	18.6	23.1	17.8	18.6	1	5.5
	MAY	15.9	14.2	16.1	13	20.4	16.7	17.3	5.7	7.5
	MEAN	17.7	18.3	17.6	14.8	18.3	15.9			

KEY:

* Sample not collected. N.B.The overall analysis was based on complete sets of data only.

APPENDIX D. Meteorological data[1] analysed during the study of the effects of climate on the picrate test.

DATE OF SAMPLE	SUNSHINE HOURS					TEMPERATURE °C									
	DAY			MONTH			DAY 0			NIGHT -1			DAY -1		
	0	-1	-2	MEAN	TOTAL	GRASS	MAX	MIN	MEAN	MAX	MIN	MEAN	MAX	MIN	MEAN
7. 3.74	3.6	5.9	0.0	2.4	67.5	4.0	12.4	0.0	6.2	4.0	2.0	3.0	7.0	1.5	4.2
18. 4.74	0.0	0.0	0.6	2.4	74.0	5.0	6.7	4.6	5.7	7.7	8.2	8.0	7.4	4.6	6.0
22. 5.74	8.0	1.9	5.7	6.2	191.0	5.3	14.6	10.1	13.4	13.6	9.0	11.3	14.6	11.0	12.8
11. 6.74	10.7	4.0	6.0	6.1	184.0	5.8	18.1	5.1	11.6	11.0	6.6	8.8	13.2	8.2	10.7
8. 7.74	2.0	0.7	7.0	5.9	177.0	10.4	20.1	11.2	15.8	12.2	10.1	11.1	17.6	13.0	15.3
6. 8.74	5.1	10.2	11.7	5.8	180.0	9.3	21.9	9.2	15.5	13.9	9.0	11.5	16.4	13.9	14.7
10. 9.74	1.5	6.8	7.5	5.9	184.0	7.1	16.8	9.5	13.2	13.0	11.4	12.2	16.6	11.9	14.3
8.10.74	0.2	0.0	0.0	4.3	129.0	8.1	11.1	6.1	8.6	8.9	5.0	7.0	11.0	10.6	10.8
5.11.74	0.5	0.0	0.0	1.6	50.0	0.1	9.3	3.2	6.3	9.0	5.7	7.4	9.8	7.9	8.9
4.12.74	5.7	1.6	2.7	2.5	74.0	5.0	8.0	3.2	5.6	7.5	5.5	6.5	12.0	6.4	9.2
7. 1.75	0.0	4.0	0.0	2.0	63.0	-2.0	5.5	2.2	3.9	10.9	6.6	8.8	11.5	6.2	8.9
5. 2.75	0.0	0.0	0.0	2.1	65.0	2.6	4.4	3.0	3.7	5.0	3.9	4.5	5.1	4.4	4.8
5. 3.75	1.9	5.6	0.2	1.8	51.0	0.7	7.0	4.6	5.8	7.9	2.7	5.3	9.9	4.7	7.3
8. 4.75	0.0	6.3	2.0	3.2	98.5	-0.9	4.2	-1.7	1.2	6.0	1.0	3.5	6.0	4.4	5.2
21. 5.75	8.4	4.0	14.2	5.4	167.6	4.0	9.3	7.3	8.3	15.1	5.7	10.4	17.2	8.7	13.0

1 Data kindly provided by the Meteorological Office, Newcastle upon Tyne.

APPENDIX D. (Data continued)

DATE OF SAMPLE	TEMPERATURE °C									
	24h -2			24h -3			MONTH			
	MAX	MIN	MEAN	MAX	MIN	MEAN	MAX	MIN	MEAN	30yr
7. 3.74	4.4	-2.6	0.9	5.5	-1.0	2.3	7.7	3.6	5.7	4.2
18. 4.74	7.9	4.3	6.2	8.6	1.5	5.1	7.1	4.2	5.7	5.5
22. 5.74	17.9	8.5	13.2	14.8	8.5	11.7	12.4	7.1	9.8	7.5
11. 6.74	12.8	6.6	9.7	11.4	7.4	9.4	13.5	8.2	10.9	9.0
8. 7.74	14.4	11.1	12.8	12.4	9.1	10.8	14.6	9.4	12.0	15.0
6. 8.74	14.5	11.2	12.9	18.5	10.6	14.6	17.3	11.2	14.3	14.5
10. 9.74	16.7	10.1	13.4	14.3	8.0	11.2	17.8	11.5	14.7	14.0
8.10.74	10.1	5.9	8.0	10.8	6.8	8.8	14.8	9.0	11.9	13.0
5.11.74	9.5	4.2	6.8	9.0	4.2	6.6	10.2	5.6	7.9	10.0
4.12.74	13.0	11.2	12.1	13.0	5.0	9.0	8.9	4.3	6.6	7.5
7. 1.75	12.0	5.5	8.8	12.0	4.8	8.4	10.1	5.5	7.9	5.3
5. 2.75	7.4	3.2	5.3	10.2	3.7	7.0	9.3	3.3	6.4	4.0
5. 3.75	9.9	4.7	7.3	10.5	2.9	6.7	6.8	2.7	4.7	4.2
8. 4.75	6.0	2.7	4.4	5.2	2.0	3.6	6.9	2.3	4.6	5.5
21. 5.75	16.6	8.0	12.4	14.6	7.4	11.0	10.1	5.7	7.9	7.5

KEY:

Day: 9am to 9pm;

Night: 9pm to 9am;

24h: one day from 9am to 9am;

0: day of sample;

-1: day prior to sampling;

-2: 2 days prior to sampling;

-3: 3 days prior to sampling;

30yr: 30 year monthly mean.

Appendix E. The status of Brassica oleracea L. subsp oleracea (Wild Cabbage) in the British Isles.

The History of Cabbage in Cultivation.

Johnson (1862) provided an extensive history of the cabbage. The ancient Romans and Greeks cultivated three varieties (Greek legend has it that the cabbage sprung from where Zeus' sweat hit the ground). Cato mentioned that it was either boiled or eaten raw. Later, Pliny reported that cabbage was going out of favour with the lower orders, due to the quantity of oil (which was becoming more expensive) required to make it palatable (presumably it was eaten raw). He mentioned several varieties, one of which, 'Halmyridia', grew on the sea-shore and was used as a vegetable on long voyages.

It has been suggested that the Romans first brought the cabbage to Britain (Gates 1950a). Subsequently the Saxons cultivated it (their second month was called Sprout-kale), as did mediaeval religious orders. It was also apparently cultivated in eastern Fife, where it was so popular that the people of the area were known as 'kail-suppers'. As a commercial crop it may have been introduced by Sir Anthony Ashley of Dorset, as late as the 16th Century.

The cabbage was also used as a medicine. Pliny recommended gouty people to live on cabbages and the water they have been boiled in. Turner (1551) mentioned the use of cabbage as a general cure for internal disorders. Gerarde (1633) similarly chronicled its healing powers. (Saarivirta, in Virtanen (1962), has shown that one of the mustard oils of cabbage has antibiotic and fungistatic activity). Thus it would appear that the cabbage has been grown in Britain for various reasons, probably since the Roman invasion.

The derivation of the cultivated cabbage is open to question, Schulz

(1936) and Gates (1950b) held the view that the range of variation in the wild cabbage, Brassica oleracea L. subsp. oleracea (B. sylvestris (L.) Miller) is insufficient to account for all the present day varieties, some of which have arisen through hybridisation between B. oleracea and other Brassica species. However, de Candolle (1824) and Bailey (1922) held the opposite viewpoint.

Brassica oleracea in the Flora of the British Isles.

B. oleracea was recorded in the earliest Floras. Turner (1551) noted it growing wild at Dover, E. Kent; Gerarde (1633), who called it B. sylvestris ('Wild Coleworts'), saw it along the northern coast of Kent and near Colchester (N. Essex). Hudson (1762) mentioned it from Cornwall. From this period the numbers of records increased until by the beginning of the 19th Century the present-day range of the species was almost covered. Watson (1847) recorded it from 12 provinces, although he only considered it likely to be native in five of these (Peninsula, Channel, Thames, S.Wales and N.Wales) and was surprised it grew wild in so few places. Later (1870) he summed up his view as, 'Denizen?.....Coast cliffs, native? Inland only as an alien ..... Wild on the western coasts of France? - N.B. Exceedingly difficult to trace the native habitats of this plant.' Watson's earlier viewpoint has in essence remained unchanged to the present day (cf. Clapham 1962), although the authors of some local Floras were not entirely in agreement. It was considered a denizen or alien in Monmouth (Wade 1970), N. Somerset (Murray 1896), Dorset (Mansel-Pleydell 1895), S. Essex (Gibson 1862), E. Norfolk (Nicholson 1914), N.E. Yorks.(Baker 1906) and Fife (Young 1936). Wolley-Dod (1937) believed some E. Sussex sites to be adventive. However, it was recorded as native in E. Kent (Hanbury & Marshall 1899), S. Devon (Keble-Martin & Frazer 1939), E. and N. Cornwall (Davey 1909) and Glamorgan (Trow 1911). Trow commented 'It was almost certainly a true native with us, or at any rate indistinguishable from one,' although Watson

had classed the Glamorgan plants as denizens. Trow's comment is interesting in the light of an observation by Syme (1863), who noted 'Red cabbage of neglected gardens at the sea-side pass back in a few generations to the condition of the wild cabbage.' I have made similar observations. It would seem that the wild derivatives of cultivated plants become morphologically indistinguishable from 'native' plants within a few years.

Through the Floras, much of the history of B. oleracea as a member of the British flora may be traced. It is immediately apparent that there was an increase in the number of records during the 19th Century, which probably coincides with the increase in plant recording at that time. This has been followed by a decline in the numbers of populations during the first half of this century. The decline is apparently continuing in most areas.

The sites of B. oleracea are nearly always associated with towns and villages, rather than being recorded 'near ....' or 'between ....'. This might suggest that it is almost exclusively associated with towns and villages. The ephemeral nature of many occurrences is equally notable; it must be presumed that short-lived populations (accounting for about one third of the total) were introduced.

Further information may be gained from a consideration of the siting and history of the present-day populations (Table I). I have visited all the extant populations except those at Llandulas (Denbigh), Flat Holm (Glamorgan) and Fortrose (E. Ross), and I have searched for many of those previously recorded. I have also received much excellent information from vice-county recorders concerning the locality and status of contemporary populations.

The population on the Great Orme (Caernarvon) was recorded as 'rare and local' by Griffith (1895). He gives the first record as 1805, when it was recorded from the north-east side, i.e. above Llandudno. The species

is now to be found all around the seaward side of the headland. The Little Orme population (first recorded by Griffith (1895) is probably derived from that at the Great Orme, possibly by the seeds being carried by sea-birds (see Gillham 1970). The Llandulas (Denbigh) population was first recorded in 1912 (Dallman 1913).

The population at Tenby (Pembroke) was first recorded in 1773 (Riddelsdell 1905), the present-day plants growing around and in back gardens, as well as along the neighbouring cliffs. Falconer (1848) queried whether the plants were '.... Truly wild?' The very local siting in association with an old town suggests that the species may have been introduced.

B. oleracea has particularly declined in Glamorgan. In the 19th Century, populations extended from Southerndown to Barry Island, including a number of towns and villages (Trow 1911), but they have now retreated westward, extending only to Nash Point. The population which became extinct most recently (probably during the 1960s) was at Barry Island (G. Ellis pers. comm. 1975).

During the 19th Century there appears to have been an almost continuous series of populations along the Cornish coast from St. Germans (E. Cornwall) around to St. Ives (W. Cornwall) (Davey 1909), although many of these are now extinct (L.J. Margetts pers. comm. 1975). There are records dating back to the 18th Century (Hudson 1762), all of which are associated with towns and villages. Again this suggests the populations are adventive.

In N. and S. Devon populations have come and gone (Keble-Martin & Frazer, 1939), although there are still large populations at Dartmouth and Babbacombe. At the former site, B. oleracea grew particularly around the castle (in 1882) at the edge of town, but now the population extends for some kilometres westward along the coast. This again suggests the plants are adventive.

The expansion of B. oleracea in Dorset is well documented. It was first recorded in 1813, on Portland, where it is now extinct. In all its other sites it was rare in the late 19th Century (Mansel-Pleydell 1895). There are now extensive populations at Durdle Door to Lulworth Cove and St. Aldhelm's Head to Winspit, as well as fewer plants at several other sites. Cabbages are cultivated alongside some these sites, suggesting a source for the wild plants.

The first record for the Isle of Wight must be pre-1616 (due to Lobel who died 1616), although it was not published until 1665 (Townsend 1883). Townsend interpreted a comment made by Lobel, which suggested that during Lobel's lifetime B. oleracea had become scarce after a former abundance. At the time of Townsend, the species was very rare, the few sitings being regarded as introductions. The only present-day population, 1 km. east of Freshwater Bay (R.P. Bowman pers. comm. 1974), may be associated with a record due to Bromfield (1860) further to the east, although there is no information on this point.

The Dover (E. Kent) population is unique in that it has been recorded as growing wild for over 400 years. This population has perhaps the strongest claim to native status, yet Dover has had extensive garrisons for centuries and has been a major route for invaders. It was also one of the main areas of Saxon settlement during the early post-Roman period. Thus cabbages may have been grown here for food since 500 A.D. or earlier, and it is still a major area for cabbage cultivation. The other E. Kent population, by Broadstairs, grows very close to cabbage fields.

It should be noted that B. oleracea is recorded as native on the corresponding chalk cliffs of the French channel coast (Rouy & Foucaud 1895, Coste 1937), although Grenier & Godron (1848) suggested it was adventive.

The Whitby and Staithes populations (N.E. Yorks.) grow around habitation.

In Whitby the plants extend up from back gardens, whilst at Staithes they grow particularly around old allotments, which the owner tells me, have been cultivated for centuries. The populations are almost certainly introductions.

The history of the Tynemouth (S. Northumberland) population is somewhat different in that it grows around an old priory and garrison. Local folk-lore has it that cabbages were cultivated by the monks and have since become naturalized (there is a local name of 'Monk's Cabbage'). Certainly they have been recorded since 1805 (Baker & Tate 1867). If this folk-lore is true, then these plants plants may be ancient escapes from cultivation.

Of the series of populations that formerly grew along the north side of the Firth of Forth, all are extinct apart from that at Crail (Fife). This latter population, first recorded in 1840 (G.H. Ballantyne pers. comm. 1974), is almost certainly introduced. It grows by the village, extending down from gardens to the shore. There appears to be some plants of very recent origin, since it is possible to identify individuals closely resembling B. oleracea L. var. capitata L. 'January King' and 'Dutch Savoy'. The Auchmithie (Forfar) population is probably also derived from garden escapes. It extends along from the village and allotments (where cabbages are still grown) to neighbouring cliffs.

Apart from the extant vice-county records, there have been populations (mainly short-lived) in Durham (Winch et al. 1805), E. Norfolk (Nicholson 1914), N. and S. Essex (Gerarde 1633, Gibson 1862), E. and W. Sussex (Arnold 1907, Wolley-Dod 1937), S. Hants. (Townsend 1883), N. Somerset (Murray 1896), Monmouth (Wade 1970), Westmorland (Wilson 1938) and Guernsey (Marquand 1901). McClintock (1975) reports that none of the Guernsey records has been substantiated.

B. oleracea has never been established in Ireland. It is not mentioned

by Praeger (1901, 1934) or by Webb (1959), although Druce (1932) recorded it from Mid Cork and Perring & Walters (1962) from E. Cork. Hooker (1870) included Ireland in the distribution of the species.

B. oleracea is also mentioned in many Floras as a casual.

To the best of my knowledge the only extant populations of B. oleracea are those in Table I, although, as Perring & Walters (1962) show, the species has been recorded from more sites.

Discussion.

In the British Isles most extant populations of B. oleracea seem to have originated from cultivation. Other authors have similarly questioned the status of northern European populations, e.g. Hegi (1919) suggested that B. oleracea growing in Heligoland was introduced (it was not mentioned in a 16th Century species list) and that other northern European populations were introductions. Gates (1950b) mentioned that Ascherson considered the true B. oleracea to be confined to the Mediterranean, the plants on the British Isles coasts being escapes from cultivation, but in contrast he (Gates 1950a, b) believed it to be native around northern European coasts.

Certainly, cabbages have grown wild in the British Isles for several hundred years. The fact that the Saxons named a month 'Sprout-kale', suggests that they cultivated cabbages. Similarly 'kail' has apparently been growing or gathered for many centuries in Fife. If B. oleracea was found in pre-Roman Britain then it should probably be classified as native. If, as seems likely, the Romans or Saxons brought the cabbage to the British Isles, then it is a denizen. Certainly most contemporary populations seem to be of recent and impermanent status. That wild B. oleracea is strongly associated with man seems to reinforce this latter point. Thus the distinction between introduced and native populations as recorded by Perring &

Walters (1962) should be regarded with some caution, since the majority of these may be introductions.

However, there is a possible complication. Many of the populations are closely associated with sea-bird colonies (Table I). There is slight evidence that B. oleracea seeds may be distributed by sea-birds (Gillham 1970). An increase in the population and 'civilization' of man in the British Isles over the last century, has been accompanied by an increase in gull populations, particularly of the herring gull (Larus argentatus Pontopp). This gull feeds on domestic rubbish and breeds on headlands and off-shore islands, often close to towns and villages. I believe the Little Orme population may have arisen by means of sea-bird dispersal. If gulls are an important factor in distribution , then the association of man and B. oleracea could be coincidence. The role of sea-birds is, I believe, non-proven, although they may well help in the distribution of seeds to guano-rich sites (Cultivated cabbages require high nitrogen levels). If this is so, it is difficult to see why wild cabbages are so scarce and on the decrease.

This appendix is published in Watsonia 11, 1976.

APPENDIX E. Table I Siting and first records of extant Brassica oleracea populations.

Locality[1]	1st Record	Present day[1] relationships to habitation	Present day[1] agricultural relationships	Proximity[1] of sea-bird colonies
Denbigh, v.c. 50				
Llandulas	1912 Dallman (1913) J.M. Brummitt (pers. comm. 1975)	by Llandulas	?	?
Caernarvon, v.c. 49				
Little Orme, Llandudno	1895	at edge of town.	none	nest-site
Great Orme, Llandudno	1805 Griffith (1895)[2]	extends from edge of town.	none	nest-site
Pembroke, v.c. 45				
Tenby	1773 Riddlesdell (1905)	within town	none	none
Glamorgan, v.c. 41				
Flat Holm	? G. Ellis (pers. comm. 1975)	?	?	?
Southerndown to Nash Point	1850 Trow (1911)	none	none	none
W. Cornwall, v.c. 1				
St. Ives	1909	within town.	none	none
Prussia Cove	1909 Davey (1909)[2]	by small village.	none	none
E. Cornwall, v.c. 2				
Fowey	1909	at edge of town.	none	nest-site
Polruan	1909	at edge of town.	none	nest-site
West Looe	1909 Davey (1909)[2]	within town.	none	none

APPENDIX E. Table I (data continued)

Locality[1]	1st Record	Present day[1] relationships to habitation	Present day[1] agricultural relationships	Proximity[1] of sea-bird colonies
S. Devon, v.c. 3				
Dartmouth	1882	extends from town.	none	nest-site
Torquay	1882	within town.	none	none
Babbacombe	1892 Keble-Martin & Frazer (1939)[2]	within town.	none	nest-site
Dorset, v.c. 9				
Durdle Door to Lulworth Cove	1895	none	none	nest-site
Kimmeridge Bay	1895	by small village.	?	none
Winspit to St.Alban's Head	1895	none	cabbage cultivated nearby.	none
Handfast Point	1895 Mansel-Pleydell (1895)[2]	none	cabbage cultivated nearby.	nest-site
Isle of Wight, v.c. 10				
Freshwater Bay	1860? (Bromfield 1860) R.P. Bowman (pers. comm. 1974)	none	derelict fields	nest-site
E. Kent, v.c. 15				
Kingsdown to Folkestone	1551 Turner (1551)	centred by Dover and St. Margaret's Bay.	derelict fields cabbage cultivation	nest-site
Stoney Bay (Broadstairs)	1903 Pittock (1903)	within town	cabbage cultivated nearby	none

APPENDIX E. Table I (data continued)

Locality[1]	1st Record	Present day[1] relationships to habitation	Present day[1] agricultural relationships	Proximity[1] of sea-bird colonies
N.E. Yorks. v.c. 62				
Whitby	1906	within town.	none	roost?
Staithes	1831	by and amongst village.	among derelict allotments.	nest-site
S. Northumberland, v.c. 67				
Tynemouth	1805 Winch et al. (1805)	within town	none	nest-site
Fife, v.c. 85				
Crail	1840 G.H. Ballantyne (pers. comm. 1975) Young (1936)	by harbour in village.	cabbages & kale grown nearby.	nest-site
Forfar, v.c. 90				
Auchmithie	1913 R. & M. Corstophine (1940 MS)	centred on village	cabbages grown close by.	nest-site
E. Ross, v.c. 106				
Fortrose	1968 U.K. Duncan (pers. comm. 1975)	by a town?	?	?

1. These data are based principally on personal observations.
2. The reference applies to all the records for that vice county.

APPENDIX F. MORPHOLOGICAL VARIATION IN B. OLERACEA

It became apparent during studies of B. oleracea populations, that there was a degree of intra- and inter-population variation in plant morphology. To study this a number of characters were measured on selected plants (chapter 4), from which a series of ratios were constructed (ratios rather than gross measures were usually used, since ratios are likely to be less subject to environmental variation). In addition, a number of other non-metric characters were also scored (Table F1). The characters finally employed, were those empirically considered to give the greatest amount of information concerning intra- and inter- population variability.

The data was summarised in terms of picrate score (Table F2), the possible relevance of which has been discussed previously (chapter 8).

The inter-population variation which was observed, suggested that the populations had become locally differentiated (populations of B. oleracea are very isolated). This may have been due to the possibly independent origin of each population, through escapes from cultivation (Appendix E). To analyse the collected information for such differentiation, the data for each individual flowering plant was treated to a cluster analysis by computer (using the Clustan 1A set of programs). Only the data for flowering plants (in total 337 individuals) was used, since these plants had survived for at least four or five years and are probably the most representative of local conditions.

The analysis used was Ward's method followed by relocation (Wishart 1969). The part-optimal solution was displayed as a dendrogram (Fig F1), although this differed little from the terminal solution (Tables F3, F4; Fig F2).

The dendrogram data is displayed in terms of regional groupings. This demonstrates that there is a degree of local differentiation. The major

Table F.1 The characters and ratios used to study morphological variation.

Character	Scale	
Lamina		
a) length to breadth	ratio	
b) shape[1]	ratio	
c) edge	0 - 6	0 = entire 6 = deeply indented
d) number of basal lobes	count	
Flower		
e) diameter	cms	
f) petal shape[2]	ratio	
g) colour[3]	1 - 4	1 = white 2 = cream 3 = light yellow 4 = yellow
Plant		
h) age	years	
i) stem diameter[4]	cms	
j) width : height[5]	ratio	
k) width : stem diameter	ratio	
l) picrate score	1 - 5	

Notes:

1. The width of the lamina at its midpoint/the width at $^1/_3$, the length from the tip.
2. Length of petal/width of petal.
3. The flower colours were scored subjectively, with no reference to standard colour charts.
4. Stem diameter at ground level.
5. The width at its widest vegetative point/ the length of stem from ground level to vegetative apex.

Table F.2 Summary of the data in terms of picrate score.

		Picrate score					Total	
	character	1	2	3	4	5	mean	C.V.%
a)	length : breadth	1.46	1.43	1.36	1.34	1.40	1.43	34.69
b)	shape	1.10	1.09	1.12	1.18	1.15	1.11	16.58
c)	edge[1]	3.00	3.00	2.73	2.25	2.17	2.88	-
d)	no. lobes	3.13	3.25	3.71	3.50	3.38	3.25	60.00
e)	flower diam.	2.83	2.71	2.79	2.85	2.98	2.81	15.66
f)	petal shape	1.34	1.35	1.29	1.40	1.40	1.34	17.16
g)	flower colour[1]	4.00	4.00	4.00	4.00	3.50	4.00	-
h)	age	5.52	5.57	4.78	4.82	5.00	5.38	51.12
i)	stem diam.	1.69	1.69	1.56	1.38	1.39	1.65	59.09
j)	width : height	1.84	2.34	2.54	2.18	1.56	2.04	90.69
k)	width:stem diam.	29.01	29.99	33.61	36.26	33.38	30.38	57.60

Notes: 1 median values.

N.B. Flowering characters are the mean for the flowering plants, whereas all the other results are based on the total data.

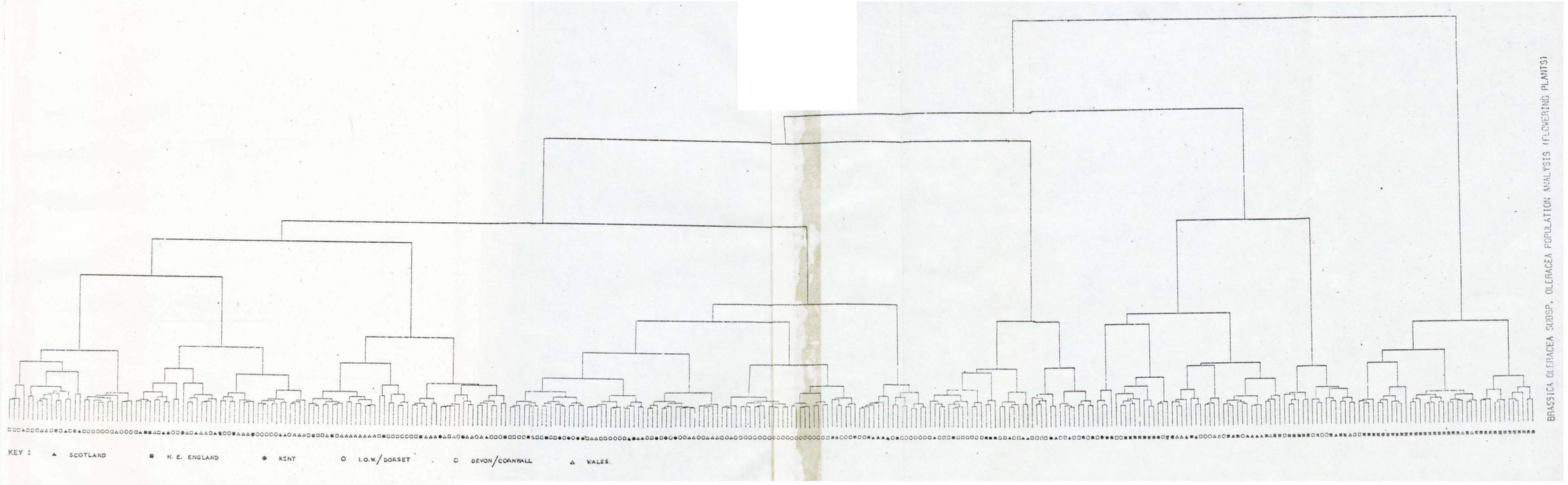


Figure F.1 The part-optimal solution of the cluster analysis, to show the relationship between the populations as geographic units.

Table F.3 The terminal solution of the cluster analysis. The values are as the percentage of each population in each of the three clusters.

Population	Cluster 1	2	3
Wales			
Great Orme	81.8	18.2	0.0
Tenby	87.5	12.5	0.0
Southerndown	75.0	16.7	8.3
Devon/Cornwall			
St. Ives	50.0	0.0	50.0
Prussia Cove	60.0	10.0	30.0
Polruan	45.0	10.0	45.0
Looe	50.0	21.4	28.6
Dartmouth	71.4	14.3	14.3
Babbacombe	52.6	15.8	31.6
Dorset/I.O.W.			
Durdle Door/ Lulworth Cove	77.8	5.6	16.6
Kimmeridge Bay	87.5	0.0	12.5
St. Aldhelm's Head/ Winspit	92.9	3.6	3.6
Handfast Point	75.0	0.0	25.0
Freshwater	100.0	0.0	0.0
Kent			
S. Foreland	52.4	28.6	19.1
N.E. England			
Whitby	8.3	25.0	66.7
Staithes	32.5	67.5	0.0
Tynemouth	15.2	84.8	0.0
Scotland			
Crail	55.6	16.7	27.8
Auchmithie	87.5	12.5	0.0
mean	62.9	18.2	19.0
coefficient of variation %	40.2	119.5	101.2

Table F.4 Cluster diagnostics, the values are as the mean values for each character.

Character	Cluster 1	2	3
a) length : breadth	1.44	1.54	1.38
b) shape	2.31	0.94	1.21
c) edge[1]	2.65	3.98	2.71
d) no. lobes	2.31	3.33	2.96
e) flower diam.	2.79	2.83	2.83
f) petal shape	1.29	1.47	1.34
g) flower colour[1]	4.00	4.00	4.00
h) age	5.92	8.62	6.88
i) stem diam.	1.65	3.02	2.11
j) width : height	1.37	1.75	1.38
k) width:stem diam.	24.07	20.36	26.93
l) picrate score[1]	1.00	1.00	4.00

Notes: 1. Median values.

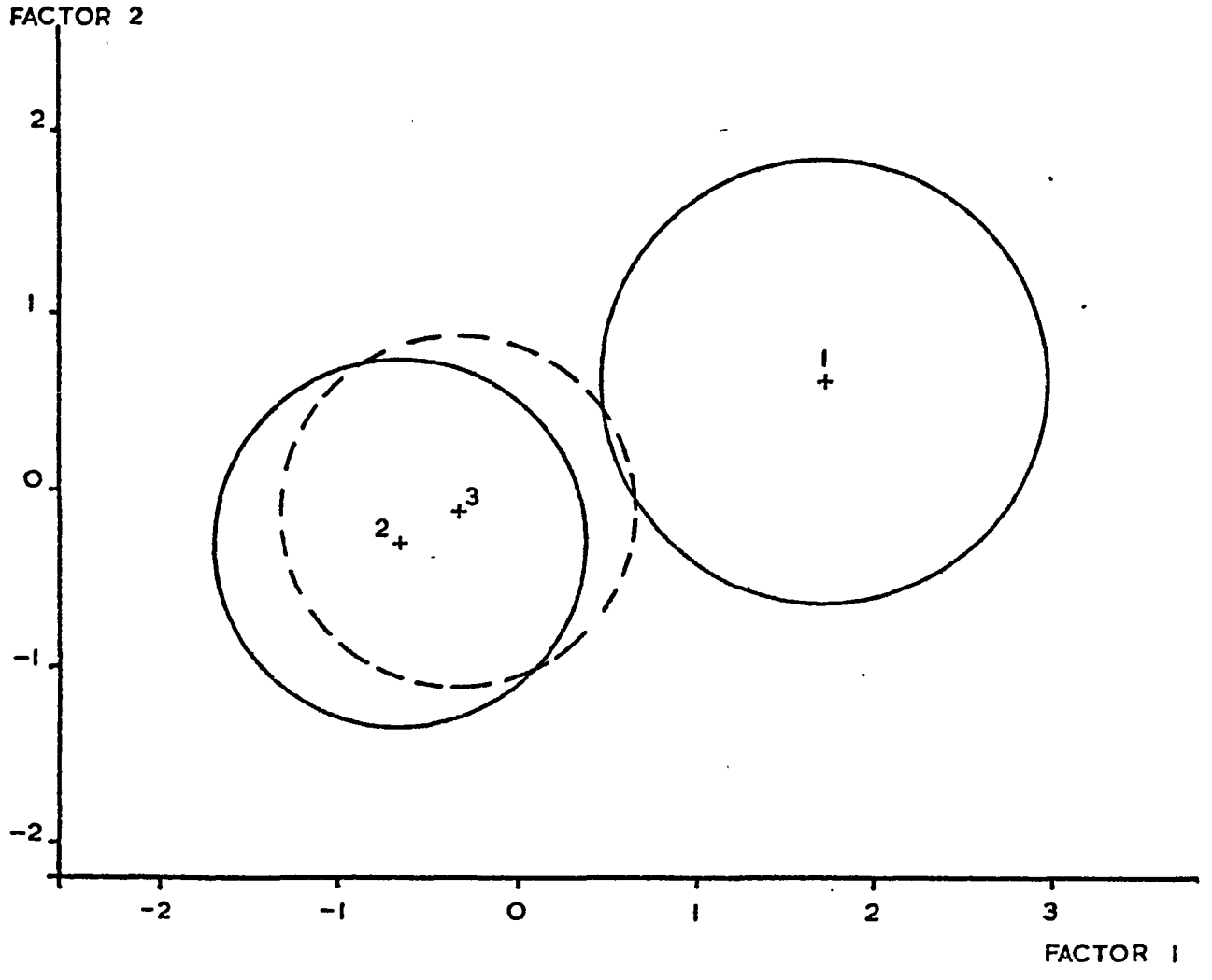


Figure F.2 The optimal terminal solution of the cluster analysis, to show the relationship between the three clusters. The clusters are displayed as circles on the first two principal components axes.

split is between the populations of north-east England (■) and the rest of the country. Various other groupings are apparent, particularly of the Isle of Wight/Dorset (○) and Welsh populations (△), although the distinctions between these groupings is not clear-cut. Certainly no clinal trend emerged; thus populations in the west do not show maximum differentiation from, for example, the Scottish populations. The results suggest that in general forces acting on the morphology of the plants are fairly similar at each site. This is perhaps to be expected considering the extreme environment in which B. oleracea is found, plants rarely being found away from maritime cliff faces.

The terminal solution confirmed this similarity between most of the populations. The optimal solution was to reduce the data to three clusters. With various exceptions the majority of the plants were found in cluster 1 (62.9%), with fewer in each of the other clusters (18.2% and 19.0% respectively). The exception was that the plants from Staithes and Tynemouth were principally in cluster 2. Of the main geographic groupings, the Welsh plants are mainly in cluster 1 with a low proportion in cluster 2. The plants from Cornwall and Devon are found in all three clusters, although mainly in clusters 1 and 3. The Isle of Wight/Dorset plants are principally in cluster 1 with a low proportion in cluster 3. The plants from Kent are largely in clusters 1 and 2, with the two Scottish populations showing variable groupings. Thus within most populations there is a certain degree of variation away from what appears to be the 'norm' of cluster 1, this variation varying locally.

Other results suggest that this may be a plastic differentiation, rather than genetic. A number of plants from Dorset, Wales and North-east England were grown in an experimental plot for 3 years. At the end of this period comparable morphological measurements were taken from them, and the data included in the cluster analysis. All of these plants were classified

into cluster 1.

A very interesting result is obtained from the terminal cluster diagnostics (Table F4, Fig F.2). Cluster 3 is differentiated on the basis of picrate score, the individuals in it being principally high picrate score plants. However, as a plot of the cluster groupings (as cluster circles) on the principal components axes 1 and 2 demonstrates, cluster 3 is a subset of cluster 1, cluster 2 being clearly differentiated from the other two clusters. Nevertheless this distinction lends weight to the hypothesis that high picrate score plants tend to be morphologically differentiated (as discussed in chapter 8).

APPENDIX G. The laboratory culture of P. brassicae

The eggs as received from the suppliers (or later from my own stock) were kept at 20°C and a 16h day. This light/temperature regime was continued after the larvae had emerged. The larvae were reared on cultivated or wild cabbage, whichever was most readily available; both were equally acceptable. Artificial media were not found to be acceptable (even when supplied as especially for P. brassicae larvae). The five instars from hatching to pupation took approximately 20 days. The pupation lasts from 14 - 21 days.

The imagines presented the greatest problems, since only specially bred laboratory strains will breed and survive under experimental conditions. The imagines were fed using artificial flowers consisting of an orange disc, with a tube containing 10% sucrose at its centre (David & Gardiner 1961b). The light quality also has to be correct before copulation will take place. Although David & Gardiner (1961a) found that only daylight gave consistent stimulation, I found that mercury vapour/tungsten illumination of 3:1 (in wattage power) was satisfactory, daylight being unnecessary. A derivative of David & Gardiner's 'Cambridge' strain was eventually obtained and used for the majority of the experiments.

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APPENDIX C. Raw data, based on discs from Tynemouth, additional data.

PLANT REFERENCE NUMBER	D1	P25	G18	A4	G8	B5	P2	G19	P24	A14	B22	G7
MARCH	20.0	11.0	17.0	15.0	20.0	14.0	9.0	17.5	22.0	15.0	18.0	16.0
APRIL	20.0	17.5	19.0	21.0	21.0	20.0	13.0	17.0	19.0	19.0	19.5	21.0
MAY	17.0	18.0	22.0	17.5	16.0	19.0	17.0	18.0	19.0	20.5	19.0	19.0
JUNE	20.5	19.5	19.0	20.5	19.5	20.5	17.5	15.0	16.5	18.0	19.5	20.5
JULY	19.5	19.5	18.5	20.5	19.5	19.5	19.0	20.5	20.5	18.0	20.0	20.5
AUGUST	11.5	11.0	10.0	22.0	12.0	14.0	10.0	*	15.0	19.0	20.0	17.0
SEPTEMBER	9.0	22.0	*	9.5	10.0	8.0	9.0	9.5	14.5	15.0	8.5	11.0
OCTOBER	13.0	15.0	12.0	18.0	15.5	14.0	13.0	13.0	15.0	18.0	16.0	19.5
NOVEMBER	17.0	14.0	15.0	24.0	15.0	16.0	13.0	11.0	14.5	16.0	20.0	17.0
DECEMBER	17.5	16.0	15.5	23.0	10.5	15.0	16.0	17.0	17.5	19.0	17.0	20.0
JANUARY	15.5	17.5	14.0	22.0	13.0	21.0	10.0	14.5	15.0	14.5	22.0	20.0
FEBRUARY	11.0	14.0	16.0	17.0	14.0	13.0	11.0	11.0	12.5	13.0	12.0	13.0
MARCH	13.0	16.0	14.0	12.0	14.0	12.0	11.0	12.0	12.0	13.0	10.5	*
APRIL	17.0	16.0	15.5	19.0	19.0	14.5	17.0	12.0	17.0	18.0	16.0	20.5
MAY	17.0	17.0	17.0	19.0	14.5	15.0	17.0	21.0	13.0	15.0	22.0	19.0

APPENDIX C. (Data continued)

PLANT REFERENCE NUMBER	B19	P5	D3	B14	44	A6	43	P7	P12	G9	P17	P16
MARCH	19.0	11.0	18.5	16.0	13.0	19.0	17.0	10.5	17.0	18.0	10.0	14.0
APRIL	15.0	18.0	21.0	21.0	21.0	21.0	15.0	18.0	19.5	20.0	19.0	18.5
MAY	17.0	16.5	21.5	18.5	18.5	18.0	15.5	20.0	18.0	18.5	*	14.0
JUNE	20.5	16.5	17.5	19.5	17.5	20.5	17.5	20.5	19.5	18.0	16.5	18.5
JULY	20.0	15.5	16.5	21.5	18.5	20.5	17.5	20.5	17.5	20.5	20.5	20.5
AUGUST	14.0	9.0	11.5	21.0	11.0	11.0	12.0	17.0	17.5	16.0	10.5	16.5
SEPTEMBER	17.0	10.0	16.0	15.0	16.5	14.5	10.5	9.5	11.0	11.0	10.0	22.0
OCTOBER	14.0	11.5	11.0	18.0	15.5	15.5	14.0	13.0	17.0	12.0	19.5	14.0
NOVEMBER	13.0	12.0	13.0	17.0	17.0	11.0	16.0	15.5	20.0	13.0	19.0	12.0
DECEMBER	21.5	21.0	17.0	20.0	19.5	13.5	13.5	17.5	21.0	21.0	15.0	15.5
JANUARY	15.0	19.0	15.0	13.0	19.0	12.0	13.5	16.5	20.5	19.0	17.5	16.0
FEBRUARY	11.5	13.0	14.0	13.0	15.0	13.0	14.0	11.0	19.0	16.5	14.0	12.0
MARCH	16.0	14.0	14.0	16.0	15.0	17.0	13.0	15.0	15.0	13.0	13.0	18.0
APRIL	16.5	16.0	17.5	21.0	18.0	18.0	18.0	16.5	19.0	20.0	17.0	17.0
MAY	19.5	19.5	17.5	20.0	18.0	16.0	17.0	19.0	19.0	22.5	14.0	17.0

APPENDIX C. (Data continued).

PLANT REFERENCE NUMBER	P11	P23	42	B10	A5	G16
MARCH	17.0	16.0	17.0	14.0	18.0	15.0
APRIL	20.0	21.0	18.0	20.0	21.0	21.0
MAY	18.0	22.0	20.0	13.0	20.0	16.0
JUNE	19.5	20.5	19.5	15.5	19.5	21.0
JULY	20.5	20.0	19.0	20.5	21.0	20.5
AUGUST	17.0	19.5	11.5	11.0	19.0	13.0
SEPTEMBER	14.0	11.0	13.0	12.0	11.0	14.0
OCTOBER	15.0	12.0	14.0	12.0	13.5	13.0
NOVEMBER	20.0	14.0	11.0	13.0	17.0	13.0
DECEMBER	20.0	21.5	15.5	12.0	21.0	19.0
JANUARY	15.5	18.0	13.0	15.0	15.5	19.0
FEBRUARY	16.0	10.0	15.5	12.0	13.0	14.0
MARCH	13.0	12.0	14.5	16.0	14.5	15.0
APRIL	18.5	16.0	18.0	18.0	19.0	19.0
MAY	17.0	15.0	15.5	14.0	19.0	18.0

Table 5.1 t-values associated with frass means.

Picrate Score		Picrate Score 1	Picrate Score 2	Picrate Score 3
2		0.76		
	P	N.S.		
3		0.99	0.002	
	P	N.S.	N.S.	
4		1.55	0.81	1.31
	P	N.S.	N.S.	N.S.

5.2) Raw data from the multiple choice experiments, using P. brassicae values as percentages.

Picrate Score 1L	x	1H
75.4		24.6
50.1		49.9
37.3		62.7
70.8		29.2
47.8		52.2
54.5		45.5
52.3		47.7
41.9		58.1
53.8		46.2

Picrate Score 1	x	2
65.7		34.3
63.3		36.7
52.8		47.2
25.5		74.5
48.0		52.0
16.8		83.2
63.2		36.8
51.9		48.1
22.9		77.1
45.6		54.4

Picrate Score 1	x	4
76.3		23.7
92.6		7.5
62.5		37.5
57.7		42.3
32.3		67.6
72.8		27.2
66.1		33.9
90.2		9.8
49.1		50.9
35.3		64.7
40.6		59.4
59.1		40.9
37.8		62.2
44.6		55.4
19.7		80.3
55.8		44.2

Picrate Score 1	x	5
43.5		46.5
35.1		64.9
56.6		43.4
69.6		30.4
44.9		55.1
43.3		56.7
48.8		51.2

Picrate Score 2L	x	2H
51.8		51.2
33.0		67.0
56.4		43.6
47.1		52.9

Picrate Score 2	x	3
38.5		61.5
58.4		41.6
59.6		40.4
98.2		1.8
47.2		52.8
70.4		29.6
69.0		31.0
63.8		36.2
78.6		21.4
70.3		29.7
79.5		20.5
66.7		33.3

L lower picrate response (higher reflectance)

H higher picrate response (lower reflectance)

5.2) Data continued.

Picrate 2	Score 4
2.7	97.3
69.9	30.1
21.3	78.7
59.6	40.4
46.8	53.2
100.0	0.0
51.5	48.5
34.9	65.1
28.2	71.8
62.2	37.9
75.2	24.8
36.3	63.7
51.1	48.9

Picrate 3L	Score 3H
43.9	56.1

Picrate 3	Score 4
58.6	41.4
18.0	82.0
39.4	60.6
45.4	54.6
92.5	7.5
77.2	22.8
55.6	44.4
38.3	61.8
54.6	45.4
41.7	58.3
27.7	72.3
66.9	33.1
24.5	75.5
30.2	69.7
57.8	42.2
22.6	77.4
27.7	72.7
50.3	49.7
69.1	30.9
44.5	55.5
44.8	55.2
70.8	29.2
62.2	37.8
10.9	89.1
53.5	46.5
63.9	36.1
21.9	78.1
31.1	68.1
2.6	97.4
44.7	55.3
23.7	76.3
44.3	55.7

Picrate 4L	Score 4H
54.0	46.0
41.9	50.1
80.3	19.7
28.9	71.1
28.2	71.8
73.0	27.0
81.5	18.5
44.1	55.9
69.0	31.0
36.4	63.6
74.2	25.8
89.3	10.7
21.2	78.8
51.5	48.5
33.9	66.1
15.0	85.0
11.7	88.3
43.9	56.1
29.4	70.6
53.4	46.6
34.1	65.9
87.1	12.9
17.4	82.6
44.0	56.0
65.4	34.6
48.7	51.3

Picrate 4	Score 5
40.9	59.1
85.7	14.3
52.1	47.9
48.0	52.0
24.3	75.7
23.1	76.9
41.7	58.3
45.1	54.9

6.1a) The raw data from the multiple choice experiments using Helix aspera, values are as percentages.

Picrate Score 1L	x	1H
7.1		92.9
38.3		61.7
86.2		13.8
11.4		88.6
5.6		94.4
26.7		73.3
41.0		59.0
13.1		86.9
23.7		71.3
28.1		71.3

Picrate Score 1	x	2
80.5		19.5
81.1		18.9
83.9		16.1
73.0		27.0
76.0		24.0
81.0		19.0
81.0		19.0
33.0		67.0
15.3		84.7
36.6		63.4
44.3		55.7
40.7		59.3
42.6		57.4
32.8		67.2
59.4		42.7

Picrate Score 1	x	3
28.2		71.8
85.6		14.4
58.2		41.8
50.0		50.0
55.5		44.5

L lower picrate response (higher reflectance)

H higher picrate response (lower reflectance)

6.1a) Data continued.

Picrate 1	x	Score 4
40.0		60.0
29.2		70.8
49.0		51.0
41.0		59.0
39.8		60.2

Picrate 2L	x	Score 2H
73.3		26.7
100.0		0.0
80.7		19.3
100.0		0.0
97.6		2.4
51.6		48.4
92.7		7.3
91.3		8.7
100.0		0.0
38.7		61.3
100.0		0.0
98.2		1.8
98.6		1.4
100.0		0.0
86.6		13.4
67.7		32.3
100.0		0.0
96.8		3.2
93.2		6.7
81.0		19.0
87.4		12.6

Picrate 2	x	Score 3
0.0		100.0
12.5		87.5
0.0		100.0
11.8		89.2
19.2		80.8
8.7		91.3

6.1a) Data continued

Picrate 2	x	Score 4
26.4		73.6
100.0		0.0
100.0		0.0
75.5		24.5

Picrate 3	x	Score 4
100.0		0.0
100.0		0.0
89.2		10.8
80.0		20.0
50.0		50.0
30.0		70.0
49.6		50.3
0.0		100.0
40.0		60.0
33.0		67.0
57.2		42.8

Picrate 4H	x	Score 4L
0.0		100.0
38.6		61.4
40.0		60.0
8.3		91.7
34.9		65.1
29.2		70.8
20.4		79.6
17.3		82.7
82.4		17.6
35.4		64.6
17.2		82.8
29.8		70.2
50.2		49.8
28.5		71.5
44.8		55.2
0.3		99.7
4.5		95.5
13.9		86.1
5.4		94.6
5.3		94.7
73.2		26.8
7.4		92.6
58.6		41.4
100.0		0.0
18.6		81.5
7.3		92.7
31.3		68.7
42.9		57.1
97.5		2.5
0.1		99.9
30.4		69.6
59.9		44.1
75.1		24.9
39.5		60.5
33.7		66.4

6.1b) The raw data from the multiple choice experiment using A. ater, values are as percentages.

Picrate Score		
1	x	3
85.9		14.1
22.9		77.1
82.9		17.1
67.7		32.3
64.9		35.2

Picrate Score		
1	x	4
1.0		99.0
67.8		32.2
60.0		40.0
70.4		29.6
84.3		15.7
56.7		43.3

Picrate Score		
3	x	4
40.5		59.5
34.9		65.1
1.2		98.8
66.6		33.3
38.4		61.6
97.2		2.8
46.5		53.5