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DEVELOPMENTAL AND PHYSIOLOGICAL STUDIES OF

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BRACKEN (PTERIDIUM AQUILINUM)

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by

G. I. McIntyre

A thesis submitted to the  
University of Glasgow for the  
Degree of Doctor of Philosophy

July, 1961

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## GENERAL INTRODUCTION

Bracken, Pteridium aquilinum (L.) Kuhn, is generally regarded as being one of the most successful of all vascular plants. Enjoying an almost cosmopolitan distribution (Tryon, 1941) it occurs extensively in both temperate and tropical regions of the world exhibiting a capacity for vigorous growth in a remarkable diversity of habitats. Nowhere, however, does it attain a greater luxuriance than on the upland pastures to the north and west of Britain where its increasing abundance during the last few generations has resulted in a progressive and serious restriction of grazing (Braid, 1934; Cmd. paper 6498, 1944; Cmd. paper 6494, 1944) and has led to its recognition as an agricultural weed of considerable economic importance.

Growing by means of an extensive and deeply buried rhizome the plant is not only well protected against adverse environmental conditions but is, at the same time, afforded a considerable degree of immunity to the effects of those treatments which have so far been employed in its control. Thus, while it is well known that defoliation, if carried out systematically over a period of several years, may achieve a useful reduction in the vigour of the plant (e.g. Braid, 1935, 1939) it is clear from more recent investigations (Conway and Stevens, 1954) that the abundant food reserves

present in the rhizome and the plant's capacity to utilise them in the production of new fronds seriously detracts from the efficiency of this treatment as a method of control and renders it economically impracticable as a means of total eradication. The results obtained by the foliar application of various toxic chemicals such as sulphuric acid and sodium chlorate have proved no more satisfactory for while these substances may kill the fronds to which they are applied the effect of such treatments on the rhizome, and thus on the growth and regenerative capacity of the plant, is no greater than that produced by cutting and has been found more costly to achieve (Braid, 1937).

Although the discovery in recent years of the herbicidal properties of 2,4-dichlorophenoxyacetic acid and other related compounds, and the early demonstration of the effectiveness of these herbicides against deep rooted perennial weeds (Hamner and Tukey, 1944) seemed to offer a more promising method of control preliminary field trials failed to give satisfactory results (Egler, 1950; Holly et al., 1952) and led to the classification of bracken as a highly resistant species (Brit. Weed Contr. Conf., 1953). Investigations by Stevens (1953), however, showed that the apparent immunity of the mature bracken plant was not shared by the young sporeling. In greenhouse experiments, which will later be more fully described, it was found that sporelings only a few months old

were severely injured when treated with certain of these compounds, the young rhizomes showing morphological responses similar to those typically induced in susceptible species.

In view of this observation by Stevens and the well established complexity of the factors which determine the effectiveness of a growth regulating herbicide (discussed by Audus, 1953 and Leopold, 1955) it was considered desirable that the effect of these herbicides on bracken should receive some further investigation. It was thus primarily with the object of undertaking this work that the present investigations were initiated.

Since it was intended that the first part of the work would take the form of greenhouse experiments some time was spent initially in establishing methods suitable for the propagation of the necessary experimental material. In addition, the writer considered it advisable, before undertaking investigations of a physiological nature, to acquire a somewhat greater familiarity with the salient features of the development and morphology of the bracken plant itself, and a period of several months was therefore devoted to general observational studies both in the greenhouse and in the field. In studying the extensive literature on bracken it was found that certain of the observations which were recorded in the course of this preliminary work had either not previously been described or were at variance with those

reported by earlier investigators. It was therefore decided that these observations on the development of the plant, although having no obvious significance in relation to the problem of its control, were of sufficient botanical interest to be worthy of further investigation.

The work which was subsequently carried out is therefore presented in three parts. In Part I an outline is given of the methods employed in the propagation of large numbers of uniform sporelings for use in experimental work. This is followed in Part II by an account of three small investigations relating to different aspects of the development and morphology of both the young sporeling and the adult plant. Finally, Part III is devoted to a study of the translocation and effects of the growth regulating herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). This work falls naturally into three sections. The first, which may be regarded as an extension of the earlier investigation by Stevens (loc. cit.), is concerned with the translocation of 2,4-D in the sporeling and its morphological and anatomical effects on the rhizome. This is followed in section 2 by a small field experiment designed to investigate the effect of the herbicide on the rhizome of the mature bracken plant. Finally, in section 3, an account is given of some preliminary work with 2,4-D labelled with radioactive carbon ( $C^{14}$ ) in which the technique of autoradiography has been used as a means of studying some of the factors

affecting the translocation of the herbicide. In a concluding discussion (section 4) the results obtained in each phase of the investigation are considered in relation to some of the problems associated with the use of growth regulating herbicides as a means of bracken control.

PART I

METHODS OF PROPAGATION

## THE GREENHOUSE PROPAGATION OF SPORELINGS FOR EXPERIMENTAL WORK

Although bracken, in common with many other species of ferns, can be easily raised from spores under greenhouse conditions preliminary investigations showed that the propagation of large numbers of sporelings possessing the degree of uniformity desirable in experimental material calls for careful attention to the changing requirements of the plant during its development combined with a rigorous process of selection. The method of propagation which was employed, and which is now described, was based to a considerable extent on the technique previously described by Schwabe (1951) but differs not only in the types of media used but in numerous other respects.

### 1. Preparation of spore cultures

Spore bearing fronds, collected during September from an area of bracken on Milngavie Moor, near Glasgow, were laid between sheets of paper in the laboratory and allowed to dry for a period of several days. The sporangia and leaf debris which were shed together with the spores were removed by passing the spores through a piece of metal gauze attached to the end of a short piece of wide glass tubing. The separated spores were then transferred to small glass vials which were corked and stored at room temperature.

(a) Sterile cultures. Prothalli were usually cultured on mineral nutrient agar contained in petri dishes and previously sterilised by autoclaving for 20 minutes at 15 lbs. pressure. The medium, prepared from "AnalaR" grade chemicals, was the same as that previously used for this purpose by Hutchinson and Fahim (1958) and had the following composition (g/l):- KCl, 0.6;  $MgSO_4 \cdot 7H_2O$ , 0.9;  $Ca(NO_3)_2 \cdot 4H_2O$ , 1.0;  $KH_2PO_4$ , 0.6;  $NaNO_3$ , 0.36; ferric tartrate, 0.06; agar agar, 20.

Methods suitable for the surface sterilisation of bracken spores were investigated by Schwabe (loc. cit.) who reported that although sterile cultures could be obtained by treating the spores with 0.36%  $HgCl_2$  germination was considerably retarded. He subsequently found that a sufficient degree of asepsis could be obtained by washing the spores in 10-12 changes of sterile water. In the present investigation a preliminary test was undertaken in which these two methods were combined. The spores were first treated for one minute with 0.1%  $HgCl_2$  and then immediately washed in 6 changes of sterile water. This treatment, which was subsequently adopted, was found to have no detrimental effect on spore germination and, although it did not give completely sterile cultures, fungal contaminants were of rare occurrence and very few cultures were lost by disease.

In sowing the spores 0.1 ml. of the final suspension in

sterile water was applied to the surface of the agar in each dish and evenly distributed with a sterilised glass spreader. The concentration of the spore suspension was previously adjusted to ensure that the cultures were of suitable density. Although preliminary observations suggested that the density of the inoculum need not be precisely controlled the prothalli in high density cultures became rapidly overcrowded and consequently could not easily be transplanted without injury.

The cultures were grown under greenhouse conditions with supplementary illumination provided during the winter months by means of a bank of 8 80 W. fluorescent ("warm-white") tubes which extended the photoperiod to 16 hours and gave an additional light intensity at the level of the plants of approximately 200 f.c. The temperature was not controlled but in the winter months could usually be maintained between 65° and 75°F. During the summer, however, considerably higher temperatures were recorded.

Spore germination was usually complete within 7 days and the prothalli attained an average diameter of 1 mm. after a growing period of approximately 2 weeks. During preliminary work it was observed that, at this early stage of development, many prothalli were fertilised by water droplets which formed on the under surface of the petri dish lids and dripped on to the plants. This early fertilisation resulted in the

production of very small sporelings unsuitable for experimental work and was subsequently prevented by placing a circular piece of sterilised butter muslin under the lid of each dish when the cultures were prepared.

(b) Soil cultures. Although prothalli were grown mainly on agar soil cultures were occasionally employed, particularly during the summer when high temperatures in the greenhouse proved unsuitable for agar cultures. The soil used was a compost consisting of loam, peat and sand in a ratio of 2:1:1 by volume, the three fractions having been passed through an  $\frac{1}{8}$  inch riddle before being thoroughly mixed. The compost, contained in 3 inch pots each covered with a petri dish lid, was sterilised by autoclaving for 20 minutes at 15 lbs. pressure. Spores were applied to the surface of the soil by means of a small atomiser. The cultures were watered from below by standing each pot in the lower half of a petri dish to which water was added as required.

Although the rate of growth of the plants on soil and on nutrient agar was not critically compared no difference was apparent from general observations. The prothalli were found to reach the stage of development at which they were normally transplanted at about the same time on both types of media. The soil cultures, although more easily prepared, had the disadvantage that their water regime required careful control. Waterlogging, if allowed to persist for several

days, frequently resulted in "damping off". The agar cultures, on the other hand, once prepared, required no further attention.

## 2. Transplanting and fertilisation of prothalli

After a growing period of 4-5 weeks the prothalli had generally attained an average diameter of approximately 2 mm. and, at this stage, were transplanted into shallow 4 inch seed pans (fig. 1) each covered with a large petri dish lid and containing compost prepared and sterilised in the manner already described. When transplanting prothalli from agar cultures a fine pointed scalpel was used and a small portion of agar was transferred with each plant so as to avoid injury to the rhizoids. Each seed pan held 40-50 prothalli spaced out at about 1 cm. apart. The growth of the prothalli was not appreciably checked by this operation and they attained an average size of 8-10 mm. after a further 2 weeks. Fertilisation was then induced by immersing the seed pans in a tray of water the level of which was adjusted so that the soil surface was just submerged. The cultures were left in this condition for 4-6 hours during which time the pots were lifted out and tilted occasionally to ensure efficient dispersal of the sperm.



Fig. 1. Transplanted prothalli, 10 days after their transference from spore cultures. (x  $\frac{1}{3}$ )



Fig. 2. Glass humidity chamber containing 5-6 week old sporelings in 3 inch pots. (x  $\frac{1}{6}$ )

### 3. Transplanting of sporelings and their selection for uniformity

Sporophyte embryos could be detected on the under-surface of a high proportion of prothalli 12 days after the cultures had been flooded, and after a further 6-8 days many of the prothalli bore young sporelings with their first frond just fully expanded. The sporelings, carefully selected for uniformity, were then transplanted into 3 inch pots containing sterilised John Innes potting compost (number 1). Initially, 2-3 sporelings were transplanted into each pot.

As observed by Schwabe (loc. cit.), the young bracken plant, at this early stage, is extremely susceptible to injury by desiccation and requires to be maintained under conditions of constantly high humidity. This essential condition was provided by the construction of glass humidity chambers (fig. 2) each sufficiently large to accommodate 42 3 inch pots. By moving apart the two sheets of glass forming the roof of each chamber the humidity could be gradually reduced over a period of several weeks until the plants were sufficiently hardened off to withstand normal greenhouse conditions.

Owing to the very considerable variability of the young sporeling - a feature which will later be described in some detail (Part II, Sect. 2) - plants possessing the degree of uniformity considered necessary for experimental work could

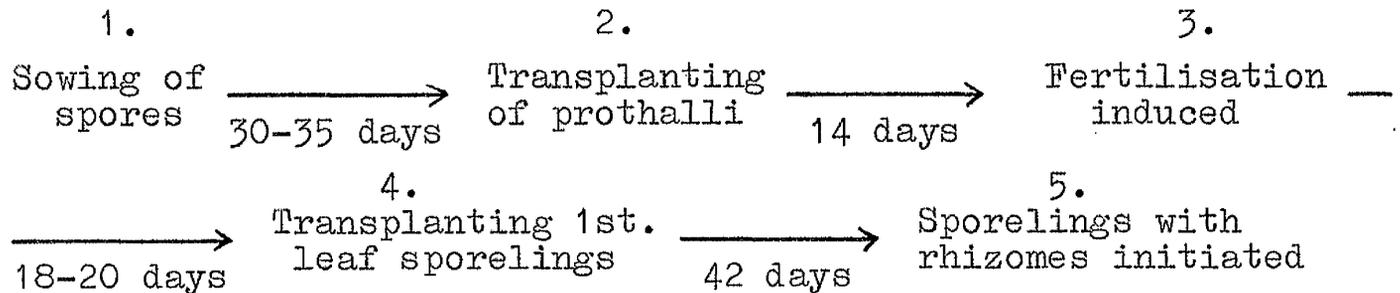
be obtained only by a careful and rigorous selection.

Thus, the initial selection made when the young sporelings were first transplanted was followed by a second selection when the plants had their second fronds fully expanded.

Finally, plants for use in experimental work were selected when they had 4-5 expanded fronds and the rhizomes had just been initiated. Such plants were then transferred to either 4 inch or 5 inch pots.

#### 4. Summary

The several stages of the propagation technique and the approximate duration of each stage may be conveniently summarised as follows:



#### A Note on the Effect of Size and Persistence of the Prothallus on the Development of the Sporeling

It was observed by Schwabe (loc.cit.) that a sporeling borne on a large prothallus showed a much greater morphological differentiation of the primary frond than one on a small (nitrogen deficient) prothallus. He also remarked that differences in prothallial size "seem to have some effect on the vigour of the sporeling produced." Both of these effects

were noted in the present work. Indeed it was with the object of obtaining very vigorous sporelings that fertilisation was delayed until the prothalli had attained a suitable size.

General observations suggested, however, that equally important in determining the size and vigour of the sporeling is the period for which the parent prothallus remains in a healthy condition. It was consistently observed that when the prothallus died or was accidentally covered with soil during transplanting or watering even after the second frond of the attached sporeling had become fully expanded the sporeling's subsequent development was markedly affected. By the time the rhizomes were initiated such plants were considerably smaller than those whose prothalli had remained in a healthy and functional condition and in consequence had to be discarded as unsuitable as experimental material. Although it is generally accepted that the prothallus is essential for the development of the fern sporeling until the first frond is expanded this marked effect of the further persistence of the prothallus does not appear to have been previously observed. Presumably it can be largely attributed to the role of the prothallus in supplying food materials to the young frond primordia and thus determining the ultimate size of the fronds produced.

P A R T I I

S O M E A S P E C T S O F T H E D E V E L O P M E N T

A N D

M O R P H O L O G Y O F T H E B R A C K E N P L A N T

SECTION 1. THE CORRELATIVE INHIBITION OF FROND DEVELOPMENT  
IN THE YOUNG SPORELING AS REVEALED BY SOME  
DEFOLIATION EXPERIMENTS

Introduction

In his ecological studies of bracken Watt (1950) observed that when a growing frond is removed by cutting the rate of growth of the associated frond bud destined to emerge in the following season is significantly increased. A similar but less pronounced effect of defoliation was apparent when the emergent frond was killed or severely injured by frost. Experiments on the effect of defoliation employed as a means of control have also served to illustrate this phenomenon for it has frequently been observed (e.g. Smith, 1928; Braid, 1935) that when bracken is cut early in the growing season before the fronds are fully expanded a second "flush" of fronds are induced to emerge.

That this effect of defoliation may not be restricted to the adult plant is suggested by the work of Albaum (1938b) who showed that the removal of the first frond produced by the young sporeling of Pteris longifolia accelerated the expansion of the second frond. Albaum also demonstrated that this defoliation stimulus could be suppressed by the application of indole-3-acetic acid to the stump of the

petiole of the excised frond. It was therefore postulated that the frond's inhibiting influence could be attributed to its production of a growth regulating hormone.

In view of the close taxonomic affinity of Pteris and Pteridium and the well established effect of defoliation in the mature bracken plant it seemed reasonable to assume that Albaum's results should apply equally well to the bracken sporeling. With the object of testing this assumption the first fronds were removed from a small number of plants and the effect of this treatment on the development of the second fronds was investigated by comparing these defoliated plants with a similar number of control plants of the same age. Careful comparisons, made at intervals over a period of 7-10 days, failed to detect any effect of defoliation on the rate of growth of the younger fronds.

Since this observation was at variance with the results of Albaum's experiments it was decided to carry out some further investigations. Thus a small number of experiments were designed, firstly, to determine whether any effect of defoliation could be demonstrated by more critical methods of observation and, secondly, to compare the effect of removing the older frond at different stages of development.

## Materials and Methods

### 1. Culture methods and environmental conditions

The plants were grown from spores on nutrient agar in petri dishes by the method already described (Part I).

A small growth chamber (fig. 3) enabled the environmental conditions to be controlled to a somewhat greater extent than was possible in the greenhouse. Illumination was provided by means of two 5 ft. 80 W. fluorescent tubes ("warm-white") which gave a light intensity of approximately 200 f.c. at the level of the plants while a photoperiod of 16 hours was automatically maintained by a time switch. A sheet of flashed opal glass forming the lid of the cabinet served to ensure that the light was evenly distributed. The temperature was not controlled and was raised by heat from the lights to a maximum of 75°F, falling to approximately 65°F during the dark period. However, although daily records showed that this range was maintained throughout most of the experiments extremes of 62°F and 81°F were recorded. Humidity was maintained at a constantly high level by lining the floor of the cabinet with filter paper which was kept in a moist condition. During the course of the investigation the cabinet was installed on a bench in the laboratory and plants were removed from it only for brief periods for the purposes of selection, defoliation, and the recording of growth data.

Four to five weeks after the spores had germinated the prothalli were transplanted into 3 inch clay pots containing steam sterilised soil of the same composition as that employed for spore and prothallial cultures (Part I). Five pots, each containing approximately 40 prothalli, were used in each experiment. Each pot was covered with a petri dish lid and was watered from below. After the prothalli had reached a size of 6-7 mm. fertilisation was induced by the method already described (Part I).

## 2. Experimental Procedure

In each of the six experiments to be described the first frond produced by the young sporeling (hereafter referred to as the "primary" frond) was removed from a small number of plants and the effect of this treatment on the development of the second frond was investigated. The experiments fall into two groups, the three experiments in each group being intended as replicates but differing in certain minor details. In experiments 1-3 the primary frond was removed when its lamina was either partly or fully expanded while in experiments 4-6 it was removed at a somewhat earlier stage of development. The general experimental procedure was similar in each of the six experiments. Thus only experiment 1 will be described in detail, the others being described only in so far as they differed from the first experiment.

A. The effect of removal of the primary frond when its lamina is either partly or fully expanded

(a) Selection and treatment of plants

Five pots of prothalli, a total of approximately 200 plants, were grown for the first experiment. Sixteen days after flooding of the cultures a large proportion of the prothalli had produced young sprogophytes. At this stage the petiole of the primary frond varied from 1-3 mm. in length, its lamina being still unexpanded. Thirty-six plants were selected for uniformity and were transplanted separately into small clay pots 1.5 inches in diameter containing the same kind of compost as that used for the prothallial cultures. The plants were then returned to the growth cabinet and after a period of 24 hours were again examined and the thirty most uniform plants selected for the experiment.

The plants were divided into two equal groups. The primary frond was then removed from the plants of one group by means of a fine pair of scissors, while the remaining 15 plants were left intact to serve as controls. The lamina of the excised frond was only partly unfolded and since previous observations had established that under similar environmental conditions the unfolding of the frond lamina occupies a period of 18-24 hours it may be concluded that the plants used in the experiment were very similar in age.

However, a few of the plants were at a slightly more advanced stage of development, the lamina of the frond having just fully uncurled. The maximum age difference within the two groups, as judged by the degree of expansion of the frond, was estimated to be approximately 12 hours.

(b) Growth measurements

Immediately after the removal of the fronds the plants of both groups were individually examined and the length of the second frond accurately determined. The method employed in these measurements was as follows: The small pot was laid horizontally on a glass slide under the low power objective of a monocular microscope (x 30 ) and its position was then adjusted so as to bring the second frond into an exactly horizontal plane. A wedge-shaped piece of plasticine fastened to the slide served to hold the pot in the required position. By adjusting the movable stage the base of the frond was made to coincide with a reference line in the ocular and the stage was then moved laterally until the line coincided with the uppermost point of the crozier, the distance moved being recorded to the nearest 0.1 mm. on the stage vernier scale. The actual points on the young frond between which measurements were made are shown in fig. 4.

Some difficulty was encountered in obtaining accurate measurements when the frond was still less than 1 mm. in length for, at this early stage of development, the lower

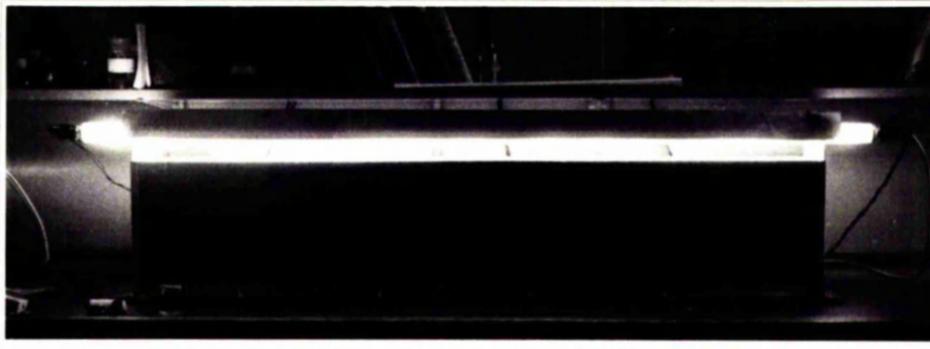


Fig. 3. Growth cabinet used for the investigation ( $\times \frac{1}{16}$ )

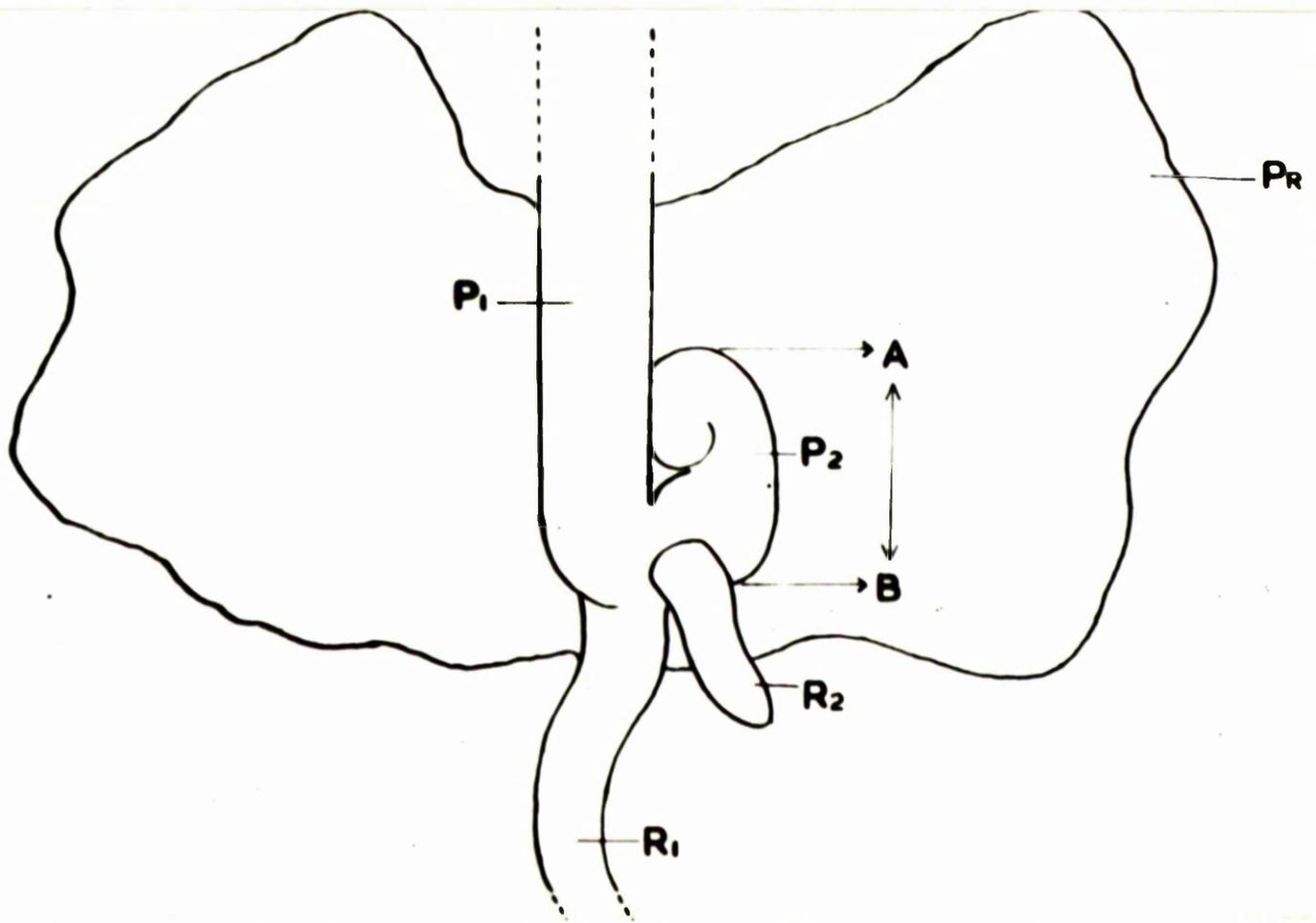


Fig. 4. Diagrammatic drawing of young sporeling and parent prothallus (PR)  
 P<sub>1</sub>, petiole of primary frond; P<sub>2</sub>, second frond;  
 A, B, reference points used in measuring the length of P<sub>2</sub>;  
 R<sub>1</sub>, primary root; R<sub>2</sub>, first adventitious root.  
 ( $\times 15$  approx.)

half of the frond was frequently found to be partly obscured by remnants of calyptral tissue. However, an examination of other plants at a similar stage of development and from which the calyptra had been removed showed that the base of the frond was consistently located at a distance of approximately 0.2 mm. above the junction of the young primary root and the stem. Since this latter point was always clearly visible it was possible to estimate the position of the frond base with a sufficient degree of accuracy. The presence of the first adventitious root (R<sub>2</sub>, fig. 4), which normally emerges when the second frond is 0.8-1.0 mm. in length, also presented difficulties of observation but, by varying the position of the plant, the base of the frond could always be sufficiently revealed for the required measurement to be recorded.

The frond was measured at intervals of 12 hours until it had reached an average length of 3.5 mm. It was generally found that, when this length had been attained, the terminal crozier of several of the fronds started to uncurl. The initiation of this new phase of development eliminated the upper reference point on the frond and no further measurements could therefore be made. Thus, it was mainly the elongation of the petiole which was actually measured although the concomitant increase in diameter of the horizontally disposed portion of the apical crozier contributed to a

slight but undetermined extent to the measurements recorded.

Experiments 2 and 3 differed from the first experiment only in the stage of development of the primary frond when it was removed from the plant. In experiment 2 it was not removed until its lamina was just fully expanded and, in experiment 3, the lamina had been fully expanded for a period of 24 hours before the frond was excised.

B. The effect of removing the primary frond prior to the expansion of the lamina

Experiment 4 was designed to investigate the effect of removing the primary frond when its petiole was still rapidly elongating and thus before the apical crozier had started to uncurl. Initially 45 plants were selected and the approximate length of the primary frond of each plant was measured under a low-power dissecting microscope by means of a pair of dividers. Thirty plants whose fronds ranged from 1.3-2.0 mm. in length were finally selected for the experiment. As before, the plants were divided at random into two equal groups. The apical crozier was then removed from the primary frond of each plant in one group with the aid of a fine pointed scalpel, the remaining 15 plants serving as controls.

At the beginning of the experiment the second frond was still entirely concealed by the ruptured calyptra and the first measurements of its length were not made until 4

days later. Thereafter its size was recorded at regular intervals as in the previous experiments.

Experiments 5 and 6 differed from experiment 4 in the following respects. In each experiment only 18 plants were used of which 9 were defoliated. The young primary frond of each plant was measured under a monocular microscope to the nearest 0.1 mm. by the method previously described. Its length immediately before it was decapitated ranged from 1.2-1.8 mm. (mean  $1.43 \pm 0.043$ ) in experiment 5 and from 1.0-1.2 mm. (mean  $1.16 \pm 0.022$ ) in experiment 6. To attain a greater uniformity in the initial stage of development between the two groups the 18 plants of experiment 6 were first arranged in pairs so that the two members of each pair possessed a very similar length of the primary frond. One member of each pair was then selected at random for defoliation.

"Student's" t-test (Snedecor, 1956) was employed in the statistical analysis of the experimental data.

### Experimental Results

The results of each experiment are expressed graphically in figs. 5 and 6 while the mean values of the measurements recorded are given in the appendix. The considerable variation in these measurements, the standard errors of which

are indicated by vertical lines in the figures, requires some comment. As will be evident from the standard errors the efforts which were made to obtain uniformity in the stage of development of the plants at the beginning of each experiment were not wholly successful. However, although the increasing variation in the lengths of the fronds at successively later stages of development can be attributed mainly to their initial lack of uniformity a study of the individual measurements recorded showed that it was also, to a lesser extent, the result of differences in their rate of elongation. Since all of the plants were subjected to similar environmental conditions it seems probable that these differences in growth were largely a reflection of the unavoidable genetical diversity of the material, although small differences in the size and vigour of the parent prothalli may perhaps have been a contributing factor. However, in spite of these several sources of variation and their effect in reducing the statistical significance of the data good agreement was nevertheless obtained between the results of experiments in which similar treatments were applied.

In experiments 1 and 2 (fig. 5) the removal of the primary frond when its lamina was only partly expanded (Exp. 1) or just fully expanded (Exp. 2) resulted in a slight

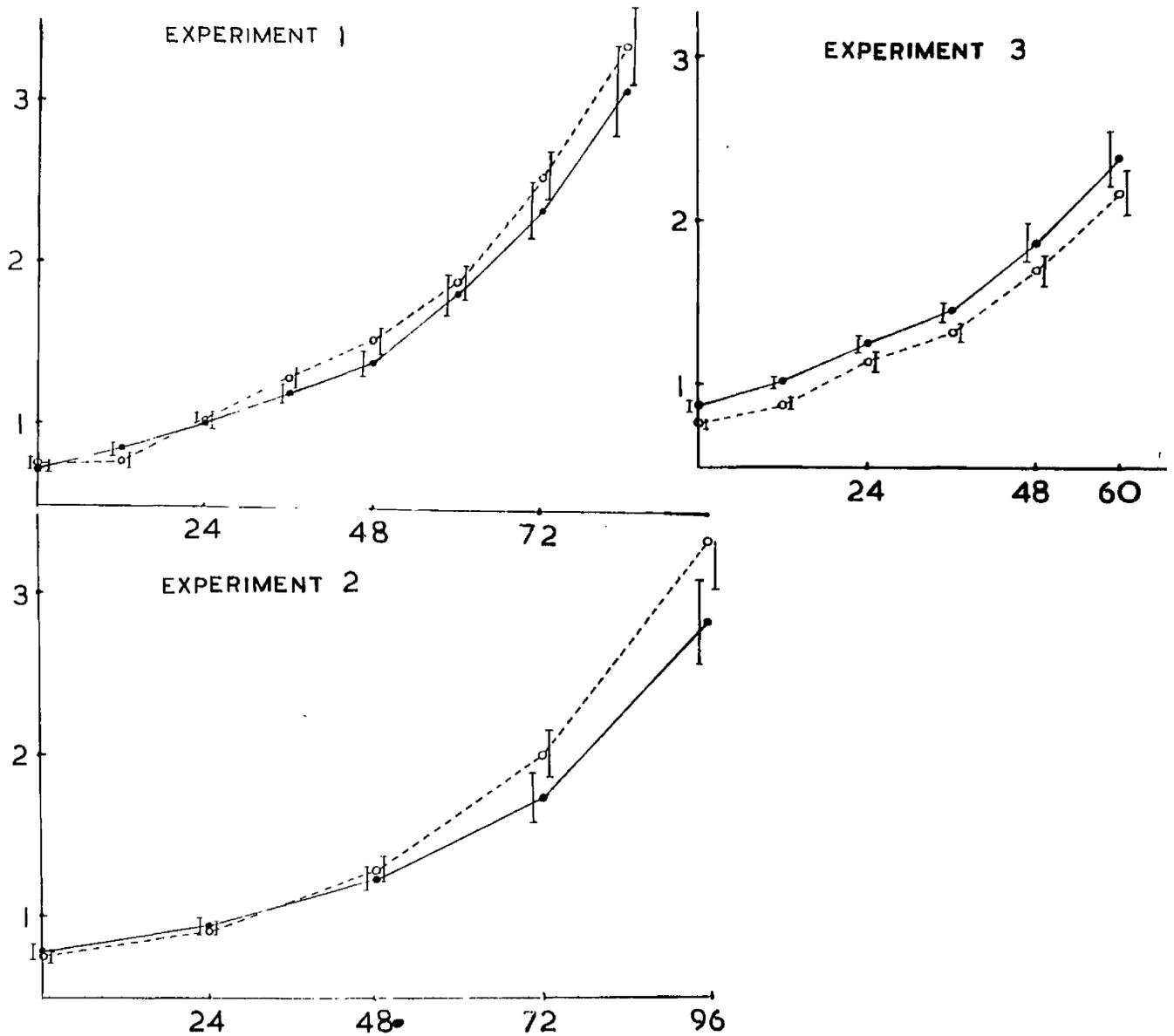


Fig. 5. The effect of removing the primary frond of the sporeling on the development of the second frond. In each graph the time after defoliation (in hours) is shown on the abscissa and the length of the second frond (in mm.) on the ordinate.

—●— = control ---○--- = defoliated plants

The primary frond was removed when its lamina was partly expanded (Exp. 1), just fully expanded (Exp. 2) and 24 hours after full expansion (Exp. 3). The vertical lines equal twice the standard errors of the means.

increase in the rate of growth of the second frond. Although this effect failed to reach the 5% level of significance the close agreement between the results of these two experiments leaves little doubt that the observed differences in growth represent a response to the treatments applied.

In experiment 3 (fig. 5), in which the primary frond was not removed until its lamina had been fully expanded for a period of 24 hours, the effect of defoliation was less apparent. However, an examination of the data suggested that the effect had merely been obscured by the larger size of the second fronds of the control plants as compared with the defoliated group at the beginning of the experiment. This conclusion was supported by the observation (see appendix) that when the second fronds of each set of plants had attained a mean length of 0.88 mm. the growth increment of the fronds of the defoliated plants during the following 12 hours was 0.27 mm. while that of the controls was only 0.15 mm. Although the absence of constant environmental conditions throws some doubt on the reliability of this comparison it seems probable that this marked difference in growth rate can be correctly attributed to the occurrence of a defoliation stimulus similar to that which was apparent in the results of the previous experiments.

A much more pronounced effect of defoliation was

evident, however, from the results of the next three experiments (fig. 6). In experiment 4 the removal of the primary frond when its mean length varied from 1.3-2.0 mm. resulted in a significant increase in the rate of growth of the second frond. This effect was shown to be statistically significant ( $P = 0.05$ ) by measurements recorded 120 hours after defoliation and attained the 1% level of significance in subsequent measurements.

A similar result was obtained in experiment 5 in which the excised frond had a mean length of 1.43 mm. In this experiment, however, the effect was not significant at the 5% level. That this was so can doubtless be attributed to the relatively small number of plants which were used and also to the considerable variation in their initial stage of development. In experiment 6 decapitation of the primary frond when its mean length was only 0.15 mm. resulted in the second fronds attaining a length which, at 105 hours after treatment, was significantly greater ( $P = 0.05$ ) than the controls. As in experiment 4 the 1% level of significance was reached during the subsequent development of the fronds.

In comparing the results of experiments 4, 5 and 6 it was noted (see appendix) that, at 108 hours after defoliation, the difference in the mean lengths of the second fronds of the defoliated and control plants was 0.19 mm., 0.25 mm. and

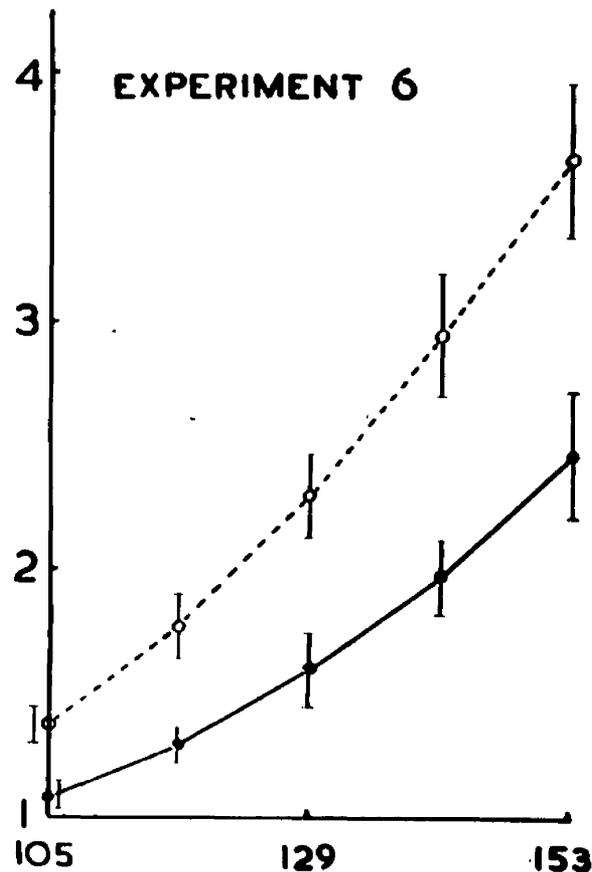
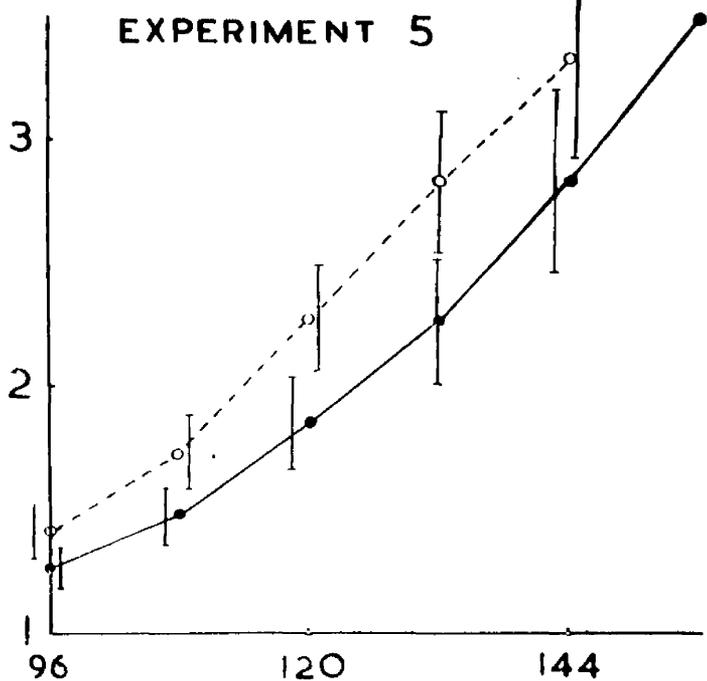
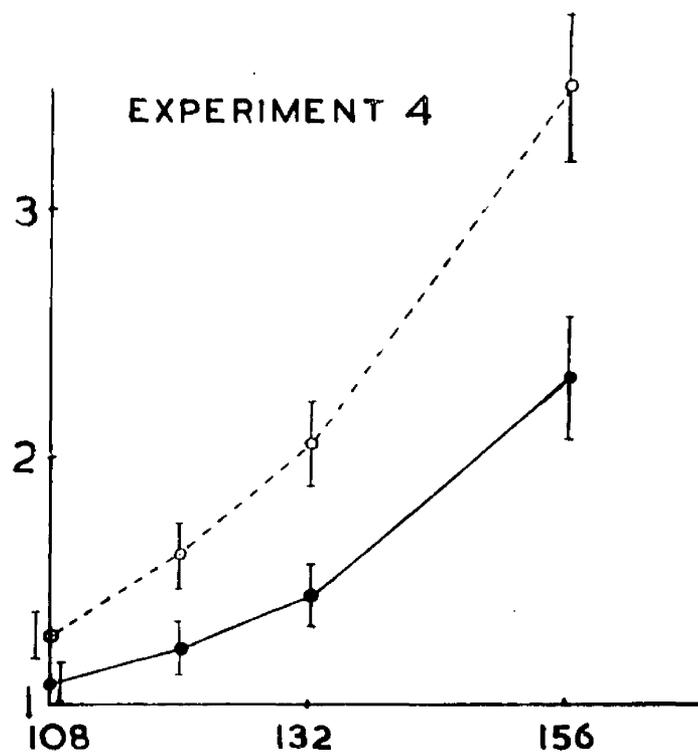


Fig. 6. The effect of removing the primary frond of the sporeling on the development of the second frond. Abscissae : time after defoliation (hours), Ordinates : length of second frond (mm.)

—●— = controls; ---○--- = defoliated plants.

In each experiment the primary frond was removed before the lamina had expanded. Its length when excised ranged from 1.3-2.0 mm. in Exp. 4, from 1.3-1.8 mm. in Exp. 5, and from 1.0-1.3 mm. in Exp. 6. The vertical lines equal twice the standard errors of the means.

approximately 0.32<sup>mm.</sup>/respectively (this difference in experiment 6 being read from the growth curve in fig. 6). These differences clearly show an inverse relationship with the length of the primary frond at the time of its removal from the plant. Its length in experiment 4 ranged from 1.3-2.0 mm. and in experiments 5 and 6 it had a mean length of  $1.43 \pm 0.04$  and  $1.16 \pm 0.03$ <sup>mm.</sup>/respectively.

### Discussion

When these results are compared with those obtained by Albaum in his experiments with sporelings of Pteris longifolia an interesting and marked divergence is immediately apparent. Although both investigations have demonstrated that the primary frond of the fern sporeling exerts an inhibitory influence on the development of the second frond the degree of inhibition recorded by Albaum would appear to be very much greater than was observed in the present investigation. In one experiment of particular interest Albaum removed the expanded lamina from the primary frond of a number of sporelings and observed that the second frond "appeared" 4 days later. He then continued to defoliate the plants, removing each successive frond as it appeared, until four fronds had been removed and the fifth had emerged. Albaum then states that throughout the whole period of the experiment "most of

the control plants had only one primary frond; in a few cases they had developed two."

The interesting feature of this experiment is the remarkable persistence of the inhibiting influence of the primary frond of Albaum's control plants. The removal of the lamina (which Albaum refers to as the "blade" and which is shown by his illustrations to have been fully expanded) caused the second frond to appear after 4 days while its retention on the plant apparently inhibited the emergence of the younger frond for a period which, although not precisely defined, is shown by a study of Albaum's data to have had a duration of rather more than 3 weeks.

In the present investigation, on the other hand, it was found that, when the primary frond of the bracken sporeling was removed after its lamina had become partly or fully expanded (Exps. 1-3) the effect of this treatment on the growth of the second frond was so slight that it was scarcely perceptible. Although it was subsequently shown (Exps. 4-6) that a much greater effect resulted from the removal of the primary frond when it was only 1-3 mm. in length and still undergoing rapid elongation the differences in the recorded mean lengths of the second fronds of the control and defoliated plants, even in these latter experiments, was equivalent to a growing period of only 12-24 hours.

It is clear from this comparison that the inhibiting influence of the primary frond as revealed by the present investigation, being apparently restricted in its occurrence mainly to that phase of the frond's development which precedes the expansion of the lamina, was of a very much shorter duration than the remarkably prolonged inhibition apparent from the results of Albaum's experiments. In seeking an explanation for this discrepancy it would seem that there are two possibilities to be considered. Either the observed difference may be a result of certain genetical differences between the species of ferns used in the two investigations or, alternatively, it may have been caused by differences in the environmental conditions under which the investigations were carried out.

The first of these hypotheses, which implies that the correlative inhibition of frond development may differ appreciably between two closely related species, is found to receive some support from the investigations by Goodwin (1937) of a similar growth correlation in species of Solidago. It was observed that in one species, Solidago sempervirens, each leaf, during its "grand period of growth" strongly inhibited the growth of the next younger leaf. On the other hand, in the related Solidago rugosa several leaves normally developed simultaneously and thus without any mutual

inhibiting effect. Some of the experimental evidence supported the view that the inhibition was controlled by auxin and it was shown that the observed difference in leaf development in the two species was correlated with the relative amounts of auxin which could be extracted from their leaves. Although there appears to have been no comparable studies involving related species of ferns there is little reason to doubt that similar specific differences with respect to this phenomenon may also occur in the latter group of plants. Certainly, it would be interesting to compare the response of Pteris and Pteridium sporelings to defoliation under the same environmental conditions.

Before considering the probable validity and the implications of the second hypothesis, namely, that the difference in the behaviour of the plants may be due to differences in the environmental conditions, it is necessary to describe briefly the method employed by Albaum (1938a) in the culture of his sporelings. Prothalli were raised from spores by a modification of the well known "Sphagnum-pot" technique, the spores being sown on the outer surface of inverted flower pots filled with Sphagnum moss and standing in water. Selected plants were removed from the sides of the pot and placed on small squares of filter paper lying on Seitz filter pads each of which was then laid on top of an inverted flower pot prepared in the same way as for the

spore cultures. During the experiment the pots were contained in a specially constructed "incubator". The temperature was controlled at  $28 \pm 1^{\circ}\text{C}$  and a high relative humidity was constantly maintained. Illumination was provided by 6 60 W. Mazda bulbs. Neither the intensity of the light nor the photoperiod is stated.

Although it is clear from this description that the conditions of temperature and illumination in Albaum's experiments differed considerably from those of the present investigation it seems unlikely that either of these environmental differences could have been responsible for the marked divergence of the results. However, another factor which, although less clearly defined, may well have been of greater significance in its effect on the sporeling's development is suggested from a consideration of Albaum's culture technique. It is apparent that the mineral nutrients which were available to the young sporeling could have been derived only from the water in which the pots were standing and from any readily soluble nutrients which may have been leached from the substance of the pots themselves and from the sphagnum moss which they contained. While the composition of the nutrient solution thus constituted is clearly a matter for speculation it would seem that such a solution may well have been markedly deficient in one or more essential mineral elements. Certainly this view is entirely consistent with

the notable lack of "vigour" of Albaum's control plants most of which produced only a single leaf during a period of more than 3 weeks. In the present investigation, on the other hand, there can be no doubt that the soil on which the prothalli and young sporelings were grown provided a supply of mineral nutrients which was adequate for normal growth.

The postulate that a difference in nutrient supply could have been responsible for the apparent difference in the degree of correlative inhibition exhibited by the plants receives some support from the recent work of Gregory and Veale (1957) who showed that, in flax, the inhibiting influence of the stem apex on the outgrowth of lateral buds could be largely controlled by varying the amount of nitrogen present in the rooting medium. At a low nitrogen level bud development occurred only when the stem was decapitated. When, on the other hand, a high level of nitrogen was provided the inhibiting influence of the stem apex was apparently eliminated, all of the buds developing to a varying extent in the intact plant. As a result of these and other similar observations it was concluded that apical dominance is primarily a consequence of internal competition for nutrients and, more generally, that "nutrition is the main factor in correlative inhibition."

If it be assumed that the mechanism underlying the observed inhibition of frond development is of a similar nature to that which is responsible for apical dominance in the flowering plants then it might be expected on the basis of these results by Gregory and Veale that the conditions of nutrient deficiency to which Albaum's plants are thought to have been subjected, by enhancing the competitive influence of the primary frond, might correspondingly extend the duration of its inhibiting effect. Conversely, when mineral nutrients were abundantly supplied, as in the present investigation, the frond's inhibiting influence might tend to be restricted to the period of most rapid growth and most effective competition. Since it is clear that these theoretical expectations are in agreement with the experimental results it would seem that the observed difference in the behaviour of the plants in the two investigations might with some justification be ascribed to differences in their nutritional status.

This hypothesis, however, assumes that a competition for nutrients is at least partly responsible for the correlative inhibition of frond development, an assumption which is at variance with Albaum's conclusions. Although Albaum suggested that the primary frond, during its early development, competed strongly for nutrients with the apex of the parent prothallus and, by so doing, caused the growth of the

prothallus to be arrested, he considered that the frond's inhibiting effect on the development of the young second frond was induced directly by auxin. As mentioned earlier, this conclusion was based mainly on the demonstration that when the primary frond was removed, the application of auxin in lanoline to the stump of the petiole inhibited the development of the younger frond.

Although this evidence is certainly consistent with a direct auxin inhibition hypothesis it can hardly be regarded as conclusive. As remarked by Dore and Williams (1956) the mere fact that the external application of auxin is capable of producing a growth inhibition similar to that which occurs in the intact plant cannot be considered as proof that the latter inhibition is auxin controlled. Clearly, the natural and the artificially induced inhibitions are not necessarily of a similar physiological nature. Furthermore, Albaum's conclusion does not agree well with the results of more recent investigations in which a similar technique has been employed. For example, it was observed by Steeves and Wetmore (1953), in a study of factors affecting frond development in adult plants of Osmunda cinnamomea, that the inhibiting effect of older fronds on the development of younger frond buds could not be reproduced merely by the application of auxin to the base of the petiole of the excised fronds. This negative result and the further observation that some degree of

inhibition was exerted by fronds which were fully expanded and had ceased to produce "significant" quantities of auxin led these workers to suggest the probability that the observed correlative inhibition was "not primarily an auxin phenomenon". It is of particular interest in relation to the hypothesis under discussion to note that it was suggested, as an alternative explanation, that the expanded frond might produce its inhibiting influence by competing for water and nutrients with the younger frond buds.

Experiments with flowering plants have also provided evidence of considerable variation in the effect on leaf growth of externally applied auxin, for although Goodwin (loc. cit.), in his experiments with Solidago spp., found that leaf development could be retarded by the auxin treatment of older leaves Jacobs and Bullwinkel (1953), who employed the same technique in their study of growth correlations in Coleus sp. failed to find any evidence of an auxin induced inhibition. Indeed, it was observed that when young expanding leaves were removed and auxin applied to the stumps of the petiole the rate of unfolding of still younger leaves at the stem apex was significantly increased as compared both with undefoliated plants and with plants which had been defoliated without a subsequent auxin application.

A possible explanation of these conflicting results is

suggested by the work of Gregory and Veale (loc. cit.) who showed that when the stem apex was removed from flax plants and auxin was applied to the cut surface the extent to which the outgrowth of lateral buds was inhibited was largely determined by nutritional factors. The degree of inhibition was found to be greatly reduced by an increase in either the nitrogen level at which the plants were grown or the photoperiod to which they were exposed. Since it was also noted that swellings developed on the stem in the region of the auxin application it was suggested that the apparent correlation between the induced inhibition and the nutrient supply was due to the diversion of nutrients from the lateral buds to the treated parts of the stem, a view which is in agreement with the so called "diversion" theory of apical dominance first proposed and well supported experimentally by Went (1936, 1939). Clearly this hypothesis could provide a plausible explanation of the inhibition of frond development which Albaum observed to result from his auxin application and one which would also be consistent with the view that his plants were growing under conditions of nutrient deficiency.

Thus, in conclusion, it would seem that, while the observed discrepancy between Albaum's results and those of the present investigation may prove to be a reflection of genetical differences between the two species concerned the

alternative hypothesis, which attributes this discrepancy to environmentally induced differences in the nutritional status of the plants, is one which is found to receive considerable support from the literature. Although this hypothesis, by its implication that nutrient competition is of functional significance in the correlation of frond development is at variance with Albaum's conclusions it is clear from the foregoing discussion that the evidence advanced by Albaum in support of his theory of an auxin induced inhibition is open to considerable criticism.

Thus while the present investigation has not contributed directly to an understanding of the mechanism of correlative inhibition in the fern sporeling - and, indeed, was not intended to do so - the results obtained, when compared with those described by Albaum, would seem to suggest the need for a more critical study of this phenomenon by means of experiments in which the probable role of both hormonal and purely nutritional factors is investigated.

Finally, certain other aspects of the present investigation are worthy of mention. In describing the response induced by defoliation it was stated by the writer that this treatment resulted in "an increased rate of growth" of the younger frond. Although convenient, this description may well be misleading for it tends to imply that the reaction induced in the younger frond was characterised merely by an

acceleration of the normal process of development. This, however, may not be the case. It is possible that the earlier emergence of the young frond resulted from a premature onset of elongation of the cells in the petiole while this response in turn may have been associated with a corresponding reduction in the meristematic phase of development. Clearly, any further investigation of this defoliation effect should seek to describe the response more precisely in terms of cellular behaviour.

Secondly, it is evident that the removal of the primary frond of the young sporeling, when only this frond is expanded, must undoubtedly produce far reaching physiological and developmental effects on the plant. Of these effects the accelerated development of the second frond may properly be regarded as merely the most readily apparent. It is possible that the latter response, although causally related to the removal of the primary frond, may prove to be more directly related to changes induced in the growth of the stem apex as a whole.

Finally, it is of interest to compare the apparent correlation of frond development in the young sporeling and in the mature plant. As previously remarked, it is well known that the cutting of bracken when the fronds are still only partly expanded increases the rate of development of the young frond buds and results in the emergence of a second

crop of fronds. It is equally well known, however, that this effect of defoliation is greatly reduced if cutting is delayed until the fronds have become fully expanded (e.g. Braid, loc. cit.). Furthermore, observations on the normal course of frond development (Watt, 1940) have shown that the young frond, which is initiated at the rhizome apex several years before its expansion above ground, (Klein, 1884) first becomes externally visible as a bud in late June and grows steadily for the remainder of the summer. Thus, its onset of rapid development from a minute primordium to a large frond bud apparently coincides with the time at which the associated frond has become fully or almost fully expanded.

These observations clearly suggest that in the mature bracken plant the emergent frond exerts its inhibiting effect on the development of the younger frond mainly during its period of rapid growth. The fact that the present investigation has shown that the same conclusion can be applied also to the primary frond of the young sporeling suggests that the mechanism responsible for the correlative inhibition of frond development, whatever may be its physiological nature, operates in a similar manner in the sporeling and in the fully mature plant.

## SECTION 2. SOME ASPECTS OF SPORELING MORPHOLOGY

In describing the methods employed in the propagation of sporelings (Part I) it was stressed that a rigorous selection for uniformity was essential if plants suitable for experimental work were to be obtained. Since this process of selection entailed the critical examination and comparison of a large number of plants at several different stages of growth it afforded an excellent opportunity of recording observations on the salient features of the sporeling's development and morphology, these observations serving as a useful preparation for the subsequent physiological studies (Part III).

When, in the course of this preparatory work, the literature relating to the bracken plant was explored it was found that, while the adult plant had been the subject of numerous morphological investigations the early stages in the development of the sporeling, although presenting a very marked and interesting contrast with the mature sporophyte, had received relatively little attention. Furthermore, a study of the few brief descriptions available (Hofmeister, 1851; Leclerc du Sablon, 1890; Jeffrey, 1899; Bower, 1923) showed that certain features of interest observed by the writer had not previously been described. It was therefore decided that

some time might usefully be devoted to the study of a few selected aspects of the sporeling's morphology with the object of making a contribution to the rather inadequate existing knowledge of the early stages of the plant's development.

In a short and general description of the bracken sporeling Bower (loc. cit.) observed that, during its early development, the young plant is characterised by the possession of a small upright stem on which is borne a rather variable number of spirally arranged juvenile fronds, and that it is by an apical dichotomous branching of this erect stem that the prostrate rhizomatous habit of the adult plant is first attained (fig. 7). The present investigation has been largely confined to these juvenile and transitional stages of development. The phyllotaxis of the fronds has been investigated and observations have been recorded on the number of fronds produced prior to rhizome initiation. Particular attention has been given to the relationship between the arrangement of the fronds and the orientation of the young rhizome branches and an hypothesis advanced by the writer to account for the observed nature of this relationship is critically examined. Finally, brief descriptions are also included of a number of other features of interest.

Shortly after this work had been completed Gottlieb (1958,<sup>1959</sup> /) published two papers dealing with the general morphology and

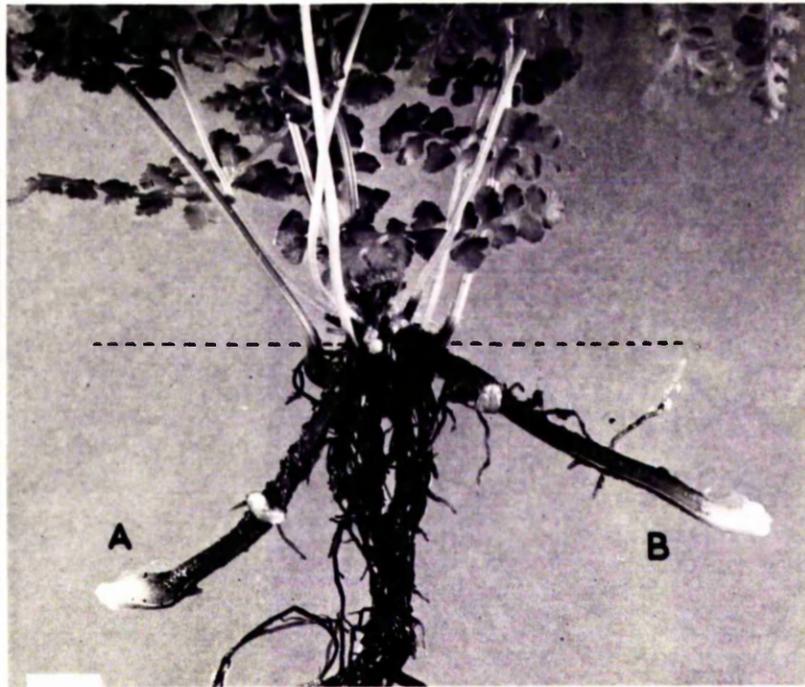


Fig. 7. A four month old sporeling grown under greenhouse conditions. The two rhizome branches (A, B) are produced by an apical branching of the erect primary stem (not visible in the photograph). Several of the older fronds borne on the primary stem have been removed to reveal more clearly the point of origin of the rhizomes. The soil level is indicated by a dotted line.

(x  $\frac{4}{5}$  approx.)

vascular anatomy of the bracken sporeling while, still more recently, some aspects of the sporeling's development were described by Dasanayake (1960). In the following account certain of the observations recorded in these recent contributions will be compared and contrasted with those of the present investigation.

#### A, Morphological Observations

So considerable is the variability of the young bracken sporeling that a study of only a few individuals may fail to distinguish the typical features of the plant's morphology from the numerous variations which occur. The following description, however, is based on a comparative study of a large number of plants raised in the greenhouse over a period of more than two years. The various morphological features which are described are illustrated by photographs of two carefully selected sporelings (Figs. 8 and 9). Each photograph is accompanied by an annotated drawing traced from the print and intended to clarify the sequence in which the young rhizomes and closely associated frond and branch primordia were produced. In these, and in all subsequent illustrations of the sporeling, the fronds are numbered in the order in which they were initiated while the two main rhizome branches are distinguished from one another according to their position relative to the fronds, Thus, the branch

arising on the same side of the stem as the 2nd. and 4th. fronds is referred to as branch A, and the other as branch B.

### 1. The phyllotaxis of the primary fronds

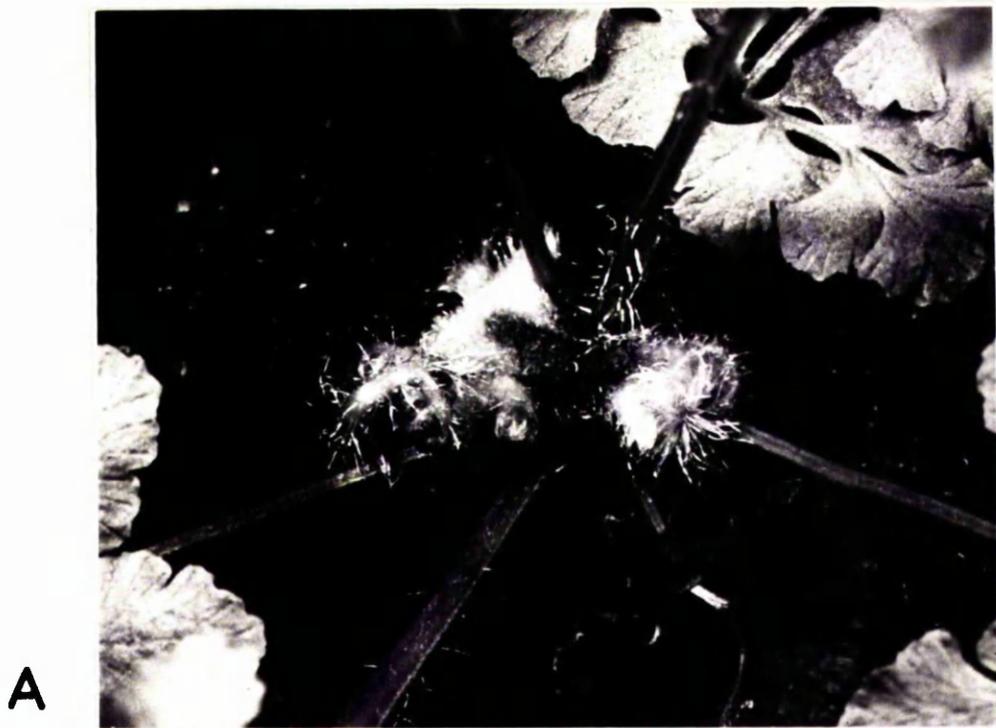
As previously mentioned, the fronds which are produced prior to the initiation of the rhizome branches are borne in a spiral arrangement on a small erect stem. These fronds will hereafter be described as the "primary" fronds and the vertical axis on which they are inserted as the "primary" stem. Although the phyllotaxis of the fronds has received but little attention Hofmeister (1851) has stated that the second frond diverges from the first "by about half the circumference of the stem". A similar view has been expressed by Gottlieb (1958) who concludes that successive fronds diverge by an angle of  $180^{\circ}$  thus constituting a  $\frac{1}{2}$  system of phyllotaxis. This conclusion, however, is clearly not supported by Gottlieb's own illustrations of transverse sections of the plant (Gottlieb, 1959, p. 95); not is it in agreement with the present observations.

A comparative study of a large number of plants indicated that the phyllotaxis, although showing considerable variation, ranges predominantly between a  $\frac{3}{8}$ ths. and a  $\frac{5}{13}$ th. system. While the similarity of the divergence angles characteristic of these two systems rendered it difficult to distinguish one

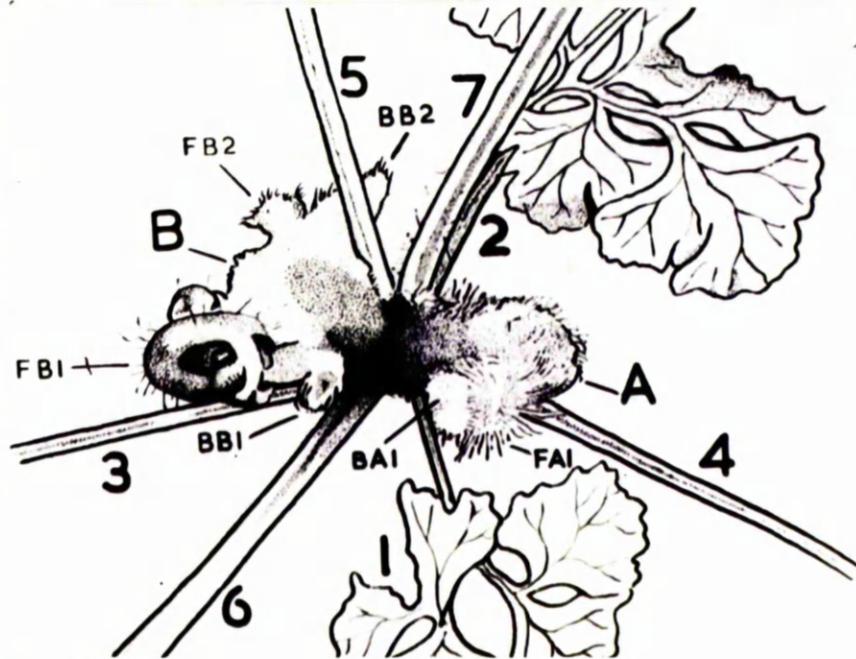
from the other merely by inspection it seemed that both arrangements were commonly represented and that plants having intermediate divergences were of frequent occurrence. The phyllotaxis of the young sporeling illustrated in fig. 8 may therefore be regarded as typical. Since this plant was photographed from above the primary stem on which the fronds are borne is completely obscured by the two well developed and horizontally disposed rhizome branches (A, B). It is nevertheless apparent that the arrangement of the fronds is either identical with or closely akin to a  $3/8$ th. system.

It will be observed, however, that the divergence angle between the 6th. and 7th. fronds is abnormally large, the relative positions occupied by these fronds being almost diametrically opposed. An explanation of this apparently abnormal divergence was provided by the anatomical investigations which are later described and which showed that the last frond to be produced immediately prior to the initiation of the rhizomes (in this instance, the 7th. frond) tends to be considerably displaced during its early development by the growth of the adjacent and closely associated rhizome branch (branch A, fig. 8). This phenomenon, which was also noted by Gottlieb (*loc. cit.*), would appear to be a characteristic feature of the sporeling's development.

Although the great majority of the plants examined possessed a phyllotaxis which corresponded closely to a  $3/8$ th.



A



B

Fig. 8. A. Photograph of a sporeling taken from above shortly after rhizome initiation. The plant has 7 primary fronds and a  $3/8$ th. phyllotaxis.  
(x 2 approx.)

B. Illustrative drawing of A. showing the primary fronds (1-7), the main rhizome branches (A, B), the frond buds borne on branch A (FA1) and on branch B (FB1, FB2) and their associated abaxial rhizome buds (BA1, BB1 and BB2 resp.). A full description of the plant is given in the text.

or a  $5/13$ th. system there was evidence of a somewhat wider range of variation. A close approach to a  $2/5$ th. system (a divergence of  $144^\circ$ ), with the 6th. frond arising almost directly above the 1st., was quite frequently observed. At the other extreme, a similar number of individuals were characterised by a reduced divergence angle estimated to be approximately  $130^\circ$ . It was clear, however, that such plants represented merely the extreme forms of a more or less continuous range of variation and that any attempt to obtain a quantitative estimate of the relative frequency with which the different types of arrangement occurred would have been too subjective to be of any real value.

#### The direction of the phyllotactic spiral

The primary fronds may be arranged in either a left or a right hand spiral. Observations suggested that these contrasted arrangements tended to occur with equal frequency. This impression was well substantiated by counts made on two different occasions. The result of these counts was as follows:--

	<u>Number of plants examined</u>	<u>Direction of spiral</u>	
		L.	R.
1.	81	40	41
2.	100	53	47

It is of interest that a similar observation has recently

been reported by Voeller and Cutter (1959) in their studies on the phyllotaxis of Dryopteris aristata. These investigators were able to show not only that clockwise and counter-clockwise arrangements occurred in a 1:1 ratio but also that differences in the direction of the spiral could be found on different buds of a single plant. It was therefore concluded that, in *Dryopteris*, this feature of the phyllotaxis is not rigidly determined by genetic factors. The present observations suggest that a similar conclusion may be applicable to the bracken sporeling.

2. The number of fronds produced prior to rhizome initiation

There is considerable variation in the numbers of fronds produced prior to apical branching of the primary stem. According to De Bary (1884), Hofmeister (loc. cit.) and Bower (loc. cit.) the number may vary from 7 - 9. Gottlieb (1959) concludes that branching "typically occurs after 8 fronds have been produced" while Dasanayake (1960) states that the number is "about 9". Thus Jeffrey's (1899) observation that rhizomes are initiated after the production of "about a dozen leaves" would seem to be inaccurate, although it is possible that this apparent delay in the onset of branching may be induced by certain unusual environmental conditions.

In the present investigation approximately 60 - 70% of the plants examined initiated their rhizomes after only

7 primary fronds had been produced. This sequence of events is exemplified by the plant illustrated in fig. 8. Although the base of the 7th. frond is not well defined in the photograph close inspection showed that this frond arose from a point well below the level of the adjacent rhizome branch. Thus FB1 and FA1 may be regarded as being the first fronds to have been produced at the apex of branch B and branch A respectively.

On the other hand, the sporeling illustrated in fig. 9 bears only 6 primary fronds. It will be observed that the fronds which are labelled FB1 and FA1 are at a similar stage of development and it is evident that they were initiated at different branch apices and thus subsequent to branching of the apex of the primary stem. The small lateral branches BB1 and BA1 which are present at the base of these fronds will be discussed later. It may be remarked here, however, that these short laterals can be assumed to have originated at the rhizome apices and immediately prior to the production of the fronds with which they are seen to be closely associated. Their presence thus provides further proof that the latter fronds were produced subsequent to rhizome initiation. It was estimated that this early branching, preceded by the production of only 6 fronds, occurred in approximately 25% of the plants examined. Finally, a relatively small number of plants, somewhat less than 10%,

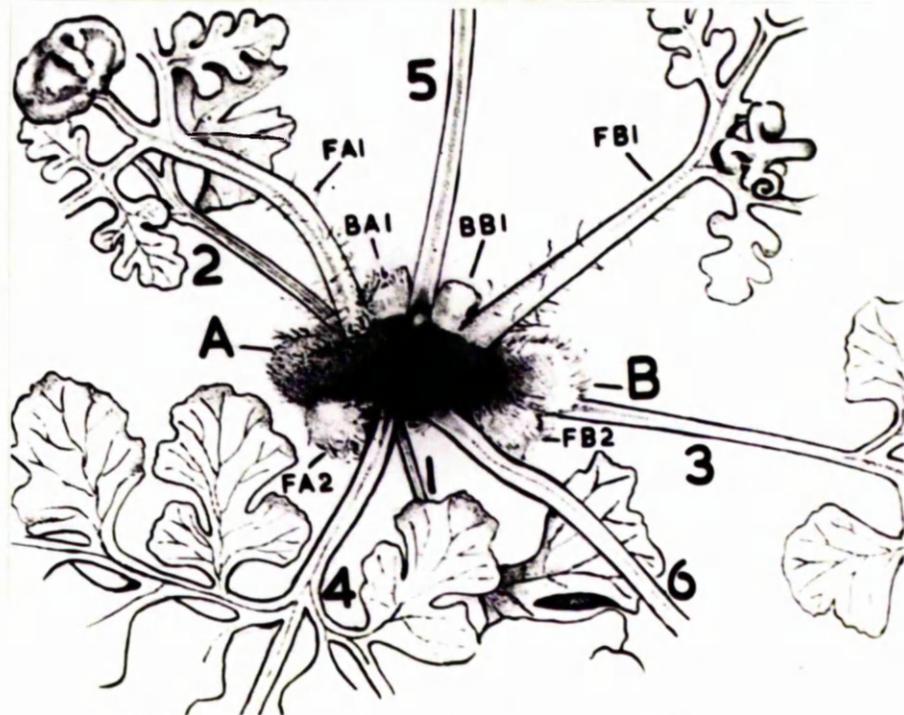
**A****B**

Fig. 9. A. Photograph of a sporeling at a similar stage of development as the one in fig. 8 but having only 6 primary fronds arranged approx. in a  $5/13$ th. system of phyllotaxis. (x 2 approx.)

B. Illustrative drawing of A. Terminology as in fig. 8 with which this plant should be compared. See text for a full description.

showed evidence of a delay in the onset of branching, 8 primary fronds being produced prior to rhizome initiation.

It is clear that these observations suggest a somewhat earlier onset of branching than was observed by the investigators previously cited in this connection. A possible explanation of this apparent discrepancy will be proposed in the concluding discussion.

### 3. The orientation of the rhizome branches with respect to the primary fronds

A comparative study of a large number of sporelings showed that the plane of branching i.e. the vertical plane passing through the longitudinal axis of the two rhizome branches, is determined by (a) the phyllotactic divergence angle and (b) the number of fronds produced prior to rhizome initiation. Variations in either of these features were found to be correlated with predictable variations in the orientation of the rhizome branches with respect to the primary fronds.

It seemed that these observations might be most readily explained on the assumption that, when branching occurs, one branch always arises in a position in the genetic spiral of the primary fronds and this diverges from the youngest frond by the phyllotactic divergence angle of the plant, the other branch diverging from the first at an angle of  $180^{\circ}$ .

This hypothesis is illustrated by the diagram in fig. 10A in which the median planes of 7 primary fronds have been drawn so as to diverge from one another by an angle of  $135^{\circ}$  i.e. the arrangement of the fronds corresponds to a  $3/8$ ths. phyllotaxis. Rhizome branch B diverges from frond 7 by this same angle while branch A diverges from B by  $180^{\circ}$ .

When this diagram is compared with the plant illustrated in fig. 8 (which also bears 7 primary fronds arranged at least approximately in a  $3/8$ th. system) it is apparent that the positional relationship between the rhizome branches and the first 6 primary fronds is closely similar in the photograph and in the theoretical diagram. That this similarity does not hold for the 7th. frond can be attributed to the fact that this frond, as previously remarked, has undoubtedly been considerably displaced during its early development by the growth of the adjacent branch apex (A). Its original position and hence its original divergence from branch B is probably represented accurately in the diagram.

A similar comparison may be made between the sporeling illustrated in fig. 9 and the diagram in fig. 10B. The phyllotaxis of this plant would seem to approach more closely to a  $5/13$ th. system (a divergence of  $138\frac{1}{2}^{\circ}$ ) and, as already observed, only 6 fronds have been produced prior to rhizome initiation. Although several of the fronds, especially the

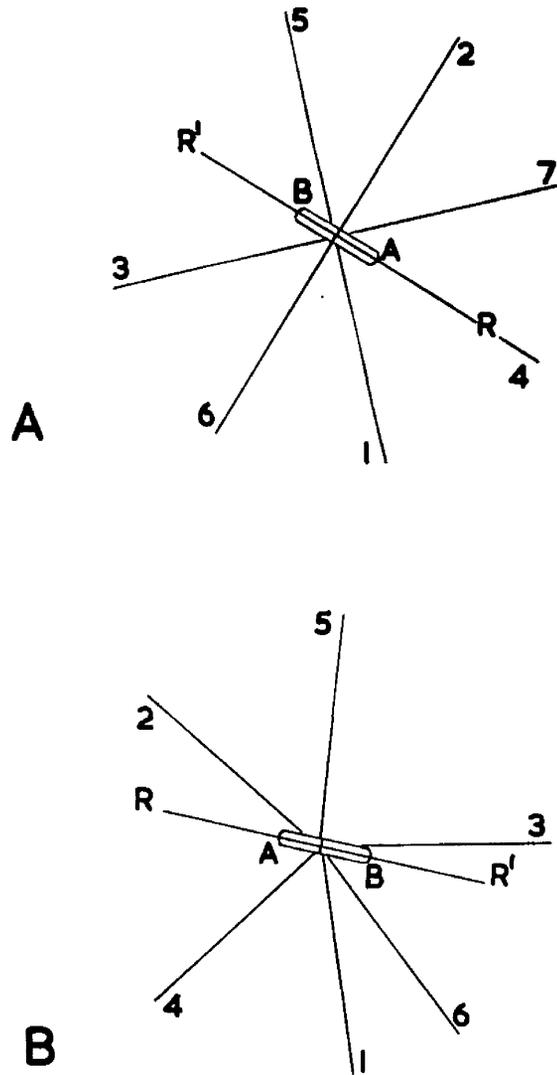


Fig. 10. Diagrams illustrating a postulated relationship between the plane of branching of the primary stem of the sporeling and the phyllotaxis of the primary fronds.

- A. Represents a sporeling with 7 primary fronds and a  $3/8$ th. phyllotaxis and should be compared with the plant in fig. 8. 1-7, the median planes of the primary fronds; A, B, the rhizome branches; RR', plane of branching of the primary stem.
- B. Represents a sporeling with 6 primary fronds and a  $5/13$ th. phyllotaxis and should be compared with the plant in fig. 9. Terminology as for A.

4th., have been pushed aside by the developing rhizomes it is clear that their positions relative to the rhizome branches and, in particular, the divergence between the 6th. frond and branch A, are in good agreement with the theoretical diagram and thus with the proposed hypothesis. That the 6th. frond, although produced immediately prior to rhizome initiation, has apparently not been displaced appreciably from its original position and has thus retained its original divergence from branch A can probably be attributed to the fact that this frond, unlike the 7th. frond of the plant previously discussed (fig. 8) arises at a point which is almost equidistant from the two branch apices subsequently produced. Thus any tendency for it to be displaced by the growth of one branch may be largely counteracted by a similar displacement resulting from the growth of the other.

This aspect of the sporeling's morphology was also investigated by Gottlieb (1959) who concluded that the plane of branching is "parallel to the earlier plane of leaf formation but is roughly perpendicular to the plane of formation of the last two leaves." This description is based on Gottlieb's view that the phyllotaxis corresponds to a  $\frac{1}{2}$  system. The present investigation has shown, however, that this view is incorrect. Thus, there is no "plane of leaf formation". Furthermore, although the plane of branching of the sporeling illustrated in fig. 8 might

well be described as being "roughly perpendicular" to a plane passing through fronds 6 and 7 this relationship obtains only because of the ontogenetic displacement of the 7th. frond. Thus, although Gottlieb correctly observed that "the foliar primordia may be displaced on the apical mound by the larger new shoot apices" she does not appear to have taken due account of this fact in her study of the relation of branching to the phyllotaxis.

#### 4. Some observations on the branching of the rhizomes

There is considerable evidence from the morphological and anatomical investigations of Velenovsky (1905) and Bayer (1903) and from later comparative studies by Bower (1923) that the buds which occur in an abaxial position at the base of the fronds of the mature bracken plant, and which were formerly believed to be adventitious in origin (Hofmeister, 1851; Sachs, 1882), originate by a dichotomous branching of the main rhizome apex and themselves give rise to the fronds with which they are associated. The adult rhizome has thus been described by Velenovsky (loc. cit.) as a "dichopodium" i.e. a false axis composed of the more strongly developed shanks of successive apical dichotomies. It may be noted, however, that this widely held view has recently been contradicted by Dasanayake (loc. cit.) who holds that the abaxial bud and its associated frond both arise from meristematic tissue at the rhizome apex and that the bud is thus "neither

adventitious nor the result of a dichotomy of the main axis."

Although no attempt was made in the present investigation to trace the origin of these controversial abaxial buds some observations on their first appearance and early development on the rhizomes of the young sporeling seem worthy of mention. According to Bower (loc. cit.) apical branching, with the consequent formation of abaxial buds, occurs at an early a stage in the development of the sporeling rhizome but is preceded by the production of several fronds at each rhizome apex. Gottlieb (1958), who appears to have been the only other investigator to have described this aspect of the sporelings development, observed that in some plants branching occurred after only 2 - 3 fronds had been produced while in others the rhizomes remained unbranched until 10 or more fronds had been initiated at each rhizome apex.

Again the present observations differ from those recorded by Gottlieb for none of the many plants examined produced more than 3 fronds at each rhizome apex before branching occurred. Furthermore, the young rhizomes of many of the plants produced branches as soon as they had been initiated at the apex of the primary stem and thus without a prior phase of frond production. This early onset of branching is evident in both of the sporelings selected for illustration (figs. 8 and 9). As previously

remarked, it may be assumed that the small abaxial buds BA1 and BB1 shown in both figures were initiated at the rhizome apices (i.e. at A and B respectively) and thus immediately prior to the production of the fronds with which they are associated. It is therefore apparent that no fronds were produced at the rhizome apices of either plant prior to the onset of branching.

A second interesting feature shown by the sporeling in fig. 8 is the marked difference in size of the two abaxial buds borne on branch B, the younger of which (BB2) is considerably larger than the older one (BB1) and, indeed, is only a little less strongly developed than the corresponding apical region of the main axis (the extreme tip of which has turned down towards the soil and is not visible in the illustration). It may also be noted that the young frond bud (FB2) associated with this larger branch is apparently borne on the branch itself. Such variation in branch development and in the position of the associated frond is a very characteristic feature of the young sporeling. At one extreme the branch may appear only as a small bud in an abaxial position at the base of the strongly developed frond (e.g. BB1, fig. 9) while, at the other, its growth may exactly equal that of the rhizome apex so that it appears as one arm of an apical dichotomy. In the latter event the associated frond may occasionally be orientated towards the

junction of the two branches but, more usually, is borne on that branch of the dichotomy which represents the bud and frequently at a considerable distance from the bifurcation of the rhizome. It is noteworthy that even where branching is only sub-equal (e.g. fig. 9, BB2) the frond is not orientated towards the main axis in spite of the fact that the main axis has developed more strongly than the branch. This observation is difficult to reconcile with Dasanayake's (loc. cit.) hypothesis that, in the mature plant, the frond is initiated at the rhizome apex and that its orientation towards the abaxial bud results merely from its subsequent displacement. On the contrary, the present observations would seem to be more consistent with the view of earlier investigators (e.g. Bower, loc. cit.) that the abaxial bud itself gives rise to the associated frond.

#### B. Anatomical Investigations

Morphological observations were supplemented by a study of serial transverse sections of a small number of selected plants. The object was to investigate the relationship between the phyllotaxis and the positions of the rhizome branches and, in particular, to assess the validity of the hypothesis proposed to account for the observed nature of this relationship. In addition, an opportunity was provided of obtaining a few accurate measurements of the frond

divergence angles and of recording some observations on the initiation of the rhizomes.

### Materials and Methods

#### (i) The selection of plants and their preparation for anatomical study

Fourteen sporelings carefully selected for uniformity were prepared for sectioning when the 4th. frond was either partly or fully expanded. Although at this stage rhizome branches could not be identified with certainty by a superficial examination of the plants previous observations had shown that branches are usually clearly distinguishable and may attain a length of 1 - 2 mm. by the time the 5th. frond is partly expanded. It was therefore considered probable that rhizome initiation had already occurred in all of the selected plants and that the young rhizome branches were merely obscured by the dense growth of hairs covering the stem apex and the associated frond primordia.

The primary fronds were removed from each plant by severing the petiole with a sharp scalpel just above the level of the stem apex, the petiole of the first frond being left slightly longer than the others to serve as a guide in the orientation of the material during the embedding process. All of the roots were cut off as close as possible to the stem. The material was then fixed in formalin-aceto-alcohol for 48 hours, dehydrated in a graded

series of tertiary-butyl-alcohol and finally infiltrated with and embedded in paraffin wax (M.P.  $54.5^{\circ}\text{C}$ ). Serial transverse sections were cut on a Cambridge Rocker microtome at a thickness of 10 - 12  $\mu$  and the ribbons attached to slides with Haupt's adhesive. The sections were stained with Saffranin O and Delafield's Haematoxylin according to the schedule by Johanson (1940) and were mounted in euparal as permanent preparations.

(ii) Measurement of frond divergence angles

Since apical branching had already occurred in all of the plants examined the primary stem apex could not be used as a reference point in the measurement of divergence angles between the primary fronds. It was found, however, that in some of the sections the median plane of two successive fronds could be drawn by eye with a considerable degree of accuracy and a measure of the divergence of these fronds thus obtained. One of the sections used for this purpose is illustrated in fig. 11. The median plane (P1) of the 4th. frond was estimated by a study of the orientation of the vascular strand in the petiole and that of the associated leaf gap in the solenostele of the stem, while the median plane (P2) of the 5th. frond could be assessed by observing the position and orientation of the vascular traces (LT.5) as they departed from the sides of the leaf gap. The plane of elongation of the endodermal cells (END) associated with the

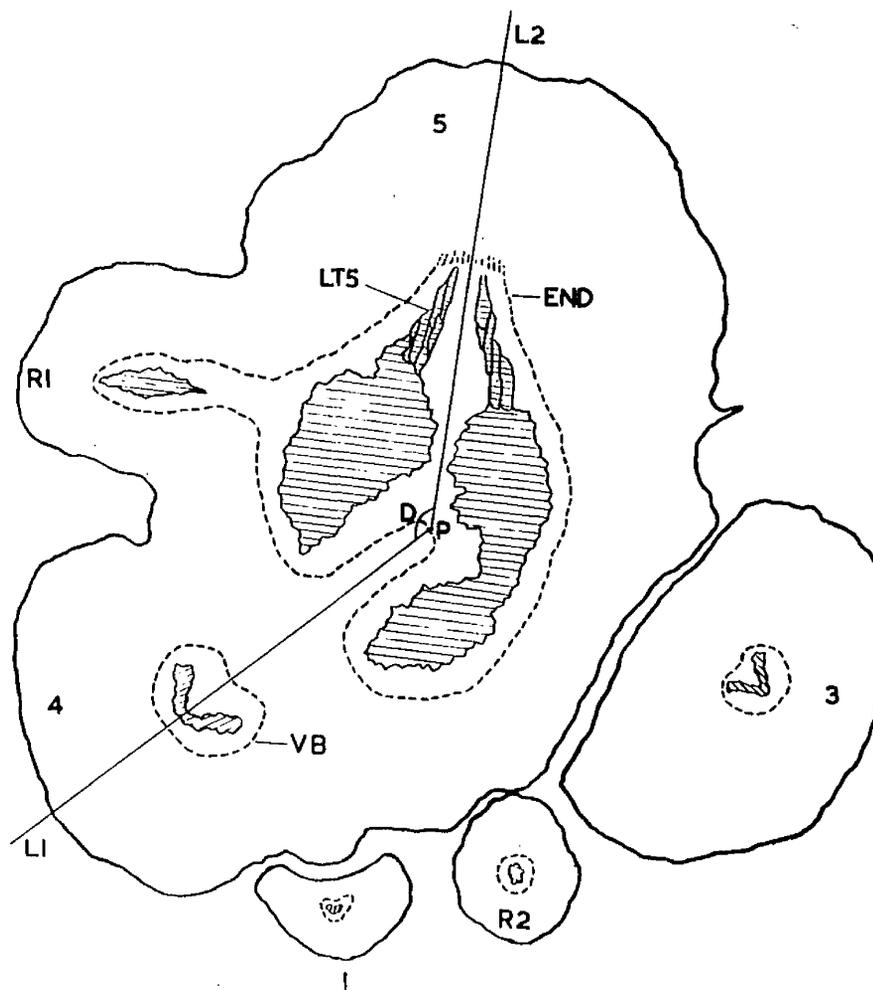


Fig. 11. A projection drawing of a transverse section of the primary stem of the sporeling to illustrate the method used in measuring the angle of divergence between successive primary fronds (as described in the text). The bases or petioles of 4 primary fronds (1, 3, 4, 5) are included. The xylem is shaded and the endodermis (END) is indicated by a dotted line. PL1 and PL2, estimated median planes of fronds 4 and 5 respectively, diverging by an angle (D) of  $135^{\circ}$ ; VB, vascular bundle in the petiole of frond 4; LT5, leaf trace to frond 5; R1, R2, roots in L.S. and T.S. respectively. (x 52)

trace of the 5th. frond also served as a guide.

The sections used in the construction of these divergence angles were projected through a modified photographic enlarger and the median planes of the selected fronds drawn on the projected image (approx. x 50).

(iii) Analysis of the positional relationship between the fronds and rhizome branches

In the study of each plant sections were projected as described above and the outline of the section, the included vascular tissue and the apical cell of each rhizome branch were drawn at a magnification of approximately x 50. It was found, in some instances, that the two rhizome branches were not orientated in the same horizontal plane and that their apices thus appeared in different transverse sections. In this event the drawing of one section was superimposed on that of the other and a composite drawing including both rhizome apices thus obtained.

(iv) Method of illustration

The projection drawings were also used to illustrate the several plants selected for discussion. The terminology employed is the same as that adopted for the illustration of the morphological observations previously described.

Observations(i) The phyllotaxis of the primary fronds as determined by the measurement of divergence angles

In attempting to obtain measurements of the phyllotactic divergence angle of the primary fronds by the method described above it was observed that many of the fronds had deviated considerably from the vertical plane in which they had been initiated. For this reason divergence angles could be measured with a satisfactory degree of precision from only a small number of the sections examined. These angles, measured from sections of six different plants, are recorded in Table 1. Since the error involved in these measurements is considered to be about  $\pm 2^{\circ}$  little significance can be attached to the apparent variation in the divergences between successive fronds of each of the first four plants in the table. The abnormal divergence of the fronds of plant number 6, however, was obviously due to the 4th. frond of this plant having been produced out of its normal position.

While it is clear that a much larger number of measurements would be required to provide a reliable estimate of the typical phyllotaxis divergence angle and of the normal range of variation the data recorded is in general agreement with the morphological observations previously described in suggesting that the divergence angle may vary from  $135^{\circ}$  to  $144^{\circ}$  with a mean value which corresponds closely to that of

TABLE 1. Angles of Divergence between the Primary Fronds  
of the Sporeling

Ref. No. of Plant	Ref. No. ‡ of Fronds	Divergence Angles	Mean Divergence per Plant
1	2 - 3	139°	140°
	3 - 4	141°	
2	2 - 3	142°	142°
	4 - 5	142°	
3	3 - 4	135°	135°
	4 - 5	135°	
4	1 - 2	138°	141°
	4 - 5	144°	
	5 - 6	142°	
5	3 - 4	156°	141°
	4 - 5	126°	
6	5 - 6	138°	138°
Mean of all divergence angles			139.5°

‡ Numbered in the order in which they were initiated.

a 5/13th. system.

(ii) Some observations on rhizome initiation

It has frequently been stated in the literature that the rhizomes of the young sporeling are initiated by an apical dichotomous branching of the primary stem, e.g. Hofmeister, 1851; Jeffrey, 1899; Bower, 1923; Gottlieb, 1958, this view being apparently based, not on any anatomical evidence, but merely on the general appearance of the two rhizome branches during their early development. Recently, however, Dasanayake (1960), as a result of anatomical observations, concluded that a true dichotomy of the primary stem does not occur. Instead, the two rhizome branches are produced simultaneously from meristematic tissue near the margin, and at either side of the apical meristem, while the stem apex itself, not being directly involved in branch formation, ceases to grow and is ultimately reduced to a parenchymatous condition.

Although it was not the purpose of the present investigation to trace the origin of the rhizome branches observations on a number of plants which had been fixed for sectioning when the 4th. frond was only partly expanded and which were found to have their rhizomes at a very early stage of development presented a picture of rhizome formation which failed to agree with either of the divergent views expressed by the investigators cited above. Two plants will serve to

indicate the nature of the evidence provided by these observations. The first, which is illustrated by the drawing in fig. 12 had apparently produced seven fronds prior to rhizome initiation and it was also evident that the 7th. frond had been considerably displaced during its development by the growth of the adjacent stem apex (A). It seemed that this apex, which was observed to possess the small three-sided apical cell characteristic of the primary stem (fig. 13), and previously described by Dasanayake (loc. cit.), was destined to give rise directly to one of the rhizome branches. This view was supported by the observation that, although it was still orientated in a vertical plane, it had produced a small frond primordium (FA1) which obviously did not occupy a position in the genetic spiral of the primary fronds. The other branch (B), which was lying in a horizontal plane and had already produced two frond primordia (FB1 and FB2) was presumed to have been initiated at the primary stem apex, probably after the production of the 7th. frond and in the position described by Dasanayake i.e. near the margin of the apical meristem.

A similar sequence of events was suggested by the sporeling illustrated in fig. 14. This plant, which will be described in greater detail in the following section, seemed, however, to represent a slightly later stage of

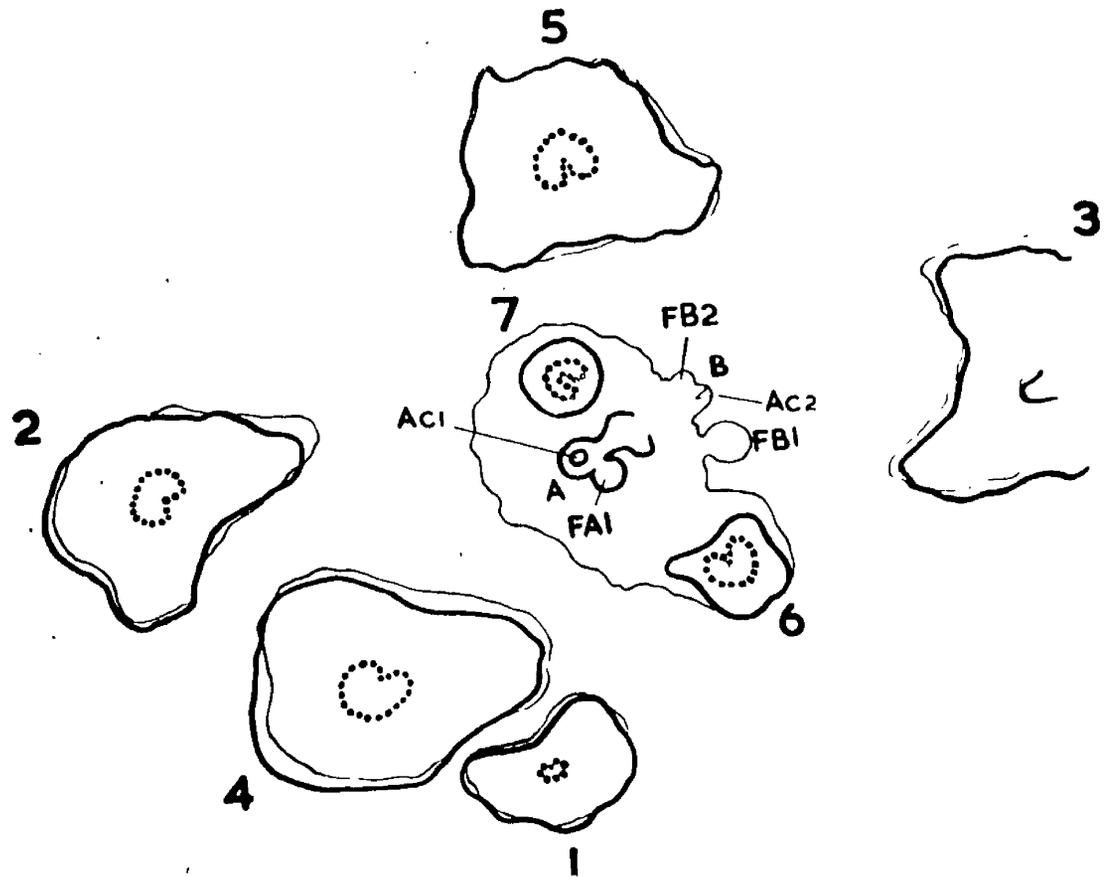


Fig. 12. Superimposed projection drawings of two transverse sections of the sporeling shortly after initiation of the rhizomes. The upper section is indicated by a heavier line. The outline of the vascular tissue is dotted. Seven fronds (1-7) have been produced prior to rhizome formation. Note that frond 7 has been displaced, probably by the growth of branch A. Rhizome branch B (Ac2, apical cell) is horizontally disposed and has given rise to 2 frond primordia (FB1, FB2). Branch A is still vertically orientated but has already produced one frond primordium (FA1). The histological details of the apex of branch A are shown in fig. 13.

(x 40)

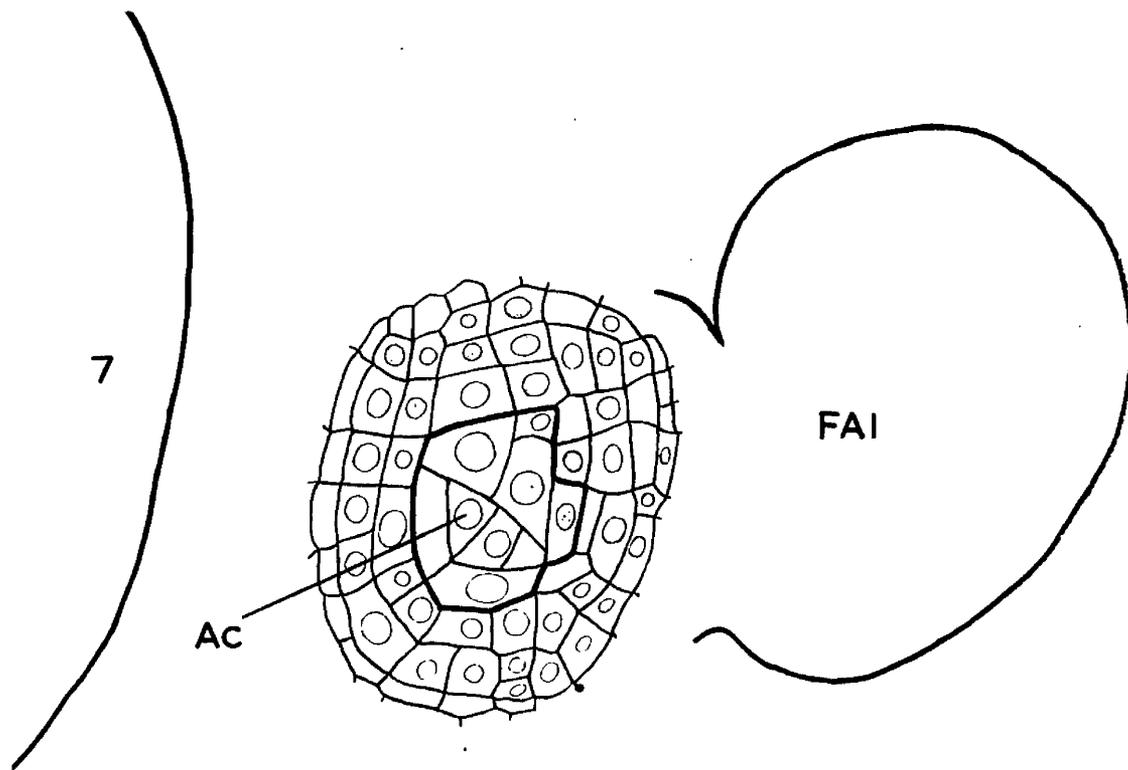


Fig. 13. Camera lucida drawing of the apical region of branch A of the sporeling shown in fig. 12. Note the small 3-sided apical cell (Ac). The cells included by a heavy line gave a staining reaction quite distinct from that of the surrounding tissue and are considered to constitute the apical meristem. The approximate positions of the youngest primary frond (7) and the first frond primordium produced at the apex of branch A (FA1) are also shown.

(x 360)

rhizome development for it was observed that the apex of branch A, which again appeared to correspond to that of the primary stem, was not orientated in a vertical plane as in the plant already described, but apparently had started to diverge from branch B and, in consequence, had been cut obliquely. A careful study of the tissues lying between the two branch apices, and between the branches of several other plants at a similar early stage of development, failed to reveal any indication of an abortive apical meristem such as might have been expected on the basis of Dasanayake's hypothesis.

All of the available evidence therefore seemed to point to the conclusion that only one rhizome branch is initiated at the apex of the primary stem and that the latter then diverges from this branch and grows out in the opposite direction, thus creating the impression that a true apical dichotomy has occurred. It must further be assumed that this postulated change in the direction of growth of the primary stem is accompanied by an alteration in the structure of its apical meristem and, in particular, that its small three-sided apical cell is replaced by, or converted into, the two-sided apical cell which is a constant feature of the growing point of the rhizome.

It need hardly be stressed, however, that since this hypothesis is at variance with the views of previous

investigators and is based on only a small number of observations it cannot be accepted without a further more detailed investigation involving a study of plants at a somewhat earlier stage of development.

(iii) The relation of branching to the phyllotaxis

The morphological observations previously described suggested that, when rhizome initiation occurs, one branch normally arises in a leaf position and thus diverges from the youngest primary frond by the phyllotactic divergence angle of the plant. The several plants which are now to be described have been selected to provide an indication of the extent to which this hypothesis was supported by anatomical observations. They will also serve to illustrate differences in the number of fronds produced prior to rhizome initiation and several other features of interest.

1. The plant which is shown in fig. 14, and to which brief reference has already been made, is illustrated by means of superimposed drawings of two transverse sections, each of which includes the apex of one rhizome branch. It had apparently produced seven fronds prior to the occurrence of apical branching and was comparable in this respect with 11 of the 14 plants examined. Sections at a lower level revealed that the 5th. frond had diverged sharply in a clockwise direction during its development and, in doing so, had caused the 2nd. frond to be slightly displaced in the same

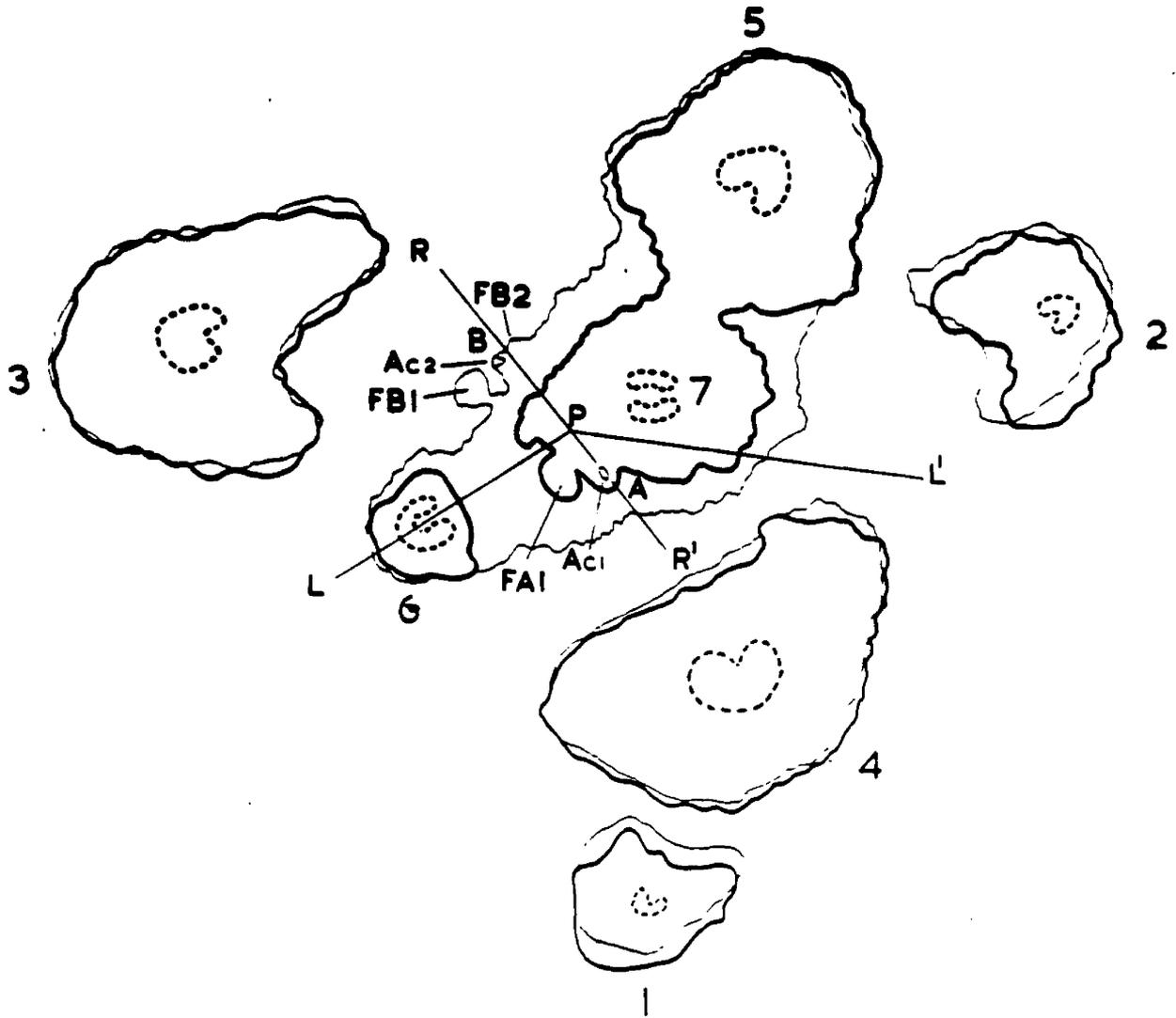


Fig. 14. Superimposed drawings of two transverse sections of the shoot apex of the sporeling shortly after rhizome initiation. Upper and lower sections are distinguished as previously described (fig. 12). The plant has 7 primary fronds (1-7). Note the displacement of the 7th. frond. Ac1, Ac2, apical cells of rhizome branches A and B respectively. Branch B is lying in a horizontal plane while branch A is not yet horizontally disposed and its apical cell is cut obliquely. P, estimated centre of primary stem; PL, estimated median plane of frond 6; PL', estimated plane of inception of frond 7; RR', plane of branching of primary stem; FA1, and FB1, FB2, frond primordia of branches A and B respectively. (x 40)

direction. The other primary fronds shown in the figure, however, with the exception of the 7th. frond, did not appear to have deviated by more than a few degrees from their original positions. Although frond divergence angles could not be measured with a satisfactory degree of accuracy a study of sections passing through the levels of insertion of several pairs of fronds suggested that the phyllotaxis corresponded closely to a  $5/13$ th. system.

In studying the positional relationship between the plane of branching and the phyllotaxis an attempt was made to locate the centre of the primary stem. It seemed that this point should correspond approximately with the point (P) at which the median plane (PL) of the 6th. frond, estimated from the orientation of the vascular tissue in the petiole, intersected the line RR' joining the apex of branch A to a point mid-way between the apex of branch B and the young frond primordium FB2 from which the latter apex was found to have diverged. That the point thus determined is not equidistant from the two branch apices can be attributed to the fact that branch A, as previously noted, is not orientated in a horizontal plane.

The hypothesis under investigation required that, in this plant, branch B should have arisen in a position in the genetic spiral and thus should diverge from the 7th. frond by the phyllotactic divergence angle. A study of the

sections in fig. 14 showed clearly, however, that the 7th. frond was not orientated towards the estimated centre of the primary stem and that it had been displaced during its development, apparently in a direction approximately at right angles to the plane of branching, by the growth of the adjacent rhizome branch (A). Owing to this effect of rhizome development, which has already been described, the original divergence between frond 7 and branch A could not be measured directly. However, on the assumption that the phyllotaxis of the plant was identical with a  $5/13$ th. system it seemed that the original median plane of the 7th. frond should be represented approximately by a line (PL') drawn to diverge from the median plane of the 6th. frond by an angle of  $138\frac{1}{2}^{\circ}$ . As can be observed from the figure the divergence between this estimated plane of inception of the 7th. frond and the median plane (PR) of branch B was found to be  $136\frac{1}{2}^{\circ}$  and thus sufficiently close to the phyllotactic divergence angle to suggest that branch B had been produced in a leaf position. However, in the absence of a more exact knowledge of the phyllotaxis this evidence cannot be regarded as conclusive.

2. Two of the plants examined were found to have produced only six fronds prior to the occurrence of apical branching. One of these plants is illustrated by the drawing in fig. 15 of a single transverse section which includes both rhizome

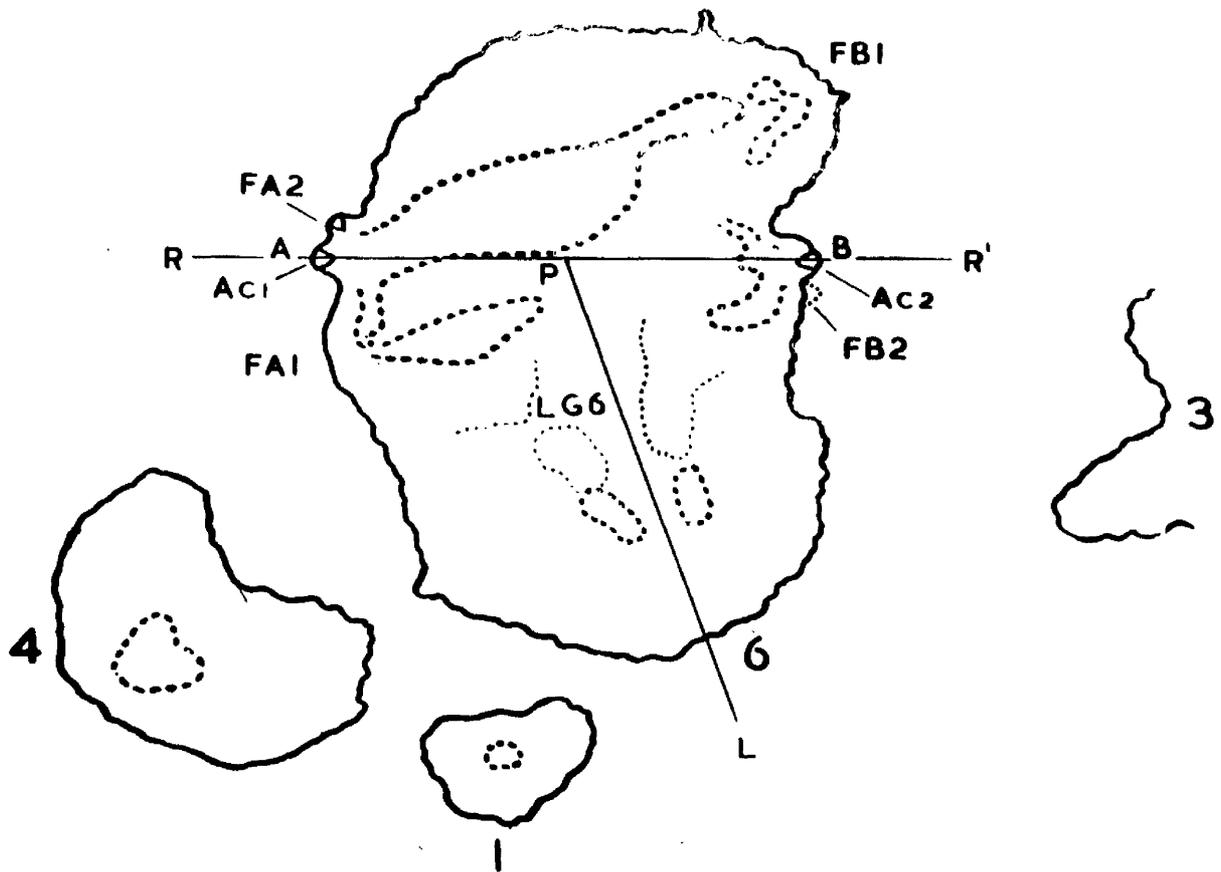


Fig. 15. Projection drawing of a transverse section of the shoot apex of the sporeling shortly after rhizome initiation. The plant has a  $\frac{3}{8}$ th. phyllotaxis and 6 primary fronds (only fronds 1, 3, 4 and 6 are shown). The two rhizome branches (A, B) are lying in the same horizontal plane. Ac1, Ac2, apical cells of branch A and branch B respectively; FA1, FA2, and FB1, FB2, frond primordia produced at the apex of branch A and branch B respectively. (FB2 is superimposed from a lower section); P, estimated centre of primary stem; LG6, leaf gap associated with frond 6; PL, estimated median plane of frond 6; RR', plane of branching of primary stem. The histological details of this section are shown in fig. 16. (x 40)



Fig. 16. A photomicrograph of the section illustrated by the annotated drawing in fig. 15 (the petioles of fronds 1, 3 and 4 are omitted).

(x 44)

apices. The histological details of this section are shown in the photomicrograph in fig. 16. The conclusion that this plant had produced only six primary fronds was clearly indicated by the fact that there was no frond present in even the approximate position at which the 7th. frond would have been expected to occur. Thus FA1 and FB1 were considered to be the first fronds produced at the apices of branch A and branch B respectively. Angles of divergence between the 3rd. and 4th. and the 4th. and 5th. fronds (Table 1, plant 3), measured by the method previously described, were both estimated to be  $135^{\circ}$ , indicating that the plant possessed a  $3/8$ th. system of phyllotaxis.

To determine whether apical branching in this plant could be interpreted in terms of the proposed hypothesis an attempt was made to measure the angle of divergence between the 6th. frond and branch A. Since both rhizome branches were orientated in a horizontal plane and since unequal growth of the two branches at this early stage of development is of rare occurrence it was considered that the centre of the primary stem should be represented with a sufficient degree of accuracy by the mid-point (P) of the line joining the two branch apices. From this point the median plane (PL) of the 6th. frond was then estimated by a study of the position and orientation of the vascular traces to the frond as they appeared in transverse section in the petiole and as

they departed at a lower level from the sides of the associated leaf gap (L.G.6).

The angle of divergence thus constructed between the median plane of the 6th. frond and that of branch A (represented by the line RP) was found to be only  $110^{\circ}$ . It therefore seemed that branch A could not reasonably be held to have arisen in a leaf position. However, further investigation revealed that the angle of divergence between the 5th. and 6th. fronds, measured from superimposed drawings of sections passing through the levels of insertion of these fronds, was approximately  $152^{\circ}$ . Although this measurement could be regarded as accurate only to within about  $\pm 4^{\circ}$  it indicated that the divergence of these fronds was abnormally large. Since it had previously been observed that the angle between the 4th. and 5th. fronds, accurately measured from the section in fig. 11, was  $135^{\circ}$  it was concluded that the 6th. frond had either been displaced by the adjacent rhizome branch or, alternatively, had not been initiated in its normal position.

Although the fact that the frond was still orientated towards the centre of the primary stem seemed to suggest that it had not been displaced a study of other plants showed that while the youngest primary frond (this usually being the 7th.) is generally displaced in a direction approximately at right angles to the associated rhizome branch it may, at the same time, become orientated, in a varying degree, towards the

axis of the branch itself. In this event the frond's divergence from the next older frond may be considerably increased but its orientation with respect to the axis of the primary stem may be but little affected. It may be noted that it was probably this dual effect of rhizome development which was responsible for the almost diametrically opposed positions occupied by the 6th. and 7th. fronds of the plant previously discussed and illustrated in fig. 8. However, the alternative hypothesis, that the 6th. frond may have been initiated in an aberrant position at the stem apex cannot be entirely excluded for observations have shown that irregularities in the phyllotaxis of individual plants are of quite frequent occurrence (e.g. plant 5, Table 1).

Thus the reason for the abnormally large divergence between the 5th. and 6th. fronds remained uncertain. Its occurrence, however, seemed to provide a plausible explanation for the failure of the 6th. frond to diverge from branch A by the angle expected on the basis of the hypothesis under investigation.

3. The plant illustrated in figs. 17 and 18 presents several interesting features. Firstly, it is noteworthy that this was the only one of the 14 plants examined which had produced as many as eight fronds prior to rhizome initiation, a fact which agrees well with the estimated frequency with which

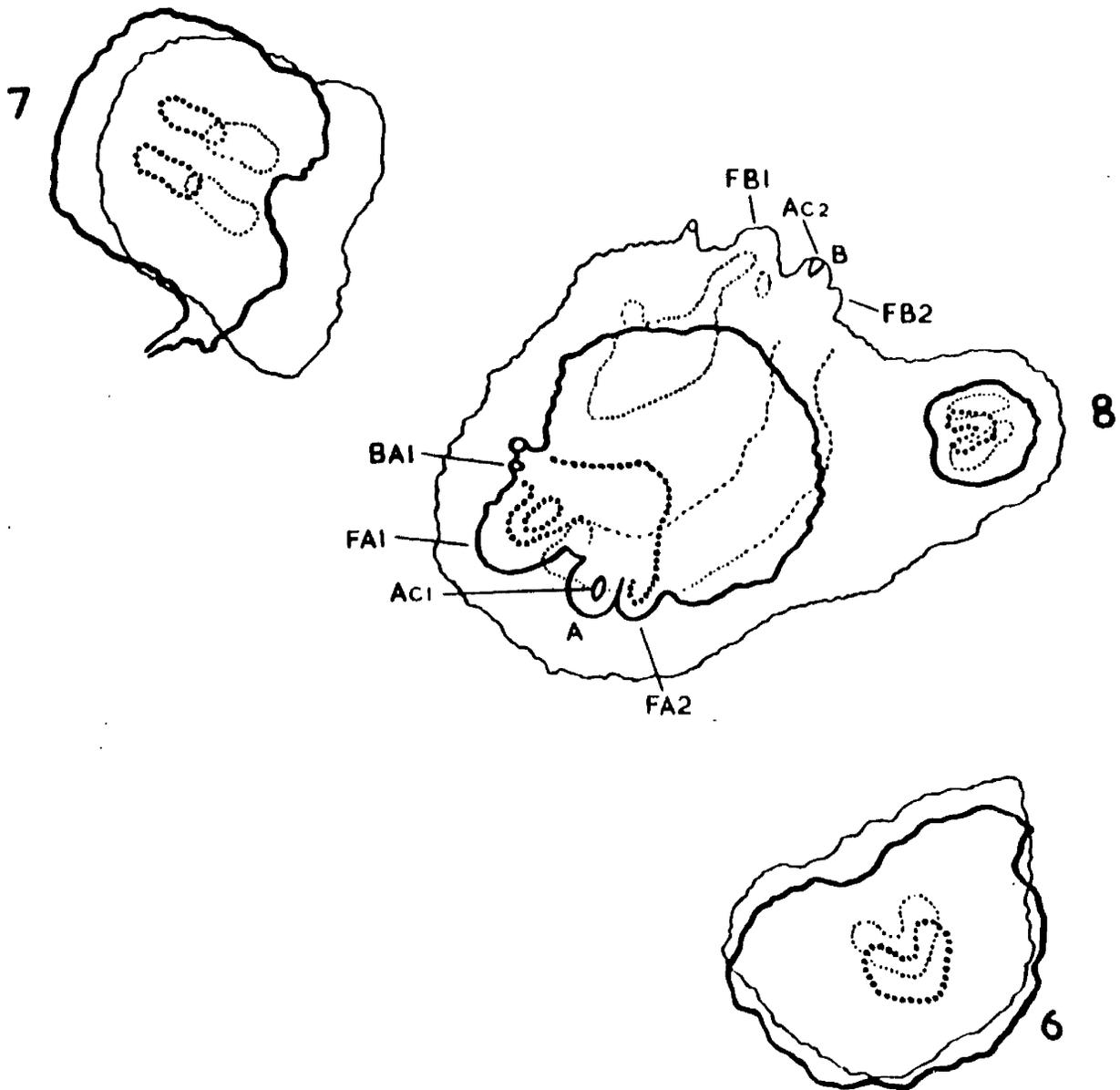


Fig. 17. Superimposed drawings of two transverse sections of the shoot apex of the sporeling. Upper and lower sections are distinguished as previously described (fig. 12). The plant has produced 8 fronds prior to rhizome initiation of which only the 3 youngest (6, 7 and 8) are shown. Note the small lateral branch (BA1) presumed to have arisen at the apex of branch A. Ac1, Ac2, apical cells of each rhizome branch; FA1, FA2 and FB1, FB2, frond primordia associated with apex of branch A and branch B respectively. The histology of the upper section is illustrated in fig. 18.

(x 37)



Fig. 18. Photomicrograph of the uppermost of the two superimposed sections illustrated by the annotated drawing in fig. 17 (the petioles of fronds 6, 7 and 8 are omitted). (x 50)

such plants were encountered in the course of greenhouse observations. A second feature of interest is the early onset of branching at the rhizome apex. The small lateral branch BA1 (figs. 17, 18) may be presumed to have been produced at the main rhizome apex (A) and prior to the initiation of the frond bud (FA1) with which it is associated. In this respect the plant is comparable with those illustrated in figs. 8 and 9 and serves to corroborate the previous observation that the onset of branching at the rhizome apex is not necessarily preceded by a phase of frond production. It may also be noted that the other rhizome (B) of this plant gave rise directly to a frond (FB1). Thus it is evident that the two branches produced at the apex of the primary stem may differ in their initial organogenetic behaviour.

Observations on the positional relationship between the plane of branching and the phyllotaxis suggested that this plant also provided support for the hypothesis under investigation. There was some evidence, however, that the position and orientation of the 8th. frond, as it appears in fig. 17 had been slightly altered as a result of rhizome development while an examination of sections passing through branch A (including the section shown in fig. 18) suggested that the main apex of this branch had diverged slightly in an anti-clockwise direction from the plane in which it had been initiated. This divergence appeared to have occurred when

the small lateral branch (BA1) was produced at the rhizome apex. Thus the original divergence between the 8th. frond and branch A could not be precisely determined. It seemed, however, from a study of the illustrated section, that their divergence must originally have been closely similar to that between the 6th. and 7th, fronds and thus to the phyllotactic divergence angles of the plant.

4. Finally, it is of interest to compare the plants illustrated in figs. 19 and 20. It was observed that both plants had produced seven primary fronds and were characterised by a similar phyllotactic divergence angle. They differed, however, in the direction of the genetic spiral, which was clockwise in one plant (fig. 19) and anti-clockwise in the other (fig. 20). When the two figures are compared it will be observed that the one presents an approximate mirror image of the other and that this relationship involves not only the fronds but is apparent also in the orientation of the rhizome branches. Such comparisons reveal very clearly the well defined correlation between the plane of branching and the phyllotaxis.

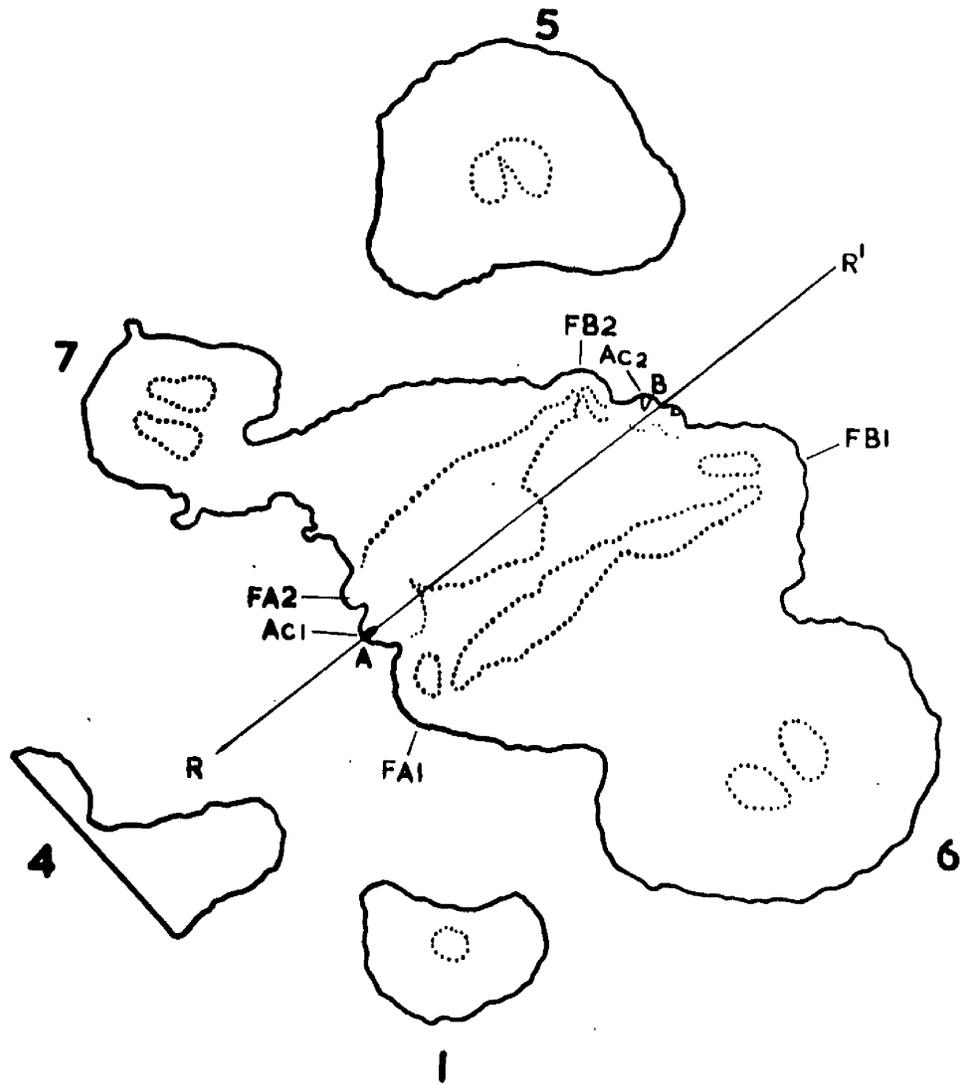


Fig. 19. Drawing of a transverse section of the shoot apex of the sporophyte. The plant has 7 primary fronds (1-7) arranged in a clockwise spiral (compare with fig. 20). Ac1, Ac2, apical cells of each rhizome branch; FA1, FA2, and FB1, FB2, frond primordia produced at the apex of branch A and branch B respectively; RR', plane of branching of the primary stem.

(x 37)

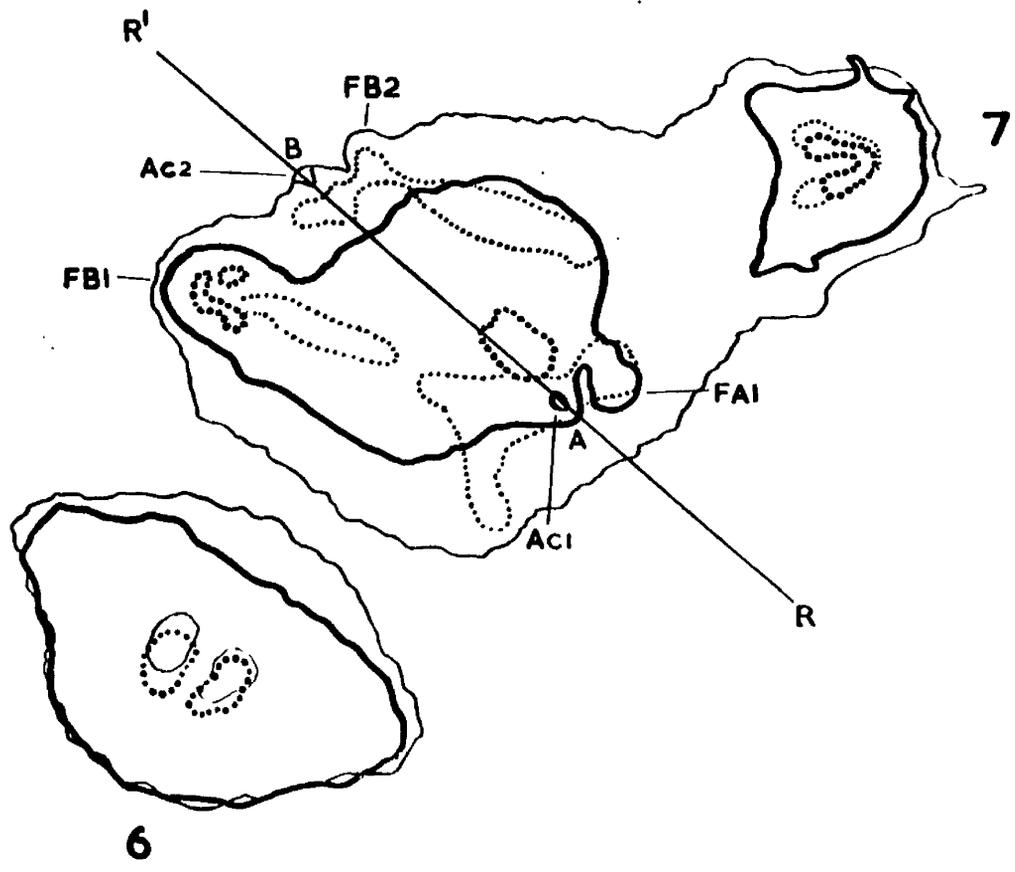


Fig. 20. Superimposed drawings of two transverse sections of the shoot apex of the sporeling. This plant should be compared with the one shown in fig. 19 from which it differs in having its 7 primary fronds arranged in an anti-clockwise spiral. It will be noted that one plant presents a mirror image of the other with respect both to the positions of the fronds (6, 7, FB1) and to the plane of branching (RR') of the primary stem.

Ac1, Ac2, the apical cells of each rhizome branch.  
 FA1 and FB1, FB2, frond primordia of branches A and B respectively.

(x 37)

## Discussion

This study of the bracken sporeling has revealed a number of interesting features which have not previously been described. At the same time, many of the observations recorded are at variance with those of earlier investigators. For example, it has been noted in the present investigation that the initiation of the rhizomes is usually preceded by the production of seven primary fronds but that quite frequently it occurs after only six fronds have been produced. In addition, observations on the early development of the young rhizomes showed that no more than three fronds are produced at each rhizome apex prior to the onset of branching and, in many instances, branching occurs as soon as the rhizomes have been initiated, that is, without a prior phase of frond production. In contrast to these observations, Gottlieb (1959) found that rhizome formation is typically preceded by the production of eight primary fronds and, furthermore, that as many as ten fronds may be produced at each rhizome apex before the first branches are initiated. It would thus appear that the onset of branching both at the apex of the primary stem and at the apices of the young rhizomes was delayed in Gottlieb's plants as compared with those on which the present observations were recorded.

A possible reason for this difference was suggested by a study of Gottlieb's data and illustrations which showed

clearly that her plants were considerably smaller than those examined in the present investigation. This difference in size was apparent not only in the length of the fronds but also in the diameters of both the primary stem and the young rhizomes and appeared to be due to differences in the methods of propagation employed. Thus the question arises as to whether factors affecting the size of the plant may be of some importance in determining the stage of development at which branches are initiated. That such a relationship may exist is suggested by the fact that, in the ontogeny of the sporeling, rhizome initiation is normally preceded by a marked radial enlargement of the primary stem (Gottlieb, 1958) which thus assumed the "obconical" form described by Bower (1923) as a characteristic feature of sporeling morphology in the Leptosporangiate ferns. It seems probable that this increase in the diameter of the stem is accompanied, as in other fern sporelings (Wardlaw, 1952) by a proportionate increase in the size of the apical meristem. Furthermore, the young rhizome apices, during their early development, undergo a similar rapid increase in diameter and again this apical enlargement is associated with the onset of branching.

While these changes in the size of the shoot apex may perhaps be of some morphogenetic significance per se they would seem to be indicative of its increasing nutritional status (Wardlaw, loc. cit.). Their evident correlation with

the onset of branching thus suggests that the latter may be determined at least partly by nutritional factors. Although this possibility does not appear to have received any critical investigation Gottlieb (1959) has observed that when sporelings are grown in nutrient culture branch production by the young rhizomes ceases "as the medium approaches exhaustion". A marked effect of nutritional factors on rhizome development has also been reported by Allsopp (1953) from his studies on the growth of Marsilea. It was found in these investigations that when the plants were subjected to conditions of carbohydrate starvation the rhizomes became considerably attenuated while the development of lateral bud meristems, which, as in bracken, are known to be apical in origin, was progressively restricted at successive nodes until finally their initiation at the rhizome apex was completely suppressed. Clearly these observations strengthen the view that in the developing bracken sporeling the increasing nutritional status of the shoot apex may be a factor of some importance in determining the stage of development at which branches are initiated.

A further question arising from the present investigation concerns the mode of origin of the rhizomes at the apex of the primary stem. Observations were consistent with the hypothesis that only one branch is initiated and that the stem apex then diverges from this branch to grow out in the

opposite direction. Although a similar interpretation of "dichotomous" branching does not appear to have been proposed for any other ferns Sachs (1882, p.177) has envisaged the possibility that the two arms of an apparent apical dichotomy may, in fact, originate in this way. He further suggests that such a form of branching might be regarded as being intermediate between a true dichotomy and a monopodium. Perhaps the strongest evidence in favour of the present hypothesis, however, is the observed relation of one branch to a leaf position. The existence of this relationship clearly suggests that the two branches differ in their mode of origin. On the other hand, if branching is truly dichotomous, as implied by Bower (1923) and other earlier investigators, then the plane of branching, being presumably determined by the orientation of the apical cell, should not be affected either by variations in the phyllotaxis or by the number of fronds produced prior to rhizome initiation. Similarly, if the two branches arise simultaneously on either side of the stem apex, as suggested by Dasanayake (*loc. cit.*) one might expect either that both branches would occur in leaf positions or that neither would do so.

In contrast to Dasanayake's hypothesis the present interpretation of rhizome formation also suggests that there may be no fundamental difference between the mode of branching of the primary stem and that of the rhizomes for, as

Dasanayake has shown, the branches of the rhizome can first be detected at the edge of the apical meristem and thus some distance behind the apical cell. The apparent difference may thus be due only to the fact that while the initial growth of the branch produced by the primary stem consistently equals that of the parent apex the branches of the rhizome are typically less strongly developed than the main axis. Even this distinction is not well defined for, as the present observations have shown, equal bifurcations of the rhizome are of quite frequent occurrence during the early stages of development.

Turning now to consider the relation of branching to the phyllotaxis, it was postulated on the basis of morphological observations that when the rhizomes are initiated one of the branches normally arises in a position in the phyllotactic spiral of the primary fronds. Although this hypothesis was well substantiated by anatomical observations the evidence obtained cannot be regarded as conclusive and further investigations are desirable. However, the validity of the hypothesis is well supported by the findings of other investigators. Bower (1910) reported that in Plagiogyria, a genus which is closely related to Pteridium, stolons occasionally appear to arise in leaf positions while Lang (1924) has described sporelings of Osmunda regalis in which rudimentary leaves were transformed directly into shoots.

Also of significance in this connection is the demonstration by Wardlaw (1949) that in Dryopteris aristata buds can be induced to arise in presumptive leaf sites when these are isolated by deep incisions from the influence of the stem apex. Later investigations (Cutter, 1956) showed that the morphological destiny of presumptive leaf primordia in this species could be similarly controlled. Evidence of this fundamental equivalence of leaf and bud positions, however, is not confined to the ferns. There is considerable support for the view that the vegetative buds (or "bulbils") of certain species of Lycopodium normally occupy positions in the phyllotactic spiral (Schoute, 1938; who reviews the earlier literature) while it has recently been reported by Cutter (1957) that in Nymphaea alba buds and leaves show a similar correspondence with respect to their arrangement on the shoot.

It might be thought, however, that if the branch produced at the apex of the primary stem of the bracken sporophyte does, in fact, occur in a leaf position then the branches subsequently initiated at the rhizome apices should show a similar relation to the phyllotaxis. If one accepts the view expressed by Dasanayake (loc. cit.), that the fronds associated with each branching of the rhizome are produced, not by the branch, but at the apex of the main axis, then it must be concluded that no such relationship exists. On the other

hand, if the branches are considered to give rise to the fronds (e.g. Bower, 1923; Webster and Steeves, 1958) and the main axis is thus regarded as a dichopodium (Velonovsky, loc. cit.) a similarity in the arrangement of the branches and the fronds is apparent. This relationship is perhaps most clearly shown by those sporelings which produce a number of fronds successively at the rhizome apex prior to the onset of branching. In such plants the branches subsequently produced might well be considered to occur in leaf positions in so far as they continue the alternate and distichous arrangement of the fronds. Furthermore, the fact that the fronds and branches are both included by the "lateral line" (i.e. the line of unlignified tissue which runs along either side of the rhizome) suggests that both are initiated in the same ~~dorsiventral~~ <sup>lateral</sup> plane at the rhizome apex.

Clearly this hypothesis, and the several other views expressed in the foregoing discussion, cannot be accepted merely on the evidence provided by the present observations. A notable feature which they have in common, however, and which provides a working hypothesis for further investigations, is their implication that the striking morphological contrast between the young sporeling and the adult plant may, in some respects, be more apparent than real. In particular, it is suggested that the factors which determine the onset of branching, the mode of origin of the branches and,

finally, the relation of branching to the phyllotaxis, may prove to be of a similar nature in both the erect and the rhizomatous phases of the plant's development.

SECTION 3. OBSERVATIONS ON FROND BUD DEVELOPMENT IN THE  
ADULT PLANT

There is evidence in the literature of a marked divergence of opinion regarding the normal course of frond bud development in the mature bracken plant. According to Klein (1884) two frond buds are normally present at the apex of the rhizome branch at the end of the growing season. The older of these, a well developed bud several centimetres in length, is destined to emerge in the following spring while the younger bud, which generally becomes visible at the rhizome apex in late summer as a rounded lateral protuberance ("höcher"), continues to develop for a further season before finally giving rise to an expanded frond. Although this account is in agreement with observations reported by later investigators (Smith, 1928; Conway and Stevens, 1954) Watt (1940), in his studies in Breckland, found that only a single frond bud was normally present in the latter part of the growing season and states that this bud, which can be detected at the rhizome apex in late June, "grows to maturity within 12 months of its first visible appearance". Recently, a similar sequence of events has been reported by Webster and Steeves (1959) from their work on bracken (var. *latiusculum*) in the N.E. United States.

In the course of a general observational study of the plant it was found that samples of rhizome dug up in September frequently showed a second frond bud at the rhizome apex. This observation appeared to agree with Klein's description of frond development but was at variance with Watt's account. However, since Watt's observations were restricted to "short shoots" (i.e. branches with an internode length no greater than 2.0 cms.) it seemed that the observed variation in the number of visible frond buds might be associated with small differences in the internode length of the rhizome branch. Further rhizome samples were therefore collected to determine whether any evidence could be obtained of a correlation between rhizome and frond bud development.

#### The Site of the Investigation

The bracken community investigated covered an extensive area of hill pasture land at Shemore Farm, near Luss (Dumbartonshire) and was growing on a S.W. facing slope at a height of approximately 1000 ft. On the top of the slope, fully exposed to the prevailing winds, the fronds were sparse and little more than 12 inches in height. At successively lower levels the density and height progressively increased and the bracken became completely dominant over an area extending upwards for a distance of several hundred yards

from the bottom of the hill. In this lower region, protected from wind by a belt of trees, and where the soil was deeper, the fronds ranged from 5 - 6 ft. in height and had an average density of 30 per square yard. It was from this region of uniform and vigorous bracken that all of the rhizome samples were obtained.

### Method

#### (i) Sampling procedure

Twenty rhizome branches were collected for examination on each of four occasions. The sampling dates were 1st. October, 22nd. October, 10th. November and 1st. December. On each occasion fronds of the current year were selected at random and were dug up together with the apical portion of the associated rhizome branch. A typical sample is illustrated in fig. 21. On the first two occasions sampling was restricted to branches having an internode length (B-C, fig. 21) not greater than 2.0 cms. Adopting Watt's (1940) terminology, these branches may conveniently be referred to as "short shoots". On the 3rd. and 4th. occasions the range was extended to include branches with internodes up to 4 cms. in length.

#### (ii) Data recorded

The following measurements, to the nearest 0.25 cms. were recorded for each rhizome sample (using the annotations

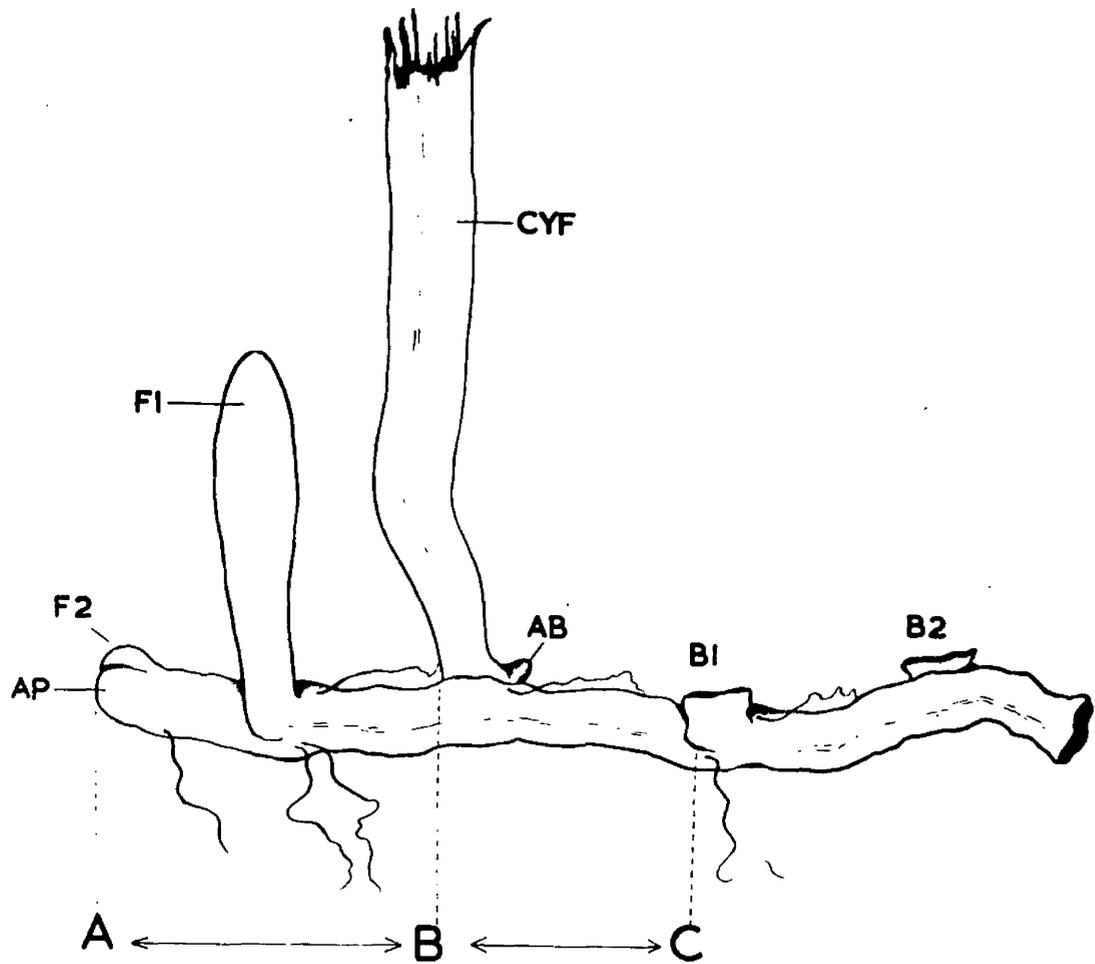


Fig. 21. Drawing of a typical rhizome sample collected for examination. CYF, base of current year's frond; F1, large bud which will give rise to next year's frond; F2, younger frond bud appearing as a lateral protuberance at the rhizome apex (AP); AB, abaxial rhizome bud; B1, B2, bases of previous years' fronds. A  $\rightarrow$  B, distance of rhizome apex from current year frond; B  $\rightarrow$  C, internode length of branch.

(approx. x 1)

of fig. 21):-

- a. Length of rhizome internode (B  $\longrightarrow$  C)
- b. Length of frond bud F1
- c. Distance from axil of current year frond to rhizome apex (B  $\longrightarrow$  A)
- d. Number of apices with a second frond bud (F2) externally visible.

This younger frond bud generally appeared as an almost hemispherical protuberance on the same level as or immediately behind the rhizome apex. Its approximate range in length was from 0.25 - 1.25 cms. but, since its junction with the rhizome was ill-defined no attempt was made to record the length of individual buds.

The rhizome branches collected on the 3rd. and 4th. sampling occasions were divided into two groups, one consisting of short shoots and the other of shoots having an internode length of 2.25 - 4.0 cms. The data were recorded separately for the shoots in each internode length category.

"Students' t-test (after Snedecor, 1956) was employed in the statistical analysis of the data in Tables 2 and 5, while the significance of the correlation coefficients in Table 3 was determined by reference to Fisher and Yate's "Statistical Tables" (4th. ed.) p. 54.

(iii) Preparations of apices for anatomical examination

To determine whether a second frond bud could be detected as a primordium at the apex of short shoots on which only a

single frond bud was present two branch apices were selected for anatomical investigation. The branches, selected at random from those in the short shoot category of the sample collected on the 1st. December, had internode lengths of 2.0 and 1.7 cms. and each bore a single frond bud (F1) at a distance of 1.5 and 2.0 cms. respectively behind the apex.

The apical centimetre of each branch was removed by a transverse cut and prepared for sectioning. In order to improve infiltration the thick felt of hairs covering each apex was carefully scraped off under a dissecting microscope with a sharp scalpel. The bulk of the material was then reduced and the internal tissues extensively exposed by cutting a slice approximately 1 mm. thick from the dorsal and ventral surfaces.

The material was fixed, dehydrated and infiltrated with wax as previously described in Section 2, p.55. Longitudinal sections were cut at a thickness of 12 - 15  $\mu$  in a plane parallel to the dorsal and ventral surfaces. They were stained in Safranin (1% aqueous) and Delafield's Haematoxylin (according to Johanson, 1940) and mounted in euparal as permanent preparations.

## Results

From Table 2 it will be observed that at least one frond bud (F1) was present at the apex of all branches examined. In the samples collected in November and December, and which were grouped according to the length of the rhizome internodes, the length of the frond bud was shown to be significantly greater on shoots with longer internodes. This relationship is illustrated by the histogram in fig. 22 from which it may also be noted that the frond buds apparently continued to grow throughout the greater part of the sampling period and thus well into the winter. As indicated in Table 3, the length of F1 also showed a highly significant positive correlation ( $P = 0.01$ ) with the distance of the rhizome apex from the current year frond (B  $\rightarrow$  A).

A second frond bud (F2) was externally visible on a small proportion of short shoots (Table 4) but was of relatively more frequent occurrence on shoots in the longer internode length categories of the November and December samples. It was found that the length of the rhizome internode and the distance of the apex from the emergent frond were both significantly greater ( $P = 0.01$ ) in shoots with F2 externally visible than in those on which only a single frond bud was present (Table 5).

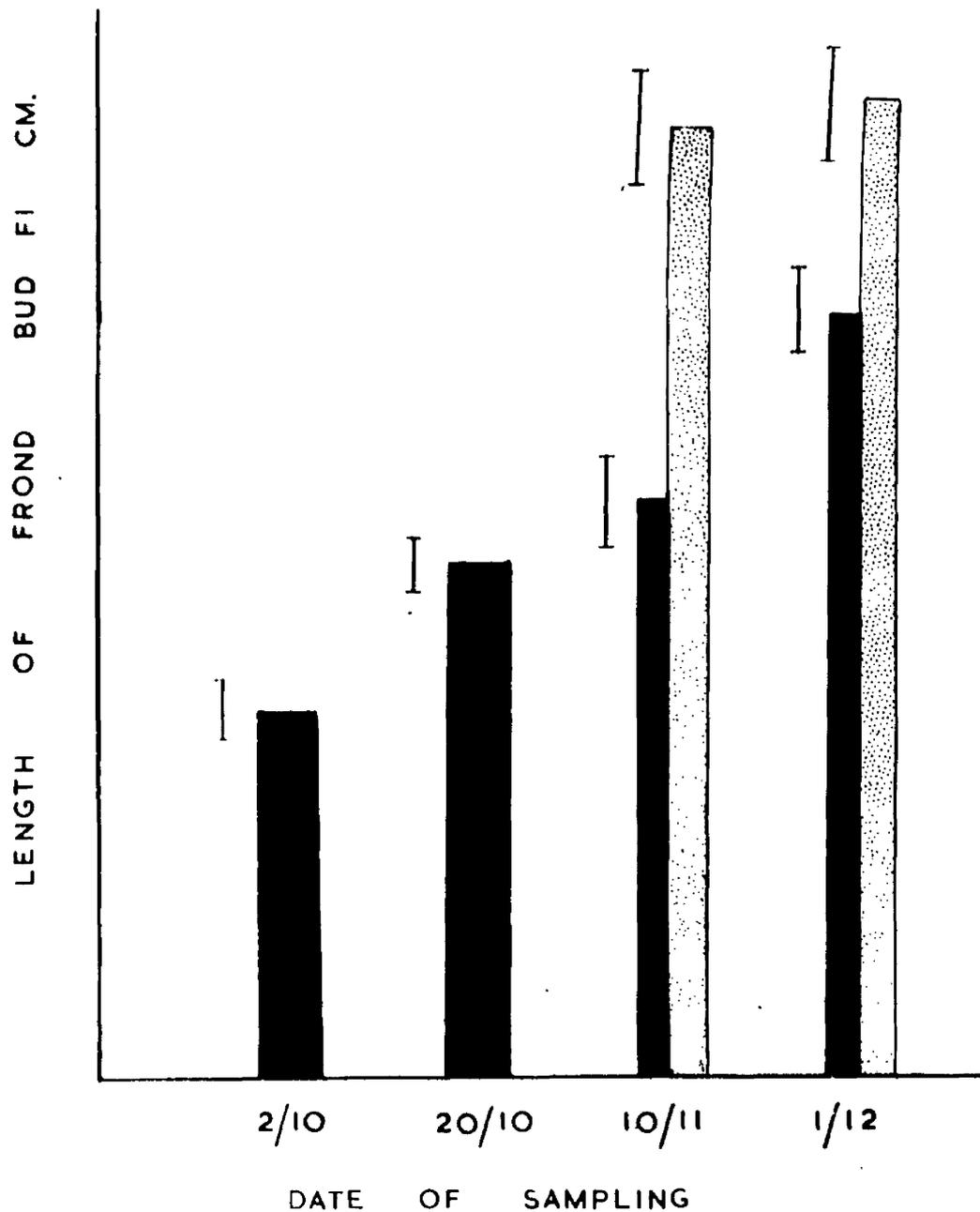


Fig. 22. Histogram showing the length of the older frond bud (F1, fig. 21) on rhizome branches collected on each sampling occasion.

■ = Buds on branches of internode length 0-2.0 cm.

▨ = " " " " " " 2.25-4.0 cm.

The vertical lines are equal to twice the standard errors of the means.

FROND BUD DEVELOPMENT IN THE ADULT BRACKEN PLANT (TABLES 2-5)

TABLE 2. Occurrence and length of frond bud F1 on shoots in different internode length categories

Date of Sampling	2/10	22/10	10/11		1/12	
Range of internode length of branches sampled (cms.)	0-2	0-2	0-2	2.25-4	0-2	2.25-4
Number of branches in each internode length category	20	20	13	7	9	11
Mean internode length (cms.)	1.47	1.60	1.61	2.82	1.61	2.81
Percentage apices with frond bud F1 differentiated	100	100	100	100	100	100
Mean lengths of frond bud F1 and standard errors	1.62 $\pm 0.285$	2.76 $\pm 0.268$	3.05 $\pm 0.478$	5.03 $\pm 0.609$	4.44 $\pm 0.506$	5.20 $\pm 0.587$
Sig. of diff. of mean length of frond bud F1 on shoots in different internode length categories	P = 0.01			P = 0.05		

TABLE 3. Correlation between length of frond bud F1 and distance from current year frond to rhizome apex

Date of sampling	Mean length of frond bud F1 (cms.)	Distance of current year frond to rhizome apex (cms.)	Correlation coefficient (r)	Significance (P) of Corr. coefficient
10/11	3.7 $\pm$ 0.419	3.12 $\pm$ 0.307	0.754	0.001
1/12	4.86 $\pm$ 0.395	3.53 $\pm$ 0.212	0.725	0.001

TABLE 4. Proportion of branch apices with a second frond bud (F2) differentiated

Date of Sampling	2/10	22/10	10/11		1/12	
Range of internode length of branches sampled (cms.)	0-2	0-2	0-2	2.25-4	0-2	2.25-4
Number of branches with a second frond bud (F2) differentiated*	1(20)	2(20)	3(13)	5(7)	1(9)	6(11)
Percentage branches with F2 differentiated	5	10	23	71	11	55

\* The number of branches examined in each internode length category is shown in brackets.

TABLE 5. The differentiation of F2 in relation to :-

- (a) Internode length (B → C)  
 (b) Distance from current year frond to rhizome apex (B → A)

	Date of sampling	Branches with F2 differentiated	Branches with F2 not differentiated	Sig.diff. P = 0.01
Mean length of internode (B - C) (cms.)	10/11	2.56 ± 0.270	1.68 ± 0.352	0.337
	1/12	2.68 ± 0.171	2.06 ± 0.234	0.465
Mean distance current year frond to rhizome apex (B - A) (cms.)	10/11	4.22 ± 0.506	2.39 ± 0.203	0.628
	1/12	4.28 ± 0.310	3.13 ± 0.213	0.497

### Anatomical observations

Several frond and branch primordia were observed at the apices of those branches selected for anatomical study. Since all of the primordia occurred in different longitudinal sections they could not be illustrated by means of a single photomicrograph. However, the diagrammatic drawing in fig. 23, constructed from an examination of a number of different sections, will serve to illustrate the primordia observed at one of the apices and their approximate relative positions while the exact relative sizes of these primordia and their histological structure is shown by the camera lucida drawings in figs. 24 and 25.

F2 was present as a well formed primordium situated at the base of a deep depression and lying at a distance of approximately 1.6 mm. from the rhizome apex. A younger frond primordium (F3) was enclosed in the same depression as the rhizome apex and at a distance of approximately 0.3 mm. from the apical cell. Both of these primordia were found to be associated with branch meristems (B2 and B3). A close examination of the apex showed a 3rd. primordium (P) at an early stage of development and recognisable as a large cell which had cut off several derivatives and which protuded slightly above the general level of the apex. Owing to the thickness of the sections this youngest primordium could not be critically examined. Its

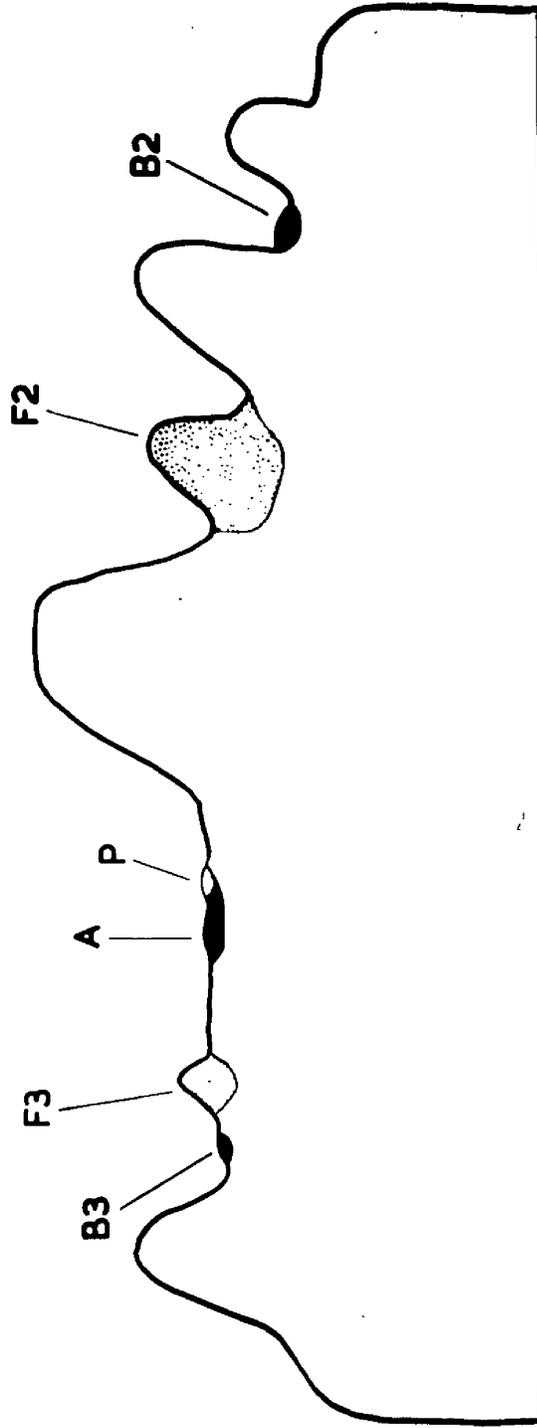


Fig. 23. A diagrammatic drawing constructed from an examination of several longitudinal sections of one of the short shoots dug up in December. The drawing indicates the approximate relative positions of the frond and branch primordia. The exact relative sizes of these primordia and their histological structure can be observed in figs. 24 and 25. F2, F3, frond primordia; B2, B3, branch meristems associated with F2 and F3 respectively; A, rhizome apical meristem with very young primordium (P).

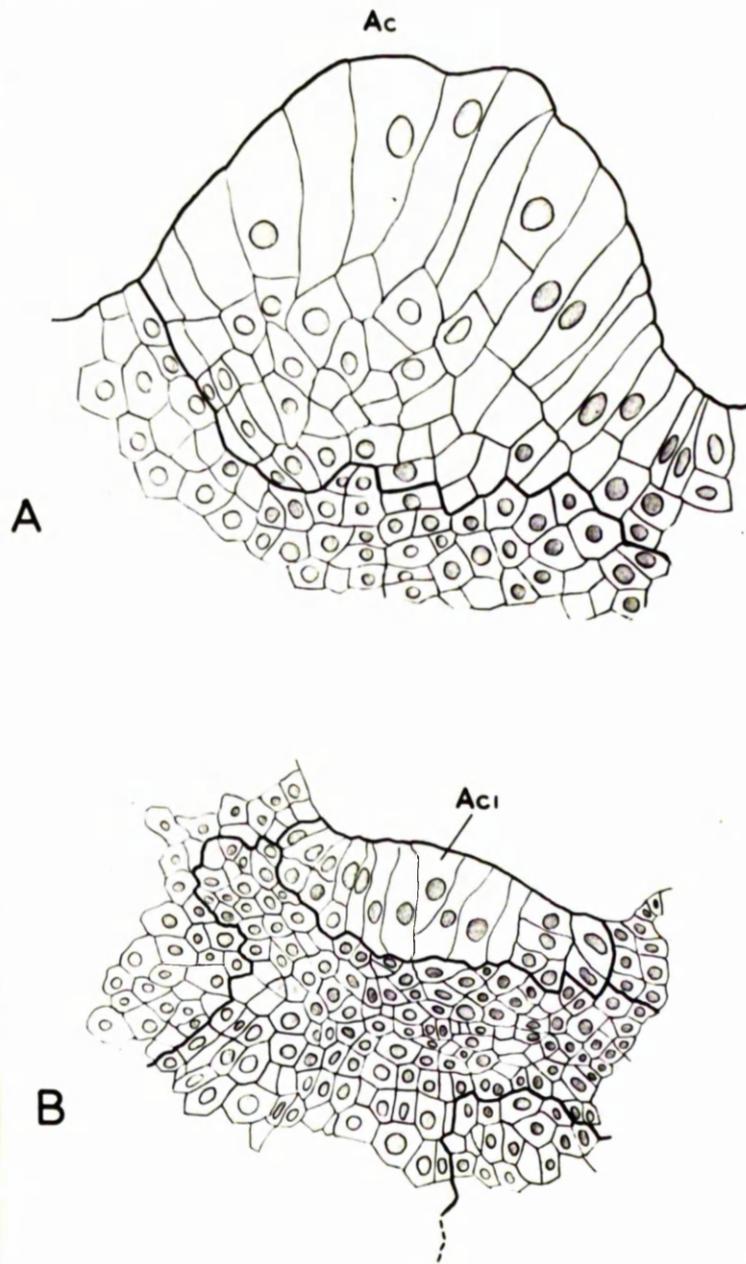


Fig. 24. Camera lucida drawings of (A) frond primordium F1 and (B) branch meristem B2 shown diagrammatically in fig. 23.

Ac, Ac1, apical cells of F2 and B2 respectively. The apical meristem of B2 and the incipient vascular tissue are delimited by a heavy line.

(x 250)

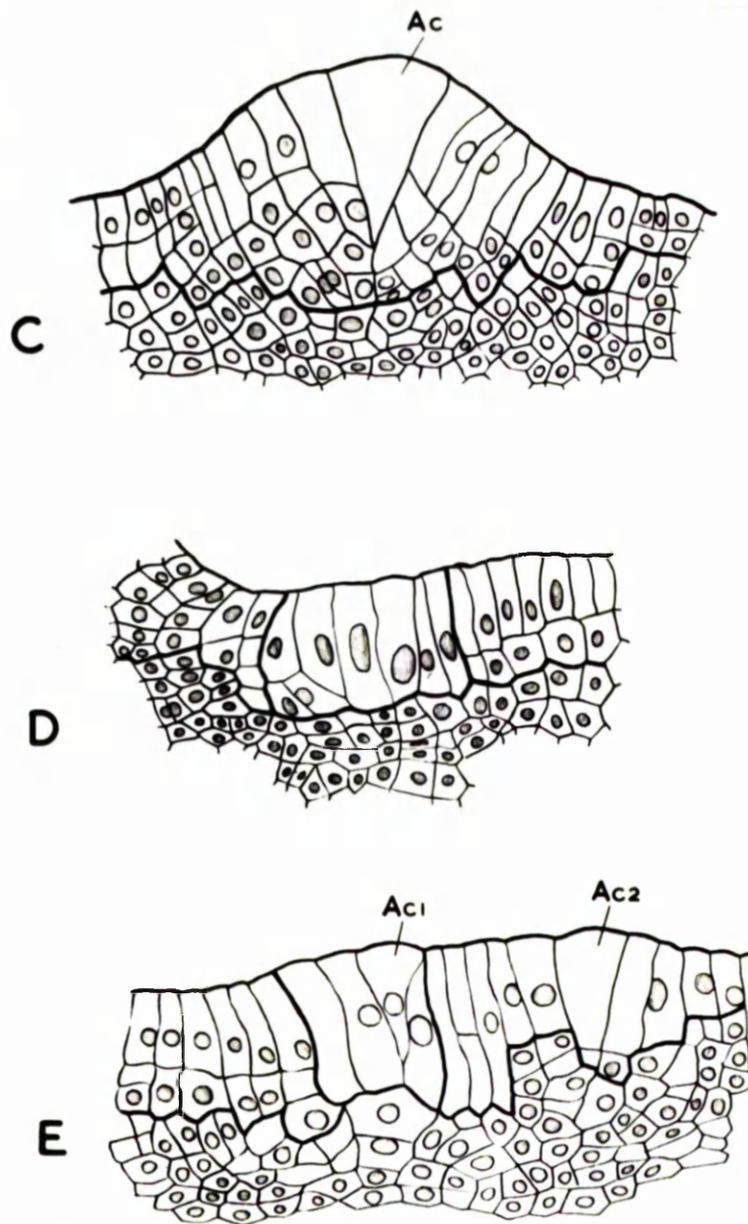


Fig. 25. Camera lucida drawings of primordia F3, B3, P and rhizome apical meristem A shown diagrammatically in fig. 23.

- C. Frond primordium F3. Ac, apical cell.  
 D. Branch meristem B3.  
 E. Rhizome apical meristem A and associated primordium P. Ac1, apical cell of rhizome; Ac2, apical cell of primordium.

In each illustration the apical meristem is delimited by a heavy line from the underlying incipient vascular tissue.

(x 250)

morphological nature is uncertain and may not yet have been determined.

The other branch apex examined also bore two frond primordia very similar in age and position to the corresponding primordia in the illustrated section. It differed from the latter apex only in the apparent absence of a branch meristem associated with F3 and of a still younger primordium (P) at the rhizome apex. It is possible, however, that the youngest primordium escaped detection.

#### Discussion

It is clear from these results that the growth of the frond bud of the mature bracken plant is closely correlated with that of the associated rhizome branch. While this correlation might have been anticipated the fact that it exists would seem to explain the apparent discrepancy between the accounts of frond bud development reported in the literature. It is evident from the present investigation that the development of the second frond (F2) from an apical primordium to an externally visible bud is correlated with the growth of the rhizome branch. It thus seems probable that Klein's account of frond development was based on a study of branches with a somewhat greater internode length than the "short shoots" to which Watt's observations were restricted.

Although this correlation does not appear to have been previously described Watt (1950) has observed that the length

of the frond bud, either in March or September, shows a highly significant positive correlation with the depth below the soil of the associated branch apex. In his earlier study of the rhizome (1940) Watt also noted that the frond-bearing shoot normally grows obliquely or vertically upwards towards the soil surface and that, as it does so, its internode length progressively decreases. Clearly these two sets of observations, when considered together, point to the existence of a positive correlation between the length of the frond bud and the internode length of the associated rhizome branch and thus may be considered to support the results of the present investigation.

However, a marked contrast with certain other observations reported by Watt is also apparent. In the present investigation it was found that at least one frond bud was present on all of the short shoots examined and that, on a small proportion of these shoots in each of the four samples, a second frond bud was present. Watt (1950), on the other hand, found considerable variation in frond bud production on short shoots carrying a current year frond. Data from samples collected in August showed that from 15-65% of such branches had not produced a frond bud. Furthermore, it may be inferred from Watt's account of frond bud development that a second bud was not present at the apex of any of the short shoots which he examined. In this connection it is

of interest to note that Conway (1958) has examined rhizome samples taken from seven different areas of bracken in the West of Scotland and has found similar evidence of a higher rate of frond bud production than that observed by Watt. Thus it seems probable that this feature may be correlated with the general vigour of the plant and that this, in turn, tends to be promoted by the climatic conditions of the West of Scotland. Certainly, in this region, the mild winters, the relative infrequency of severe frost in late spring and the high summer rainfall are all factors which would seem to favour the growth of bracken. In the Breckland, on the other hand, the more continental type of climate combined with a porous and infertile soil provide conditions which are considered by Watt (1936) to militate against the vigorous growth not only of bracken but of many other species.

## SUMMARY OF PART II

SECTION 1. It has been shown that the primary frond of the bracken sporeling exerts a retarding influence on the growth of the second frond. This effect could be clearly demonstrated by removing the primary frond during the phase of rapid extension of the petiole but it was much less apparent if defoliation was delayed until the lamina of the frond was almost fully expanded. These observations are contrasted with those reported from similar experiments with sporelings of Pteris longifolia which showed the inhibiting influence of the primary frond to be of much greater duration.. A comparison of the investigations provided some evidence that nutritional factors may be involved in this phenomenon.

SECTION 2. Several aspects of the morphology of the sporeling have been investigated. The phyllotaxis of the fronds produced prior to rhizome initiation is extremely variable but is found to range predominantly between a  $3/8$ th. and a  $5/13$ th. system. Clockwise and anti-clockwise arrangements occur with equal frequency. The number of fronds is also variable, ranging from six to eight. Anatomical observations on the mode of origin of the two rhizome branches suggested that only one branch is initiated at the apex of the primary stem and that the other is produced by the continued growth

in a diverging direction of the stem apex itself. This interpretation is at variance with that of earlier workers and, being based on only a small number of observations, requires confirmation. The observed relation of branching to the phyllotaxis was consistent with the view that one of the branches normally arises in a leaf position. This hypothesis was well supported by anatomical observations. The onset of branching at the apex of the young rhizome may be preceded by the production of one to three fronds but frequently occurs before any fronds have been produced. These various observations are compared with those of previous investigators.

SECTION 3. In the adult bracken plant the growth of the frond bud has been shown to be closely correlated with the growth of the associated rhizome branch. It is suggested that this fact could explain the lack of agreement between accounts of frond bud development reported in the literature.

PART III

STUDIES ON THE TRANSLOCATION AND

EFFECTS OF THE

GROWTH-REGULATING HERBICIDE,

2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

SECTION 1. THE TRANSLOCATION OF 2,4-D IN THE SPORELING AND  
ITS MORPHOLOGICAL AND ANATOMICAL EFFECTS ON THE  
RHIZOME

I. Introduction

The background to the present investigations has already been briefly outlined in the General Introduction where reference was made to reports in the literature of the ineffectiveness of growth-regulating herbicides as a means of bracken control. It was also noted, however, that later investigations by Stevens (1953) had shown these herbicides to be capable of causing severe injury and the production of characteristic morphological responses when applied under greenhouse conditions to the young sporeling. Since Stephen's observations are of interest in relation to the present investigations and, indeed, were primarily responsible for their initiation, her experiments will now require to be more fully described.

Two experiments are reported. In the first of these sporelings grown in pots in the greenhouse were sprayed with solutions containing various concentrations of the sodium salt of 2-methyl-4-chlorophenoxyacetic acid (MCPA). When applied at a concentration of 250 p.p.m. the herbicide induced a marked swelling and contortion of the rhizome

apices from which dense clusters of roots were produced while, at a concentration of 1000 p.p.m. the apices, in some instances, were killed. It was remarked, however, that since the spray had been applied both to the fronds and to the soil foliar absorption may have been accompanied by uptake through the roots and rhizome. Thus, in a second experiment, in which the sodium salt of 2,4-D was used, the herbicide was applied only to the fronds but in a number of different ways. Treatments in which the apical region of the frond, either intact or decapitated, was dipped into the herbicide solution (for 48 hours) resulted in the death of the treated part of the frond and in well marked morphological responses at the rhizome apices. On the other hand, when the solution was applied as a single drop either to the cut surface of the rachis or to the intact surface of a pinna morphological effects were restricted to the treated fronds. The cut surface treatment induced a curvature of the petiole of the frond while applications to the pinna caused a reduction in the size of the more apical pinnae which expanded subsequent to treatment. Neither of these droplet applications produced any visible effects on the rhizome.

While these experiments were of considerable interest in demonstrating that the bracken rhizome, at least during its early development, is not immune to the toxic effects of

growth regulating herbicides they raised an important question concerning the translocation of the herbicide within the plant. Earlier fundamental studies on the mode of action of 2,4-D (e.g. Mitchell and Brown, 1946; Weaver and De Rose, 1946; Rice, 1948; Linder, Brown and Mitchell, 1948), in which the bean (Phaseolus vulgaris) was used as the test plant, showed that when the herbicide is applied as a droplet to the intact surface of a leaf its subsequent translocation out of the leaf occurs in living tissues and apparently in association with the movement of natural assimilates. It may be presumed that the phloem is the tissue which is actually involved. On the other hand, there is equally good evidence that when 2,4-D is taken up by the roots some of the herbicide is carried up into the shoot in the xylem under the influence of the transpiration stream (Mitchell and Brown, loc. cit.; Weaver and De Rose, loc cit.). Furthermore, basipetal movement out of a treated leaf may also occur in the xylem if the blade of the leaf is removed and the cut end of the petiole is immersed in the herbicide solution (Weintraub and Brown, 1950).

A consideration of these facts in relation to Stephen's<sup>v</sup> experiment suggests that those several methods of application which produced injurious effects on the rhizome may also have resulted in the entry of the herbicide into the xylem and its distribution within the plant under the influence of the

transpiration stream. Thus, even when the "dipped" frond apex was left intact its death during the treatment period might be expected to have permitted xylem penetration to occur. On the other hand, when the solution was applied as a drop to the intact surface of the frond - a method of application which would seem likely to have precluded the occurrence of extensive xylem transport - the rhizomes were apparently unaffected. There was therefore no conclusive evidence from Stephen's results that the 2,4-D could be extensively translocated in toxic amounts in the living tissues of the plant and thus in the manner which is essential for the successful treatment of deep-rooted perennial weeds. However, it is also apparent that the dosage applied by the droplet treatment, although not precisely stated, was considerably less than by the other methods employed and, in consequence, the amount of 2,4-D reaching the rhizome may well have been insufficient to produce a visible response. Furthermore, it is well known that the penetration of 2,4-D into leaves and its subsequent translocation within the plant may be greatly affected by a large number of different factors (discussed by Leopold, 1955). The results obtained in a single experiment in which only one dosage was applied and under one set of conditions could not therefore be regarded as conclusive.

Thus, in the experiments described below the effect of foliar applications of 2,4-D on the rhizome of the bracken sporeling have been investigated and an attempt has been made to determine which tissues are involved in the translocation of the herbicide. In addition, the morphological and anatomical responses induced at the rhizome apices have been studied in relation to the dosage applied. The first part of the investigation was carried out during the autumn of 1956 while the subsequent observational studies were delayed until the spring of the following year when the higher light intensity enabled plants of greater size and vigour to be obtained.

## II. General Methods

### i. Propagation and selection of plants

Plants were raised in the greenhouse from spores by the methods previously described (Part I). Despite careful selection of the sporelings for uniformity there was still some variation in their size and stage of development, the extent of which will be indicated in the description of each experiment. The selected plants, raised from the first leaf stage in three inch pots, were transplanted into either four or five inch pots before the experimental treatments were applied.

ii. The formulation of the herbicide

2,4-D, as the parent acid, was formulated by dissolving 100 mgs. in 0.5 gms. of melted "Carbowax 1500". This solution was then added with vigorous stirring to 200 ml. of distilled water to give a uniform dispersion of the herbicide. This type of formulation was first used by Hamner and Tukey (1944) who showed it to be effective in the eradication of bindweed and is considered by Crafts (1956b), on theoretical grounds, to be particularly well suited for the control of deep-rooted perennial weeds. Carbowax 1500, a mixture of polyethylene glycols, serves primarily as a solvent for the sparingly water soluble acid. It has also been shown, however, to increase considerably the amount of 2,4-D absorbed by the plant. It is presumed that, because of its hygroscopic nature, the rate of drying of the drops on the leaf surface is retarded and penetration of the herbicide is thereby enhanced (Mitchell and Hamner, 1944; Rice, 1948). The phytotoxicity of this substance has been investigated by Mitchell and Hamner (loc. cit.) who found it injurious to tomato leaves when applied in aqueous solution at concentrations of 9% or greater and to injure bean leaves at a concentration of  $\geq 5\%$ . Since it is well known that injury to the leaf tissues tends to restrict translocation of growth regulating herbicides the effect of carbowax on the fronds of the bracken sporeling was investigated in a small

preliminary experiment. When applied in water at a concentration of 3% to fully mature fronds a slight necrosis of the treated part became apparent after 36 hours. No visible injury was produced, however, by concentrations of 2.5% or less. Thus, the 1% solution used in the formulation of the herbicide was well below the threshold of toxicity.

iii. Method of application

In each experiment the 2,4-D solution was applied to the fronds as one or more 20  $\mu$ l. droplets by means of a pipette having a total capacity of 0.2 ml. and graduated in divisions of 4  $\mu$ l. It was estimated that the error involved in the application of each droplet by this method was of the order of  $\pm 2$   $\mu$ l. (i.e.  $\pm 10\%$ ).

It has been shown that the response induced by a given amount of 2,4-D may be affected by the concentration of the solution and also by the droplet size (Smith, 1946). For this reason the dosage was varied in the present experiments by varying the number of droplets applied, the volume of the drops and the concentration of the solution being held constant.

All applications were made to fronds which were fully expanded and to parts of the frond which were apparently fully mature.

iv. Environmental conditions

The greenhouse conditions under which the experiments

were carried out were similar to those previously described (Part I). A record of the conditions of temperature and humidity at the time that the treatments were applied and for a short period thereafter is given in the description of each experiment.

### III. Experiments and Observations

#### 1. The effect of foliar applications of 2,4-D on the rhizome of the sporeling

Twelve sporelings were selected for a preliminary experiment at a stage when the sixth frond was almost fully expanded and the young rhizomes, which ranged from 0.75 - 1.75 cms. in length, were just beginning to turn down into the soil (fig. 26).

Four plants were treated with a single drop of solution (i.e. 10  $\mu$ gms. 2,4-D) while the other four received two drops each (20  $\mu$ gms.), the drops being applied to the basal pinnule of one or both members of the first pair of pinnae of the fifth frond. The remaining four plants served as controls and were treated with a 1% aqueous solution of carbowax.

The treatments were applied at mid-day during a period of warm sunny weather. The temperature in the greenhouse was 82<sup>o</sup>F and the relative humidity was 33%. During the following six hour period the temperature ranged from 76 - 84<sup>o</sup>F and the humidity from 31 - 37%.



Fig. 26. Experiment 1. One of the sporelings selected for the experiment illustrating their stage of development. (x 2/5)

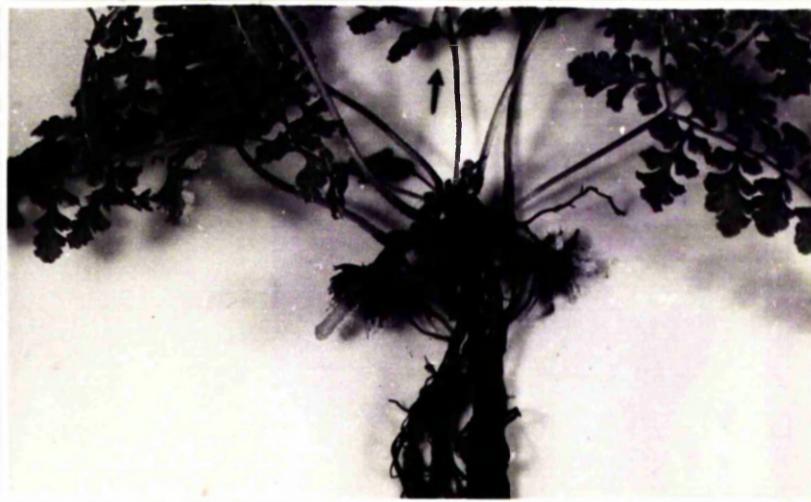


Fig. 27. Experiment 1. A sporeling 19 days after treatment with 20  $\mu$ gm. 2,4-D. Several primary fronds have been removed to reveal the treated frond (arrowed) more clearly. Note the dense cluster of roots which have been produced close behind each rhizome apex. Both branches have resumed normal apical growth. (x  $\frac{1}{2}$ )

The droplets of solution dried rapidly and after one hour only a thin film of carbowax remained on the leaf surface. Four hours after treatment a slight curling of the margins of the treated pinnules was observed and, at the end of 24 hours, a slight necrosis and withering of the treated parts had become apparent. The control plants showed no effect of the carbowax treatments. Swellings were visible at the rhizome apices of several of the plants five days later. By this time, however, most of the rhizome apices were below the surface of the soil and their appearance was not recorded.

All of the plants were removed from their pots and examined 19 days after treatment. Both of the rhizome branches of seven of the eight treated plants showed morphological effects of a similar nature to those described by Stevens. All of the affected plants, one of which is shown in fig. 27, had produced clusters of roots in the vicinity of the rhizome apices. This rooting response was accompanied in several plants by a marked swelling and contortion of the apical region, the outer tissues of which had split open to a varying extent along the plane of the "lateral line" (the line of un lignified tissue on each side of the rhizome). These various effects were much more pronounced in the plants which had received 20  $\mu$ gms. of the herbicide.

Two features are particularly worthy of note. Firstly, although comparison with the control plants suggested that rhizome development had been considerably retarded by the treatment, it was evident that normal apical growth had, in all cases, been resumed. The growth inhibition thus appeared to be of a transitory nature. Secondly, it was apparent that the two rhizome branches of each plant had been affected to a similar extent suggesting that the 2,4-D had been equally translocated into each branch.

2. The tissues involved in the translocation of the herbicide

In discussing Steven's experiments (p. 94 ) it was suggested that the methods of application employed may have permitted some of the herbicide to enter the xylem and thus to be translocated under the influence of the transpiration stream. It seemed, however, that the treatments applied in the experiment previously described should have restricted the basipetal movement of the herbicide largely or entirely to the phloem. Nevertheless it was considered desirable that this assumption should be tested experimentally.

Owing to the nature of the vascular anatomy of the bracken frond the tissues involved in the translocation of the herbicide could not be investigated by means of the conventional ringing technique. A preliminary experiment was therefore carried out to determine whether a segment of

the frond petiole could be killed by treatment with steam in such a way as to avoid causing injury to the water conducting tissues. This method, which has been used for a similar purpose by previous investigations (e.g. Mitchell and Brown, 1946) proved to be satisfactory and was then used to determine whether a continuity of living tissue was necessary for the translocation of the herbicide.

Four sporelings, each with five fully expanded fronds and with their rhizomes just initiated, were selected for the preliminary experiment. The apparatus used for steaming the petioles consisted of a conical flask fitted with a rubber stopper through which was inserted a right angled piece of glass tubing the end of which had been drawn out to form a nozzle 1.5 mm. in diameter. The fourth frond of each plant was selected for treatment and was supported by a Y-shaped piece of wire pushed into the soil. The water in the flask was heated and when a steady jet of steam was issuing from the nozzle the mid point of the petiole of each frond in turn was held close to the mouth of the nozzle for 8 - 9 seconds. At the end of this period a slight yellowing of the treated part was evident. The injured region of each frond was then immediately covered with a thick layer of vaseline, to prevent loss of water through the killed tissues. The four treated plants were left in the greenhouse overnight. When examined the following morning, 18 hours after steaming, the tissues of the treated parts were found to be severely injured but

the fronds appeared perfectly healthy and showed no sign of wilting (fig. 28).

One of the ringed fronds was removed and hand sections were cut through the treated parts. An examination of these sections showed all the living tissues to be brown in colour and completely disorganised. No injury to the xylem elements could be detected.

Twenty-four hours after treatment two of the remaining plants were removed from their pots and their roots washed free from soil. They were then suspended on wire supports with their roots dipping into a 1% aqueous solution of eosin. An unringed plant of the same age was similarly treated and served as a control. Bright sunshine and a low relative humidity provided conditions conducive to rapid transpiration. After only 20 minutes the veins of all the fronds had become distinctly coloured with the dye. This effect was observed to be produced just as rapidly in the ringed frond as in the untreated ones.

Since the technique thus appeared to be satisfactory an experiment was then designed to investigate the effect of ringing on the translocation of 2,4-D. Sixteen sporelings each with 6 - 7 fully expanded fronds and with rhizome branches ranging from 0.6 - 1.6 cms. in length were selected for the experiment and were divided at random into four equal groups. The mid-point of the petiole of the fifth frond of each plant

in two of the groups was treated with steam and, after 24 hours, 2 20  $\mu$ l. drops of the 2,4-D solution (i.e. 20  $\mu$ gms. 2,4-D) were applied to the fronds of half of the plants, a single drop being placed on one of the basal pinnules of each member of the first pair of pinnae. The same dose was also applied to the corresponding frond of the unringed plants in one of the other groups. Since it was uncertain whether the plants had been sufficiently "hardened off" by the time they had reached the required stage of development they were retained throughout the experiment in the humid frames in which they had been raised. The humidity within the frames rarely fell below 90% during the period of the experiment. When the herbicide was applied the temperature was 65<sup>o</sup>F and varied only from 65 - 67<sup>o</sup>F during the following 18 hour period.

After 24 hours one of the ringed fronds which had been treated with 2,4-D became slightly wilted. All of the other ringed fronds, however, were of normal appearance. To avoid the possibility of the droplet being dislodged the wilting frond was severed from the plant at the base of the petiole. At the same time the corresponding frond was removed from all of the other plants.

Five days later the rhizome apices of two of the unringed, treated plants were observed to be distinctly swollen and contorted. None of the other rhizome apices still visible above the soil showed any abnormalities.

All of the plants were removed from their pots and examined four weeks after treatment. Three of the 2,4-D treated plants which had not been ringed (fig. 29) had obviously been affected by the herbicide. Both rhizome apices of one of the plants (A) were dormant and slightly swollen and small fissures could be detected in the outer tissues. The apices of another plant (B) were completely obscured by a dense growth of roots while those of the third (C) were grossly swollen and contorted. One plant (D) was apparently unaffected. The ringed plants from the treated and control groups all had well developed rhizomes of normal appearance (figs. 30, 31) with the exception of one plant (A, fig. 31) a branch of which had become diseased at an early stage of development and had decayed during the experimental period.

Thus, despite the failure of one of the unringed plants to show any effects of the herbicide, the results support the previous assumption that the downward movement of 2,4-D is restricted to the living tissues of the plant.

### 3. The morphological nature of the induced response and its relation to the dosage applied

The pronounced morphological effects induced by 2,4-D at the rhizome apices seemed likely to prove useful in later investigations as a means of studying some of the factors affecting the translocation of the herbicide. An experiment

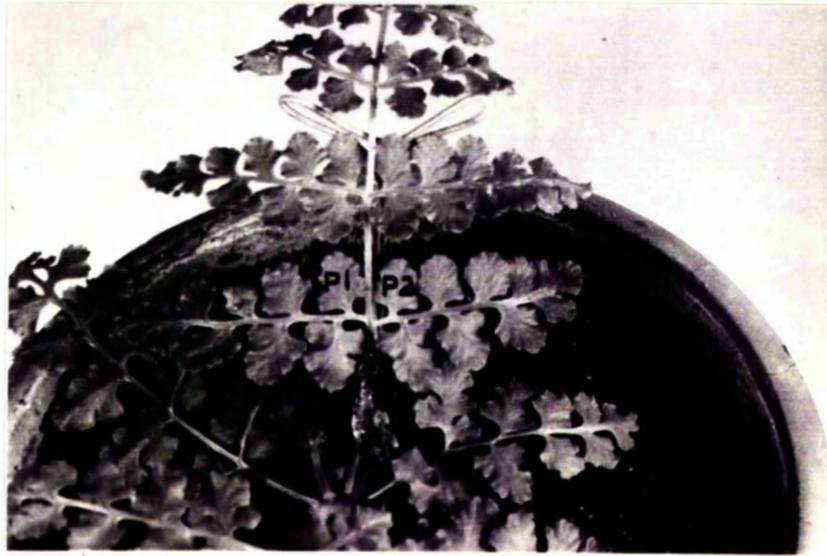


Fig. 28. Experiment 2. A sporeling frond which had been ringed by a brief exposure of a segment of the petiole to a jet of steam, illustrating the healthy appearance of the frond 18 hours after treatment. The killed region of the petiole is covered thickly with vaseline to prevent desiccation. P1, P2, pinnules to which the drops of 2,4-D solution were later applied. (x  $\frac{3}{4}$ )

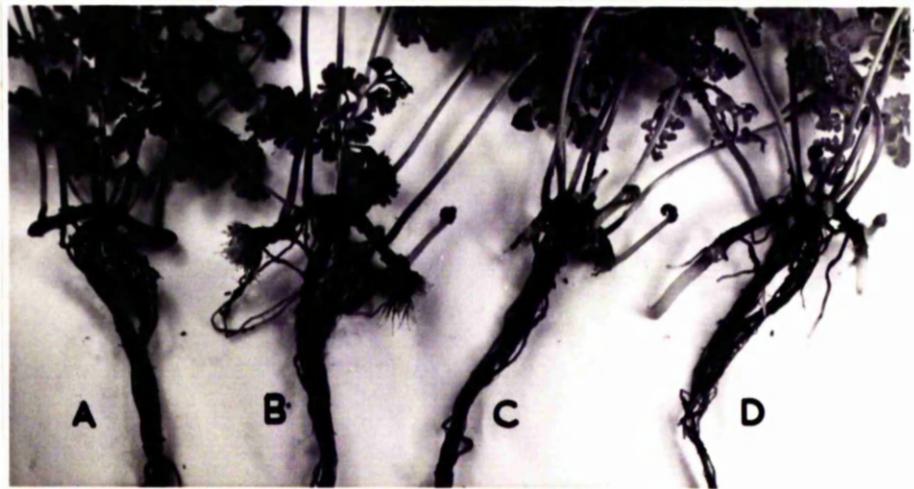


Fig. 29. Experiment 29. 2,4-D treated, unringed plants showing the effects induced at the rhizome apices (as described in the text). (x  $\frac{1}{2}$ )

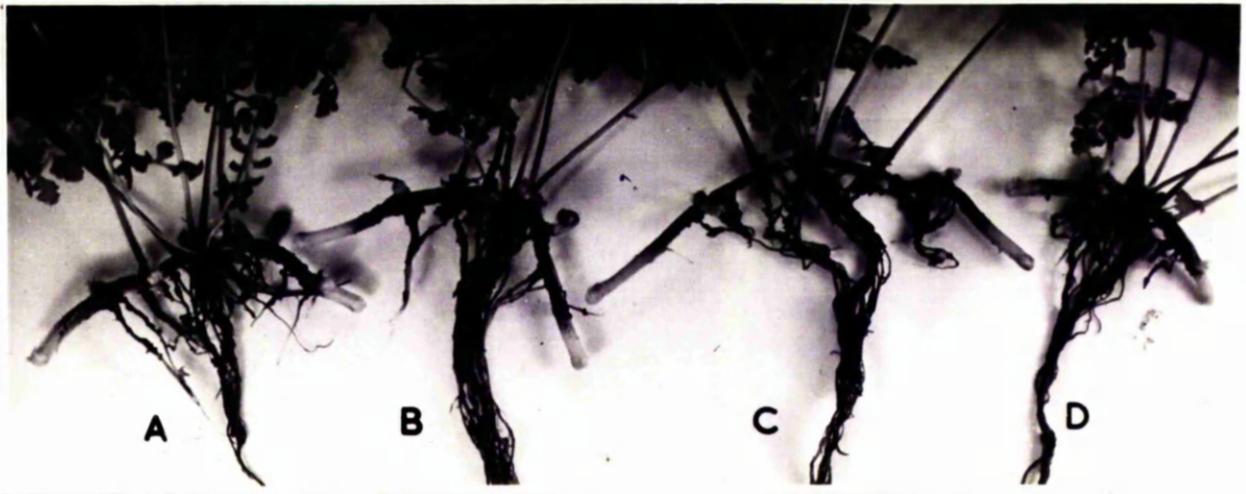


Fig. 30. Experiment 2. 2,4-D treated plants which had the treated frond ringed prior to the application of the herbicide. The rhizomes are well developed and of normal appearance. (x  $\frac{1}{2}$ )

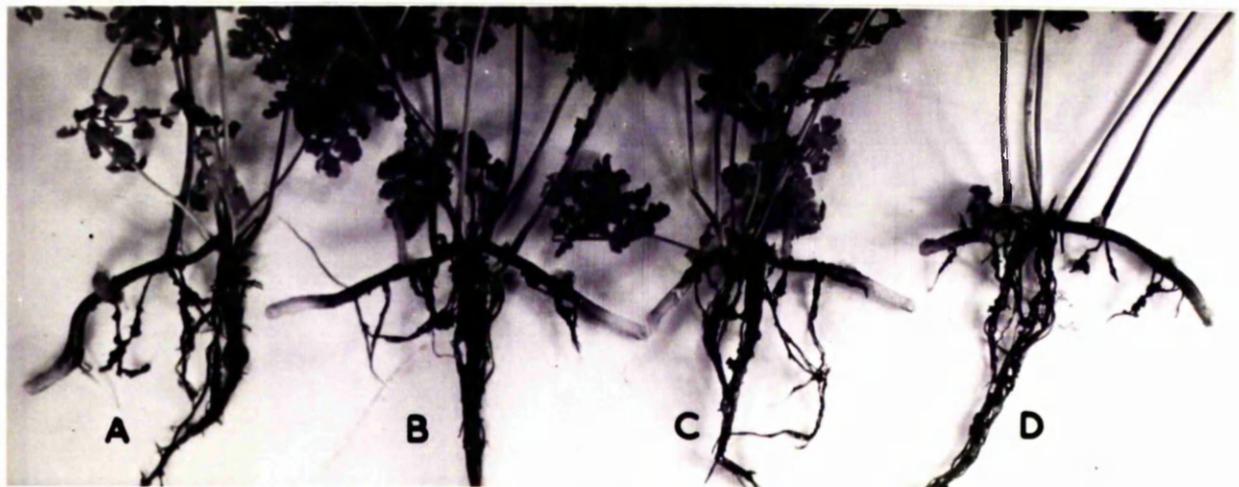


Fig. 31. Experiment 2. Plants which had one frond ringed but which received no herbicide treatment. With the exception of plant A (see text) all of the rhizomes are of normal appearance. (x  $\frac{1}{2}$ )

was therefore undertaken to examine these responses in greater detail and to relate the nature of the response to the dosage applied.

It was considered probable that the variation apparent in the results obtained in each of the previous experiments was due mainly to differences in the stage of development of the treated plants and, in particular, to small differences in the lengths of the rhizomes. Thus, in an attempt to obtain more uniform material for use in the present experiment a total of 150 sporelings were initially transplanted from spore cultures and were carefully selected for uniformity during their development. Of the twenty plants finally selected each had five fully expanded fronds and had produced two rhizome branches which were exactly equal in length. Although the total range of variation in rhizome length was from 0.3 - 1.2 cms. (mean =  $0.71 \pm 0.05$  cms.), the branches of fifteen of the twenty plants lay within the narrower range of 0.5 - 0.8 cms.

As in the previous experiment the plants had not been fully "hardened off" and were therefore retained in the humid propagation frames throughout the experiment.

Each plant was numbered and assigned at random to one of four groups. The 2,4-D solution was applied to the 4th. frond of each plant in three of the groups, the application being made as a 20  $\mu$ l. drop to each member of the first two

pairs of pinnae (Group 1); to each member of the first pair of pinnae (Group 2); and to one member only of the first pair of pinnae (Group 3). The amounts of 2,4-D applied were thus 40  $\mu$ gms., 20  $\mu$ gms. and 10  $\mu$ gms. respectively. In each case the droplet was placed on one of the basal pair of pinnules. The controls were each treated with a single drop of a 1% aqueous solution of carbowax.

The temperature within the frames when the herbicide was applied was 71<sup>o</sup>F and the relative humidity was 90%. During the following 9 hour period the temperature ranged from 68 - 74<sup>o</sup>F and the humidity from 89 - 93%. Under these conditions evaporation of the droplets was considerably retarded and was not complete until approximately 24 hours after treatment. By this time the leaf tissue under each drop showed signs of injury and after a further two days many of the treated pinnules were completely withered. To prevent these pinnules from falling on to and thus contaminating the soil all of them were removed 3 days after the treatments had been applied.

After 21 days the plants were removed from their pots and examined. In recording the nature and distribution of the effects on the rhizome the two branches of each plant were distinguished from one another by means of the terminology previously adopted for this purpose (Part II, Sect. 2), i.e. the rhizome produced on the same side of the primary

stem as the 2nd. and 4th. fronds is termed branch A, and the other rhizome, branch B. It will therefore be noted that branch A is borne on the same side of the primary stem as the treated frond while branch B is on the other side distant from it.

Two plants were selected from each experiment to indicate the nature of the dosage-response relationship and are shown in figs. 32 - 35. All of the five plants treated with 40  $\mu$ gms. of 2,4-D showed very severe morphological effects at the apices of both rhizome branches. The apical growth of both branches of one of these plants (1, fig. 32) had been completely inhibited, the two apices being considerably swollen, almost bulbous in shape, and dark green in colour. Examination under a hand lens showed that the outer tissues had been sloughed off and had partly disintegrated. The green coloration of the inner tissues thus exposed was due to the presence of chlorophyll in the tips of a large number of root primordia the growth of which had apparently been inhibited. Of the remaining four plants only branch A showed a similar complete inhibition of rhizome and root development, the apex of branch B being either wholly or partially obscured by a dense growth of short roots (2, fig. 32). Several of these branches exhibited signs of renewed apical growth.

Of the plants which had received 20  $\mu$ gms. of the herbicide

two had produced a dense cluster of roots on both branches (2, fig. 33). It was noteworthy that branch B of both of these plants had resumed normal apical growth, the region of root proliferation being located at a distance of approximately one centimetre behind the growing apex, while the apex of branch A was still apparently inactive and completely obscured by roots. Two of the other plants showed a restriction of root proliferation to branch A and no apparent effect on branch B (1, fig. 33). Both branches of the remaining plant in this treatment were of normal appearance.

The application of only 10  $\mu$ gms. of the herbicide resulted in the production of a small cluster of roots on branch A of four of the five plants so treated (1, 2, fig. 34). It was noted, firstly, that these roots were smaller in number and greater in length than those induced by larger dosages and, secondly, that the apices of the rhizomes were growing vigorously and were of normal appearance. No morphological responses could be detected on either branch of the remaining plant in this treatment.

All of the control plants, two of which are shown in fig. 35, had well developed rhizomes of normal appearance.

The inhibiting effect of 2,4-D on rhizome development is shown by the data in Table 6. It is clear that the inhibition of growth was greater in branch A and that the degree of inhibition of both branches increased with the dosage applied.

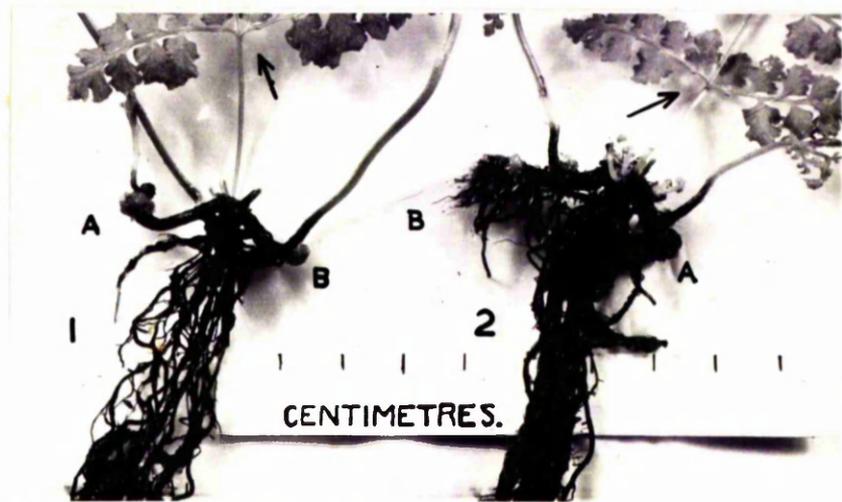


Fig. 32. Experiment 3. Sporelings which were treated with 40 µgm. 2,4-D showing the effects induced at the rhizome apices (described in text). Treated fronds arrowed. All of the other primary fronds have been removed.

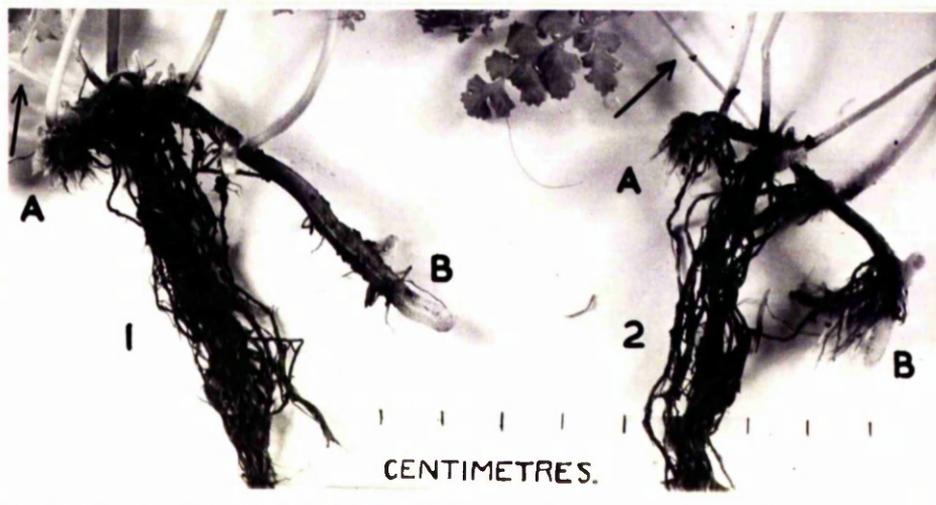


Fig. 33. Experiment 3. Sporelings which received 20 µgm. 2,4-D showing the effects induced at the rhizome apices (described in text). Petioles of treated fronds arrowed.

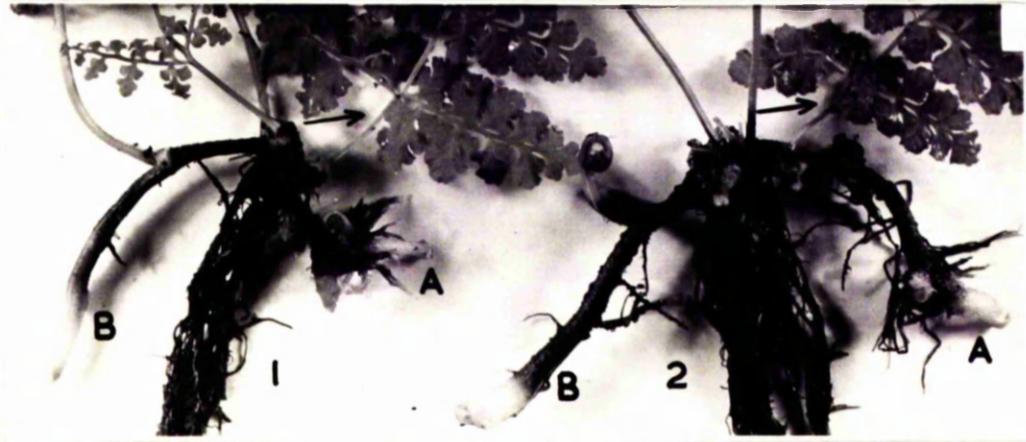


Fig. 34. Experiment 3. Sporelings which received 10  $\mu\text{gm}$ . 2,4-D showing effects induced at the rhizome apices (described in text). Treated fronds arrowed.  $(\times \frac{2}{3})$

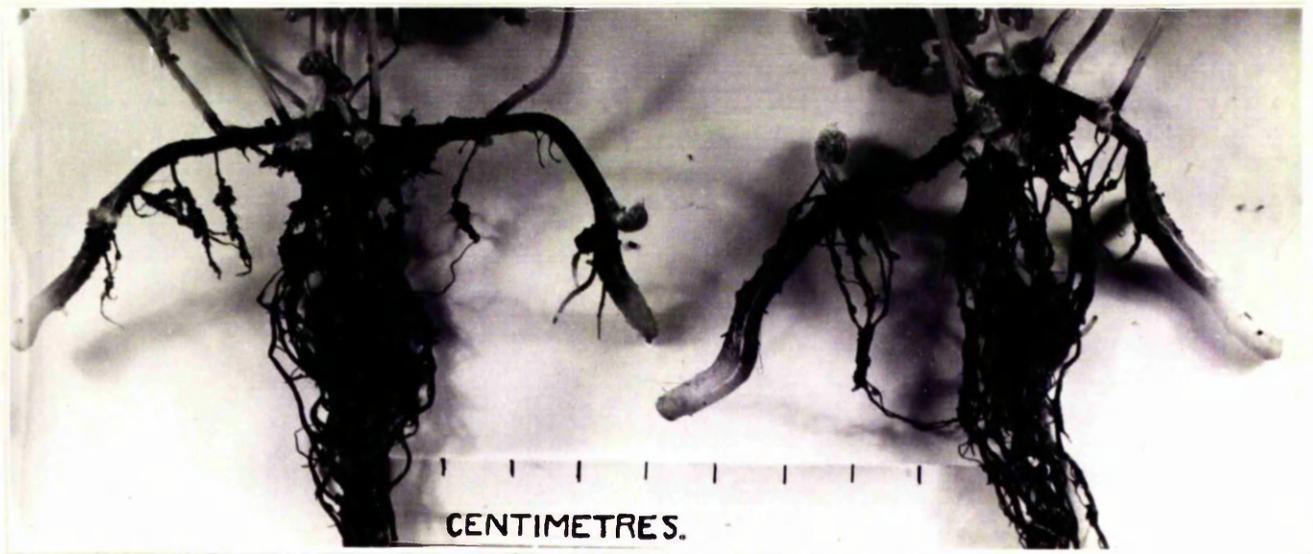


Fig. 35. Experiment 3. Untreated (control) plants with well developed rhizomes of normal appearance.

TABLE 6. The Effects of 2,4-D on the Rhizome of the Sporeling

Dosage μgms. 2,4-D	<u>Effect on growth of rhizome</u> Mean increase in length of rhizome branches (cms.) as percentage of control †		<u>Distribution of morphol- ogical effects</u> Number of plants showing effects on:-		
	Branch A	Branch B	Both branches	Branch A only	Neither branch
40	23.0 (1.14 ± 0.19)*	41.6 (1.98 ± 0.37)*	5	-	-
20	66.1 (3.28 ± 0.82)	95.5 (4.66 ± 0.47)	2	2	1
10	74.2 (3.68 ± 0.65)	97.5 (4.76 ± 0.43)	-	4	1
0	100 (4.96 ± 0.41)	100 (4.88 ± 1.00)	-	-	5

† The actual mean lengths and their standard errors are shown in brackets.

\* Significantly different,  $P = 0.01$ .

Although only 40  $\mu$ gms. of 2,4-D produced a statistically significant effect ( $P=0.01$ ) a comparison of these differences in rhizome development with the morphological observations shows them to be closely correlated with the observed differences in the rate of recovery of the affected apices.

#### 4. Anatomical Effects

Several of the severely affected apices from those plants which had been treated with 40  $\mu$ gms. of the herbicide in the previous experiment were selected for anatomical study. The material examined was obtained from the two plants shown in fig. 32 and consisted of the apical 3 mms. from both branches of plant 1 and a similar sample from branch A of plant 2. The external appearance of these apices has already been described. For the purpose of comparison apices were also fixed for sectioning from each branch of one of the control plants from the same experiment. The general procedure adopted in the fixing, dehydrating and staining of the material was the same as that previously described (Part II, Sect. 2, p.55 ). Serial transverse sections were cut at a thickness of 12  $\mu$  and, after staining, were mounted in euparal as permanent preparations.

A section taken from branch B of plant 1 (fig. 32) at a distance of 1.2 mm. behind the apex is shown in fig. 36 and has been selected to illustrate the more notable of the histological effects of the herbicide. This section may be

compared with the one shown in fig. 37 which was taken from a similar position at the rhizome apex of one of the control plants (fig. 35).

The most striking feature is the very large number of root primordia which have been produced around the outer edges of the vascular bundles and which are directly exposed at the surface of the rhizome as a result of the rupture and disintegration of the outer cortical tissues. So numerous were these induced roots that they formed an almost continuous sheath around the rhizome for a distance of 2.0 to 2.5 mm. from the apex. The tissues from which the roots had been initiated could not be determined with certainty but a study of sections immediately behind the apex where the roots, although equally as numerous as in the illustrated section, were at a somewhat earlier stage of development, suggested that they had arisen in either the pericycle or endodermis.

This remarkable stimulation of root formation, although clearly the most characteristic effect of the herbicide, was accompanied by a number of other well defined histological reactions. As can be observed from fig. 36 there is evidence of a pronounced radial elongation of the inner cortical cells, some of which also show a slight thickening of the cell wall. Abnormal cell enlargement is also apparent in the pericycle but in certain parts of this tissue the cells have remained relatively small and appear to have undergone some proliferation.

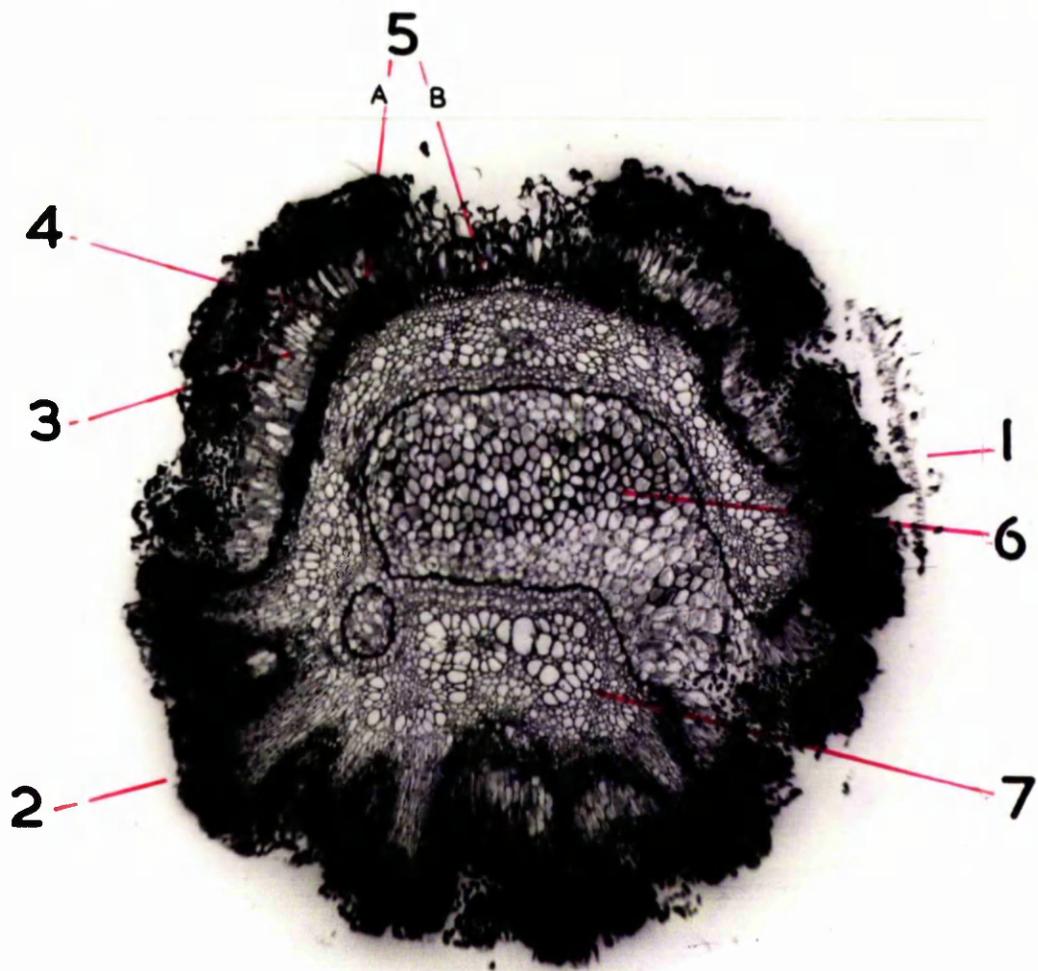


Fig. 36. Photomicrograph of a transverse section 1.2 mm. behind the apex of branch A of the sporeling (1) shown in fig. 32, illustrating the various histological effects of the herbicide. Dosage, 40  $\mu$ gm. 2,4-D; treatment period, 3 weeks.

1. Fragment of epidermis. Note that most of the epidermis and outer cortex has been sloughed off. 2. Root primordia forming an almost continuous sheath around the apex. 3. Radially elongated cells of inner cortex, some showing a slight thickening of the cell wall. 4. Endodermis. 5. Regions of cell enlargement (A) and proliferation (B) in the pericycle. 6. Sclerotic cells in central region of the pith. 7. Fully mature metaxylem of large vascular bundle. Note also the abundant deposition of tannin in the root primordia (2), endodermis (4) and pericycle (5) and, to a lesser extent, in the elongated inner cortical cells (3). (x 30)

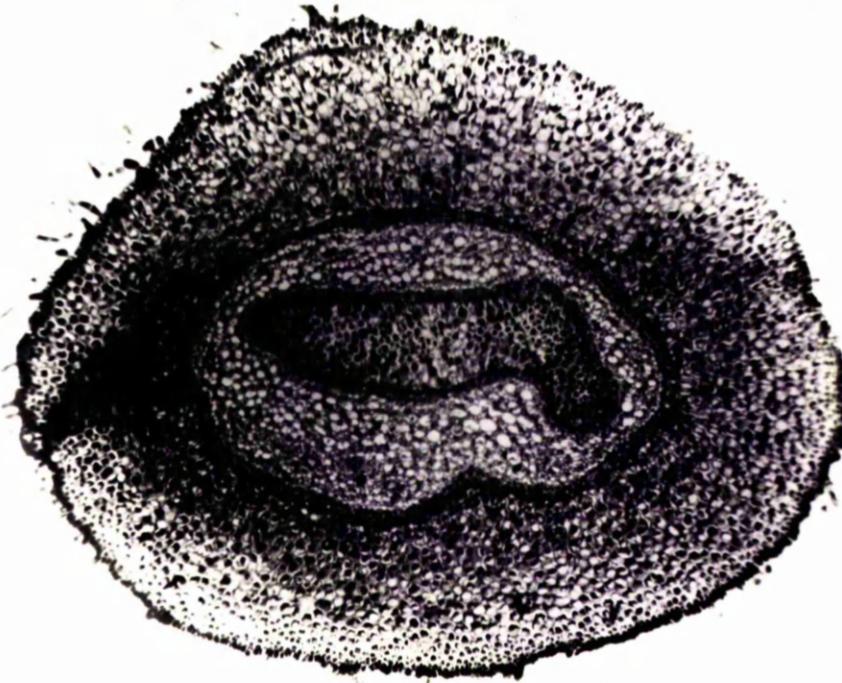


Fig. 37. Photomicrograph of a transverse section approximately 1.2 mm. behind the rhizome apex of one of the untreated plants shown in fig. 35 for comparison with the section in fig. 36. Note the relatively immature condition of the tissues as compared with those of the affected apex. (x 30)

In the central region of the pith the parenchymatous cells have become markedly sclerotic with thickened walls of a light brown colour which contrasted with the bright red of the lignified xylem elements and is suggestive of a suberin deposit. Finally, and particularly striking, is the extensive deposition of a red staining granular material which is assumed to be of a tanniniferous nature and which is present in great abundance in the outer, exposed tissues of the root primordia, in the endodermis and pericycle and, to a somewhat lesser extent, in the elongated cells of the inner cortex.

While these various effects are largely responsible for the very marked difference in the general appearance of the figured sections of the treated and control plants a comparison of these sections shows that they also differ considerably in the degree of maturation of the tissues. It was observed that all of the tissues in this region of the affected apex, apart from those of the root primordia, were fully mature while those of the control apex, with the exception of the protophloem fibres (which are not clearly visible in the figure) were in a relatively immature condition. It would seem that this difference can be attributed to the continued maturation of the tissues of the affected apex after further apical growth had been arrested by the herbicide.

Finally, there was evidence of a profound effect on the course of differentiation of the tissues in the apical region

and on the apical meristem itself. In the following serial sections towards the apex from the level of the illustrated section an interesting series of changes in the vascular morphology was observed. Firstly, the free ends of the large C-shaped meristele became united with the consequent formation of a typical solenostele. This was followed by a progressive diminution in the diameter of the pith which passed through a stage of which it was represented only by two or three sclerotic cells surrounded by the endodermis before finally fading out completely at a distance of approximately 0.2 mm. behind the apex. At this level the rhizome had become protostelic, the central region being occupied by a solid mass of rather weakly lignified scalariform tracheides surrounded by phloem and endodermis. Finally, as the extreme apex was approached, the lignified elements were gradually replaced by large thin walled parenchymatous cells. A careful examination of this apical parenchymatous tissue failed to reveal the presence of the rhizome apical cell or, indeed, any evidence of an organised apical meristem. Thus it appeared that the apical meristem had lost its characteristic structure and staining reaction and had been reduced to a parenchymatous condition.

It may be noted that a similar sequence of anatomical changes were observed in both of the other affected apices. In one of these, however, several sections from the extreme

tip were accidentally discarded and thus the fate of the apical meristem, in this instance, was not determined.

#### IV. Discussion

It is evident from the results of this investigation that 2,4-D, when applied to the frond of the bracken sporeling, can be translocated basipetally in amounts sufficient to cause severe injury to the young rhizomes. The fact that Stevens failed to obtain evidence of translocation when a similar method of application was employed suggests that the dosage applied, and which is described merely as "a single drop" of a 1000 p.p.m. solution, was not sufficient to produce a clearly defined morphological response at the rhizome apex. It is also possible, however, that the difference in the nature of the formulations may have been of some importance for there is evidence (Mullison, 1951; Crafts, 1956b) that the sodium salt of 2,4-D, the derivative used by Stevens, is less effective than the parent acid. Moreover, if Steven's formulation did not include an hygroscopic agent to retard the drying of the droplets then the low aqueous solubility of the salt may have seriously restricted the period available for the penetration of the herbicide. On the other hand, in the present investigation, it seems probable that, even after drying was complete, the 2,4-D continued to be absorbed from the residue of carbowax

deposited on the surface of the frond.

An interesting feature of the present investigation was the evident correlation between the distribution of the herbicide within the rhizomes, as indicated by the distribution of its morphological effects, and the position of the treated frond relative to the two branch apices. In the first two experiments when the 2,4-D was applied to the 5th. frond, which normally occurs in a position almost mid-way between the two rhizome branches, the morphological responses induced at each branch apex were of a similar nature. On the other hand, in Experiment 3, where the application was made to the 4th. frond, the herbicide had apparently been translocated in a relatively greater amount into the branch which had been produced almost directly above the insertion of the treated frond (i.e. branch A) than into the branch which had arisen on the opposite side of the primary stem (branch B). [These morphological relationships are clearly illustrated by the sporelings shown in figs. 8 and 9, Part II, p. - 7. In this experiment the morphological responses were found to be restricted to branch A when only 10  $\mu$ gms. of the herbicide were applied. Although some of the plants receiving 20  $\mu$ gms. and all receiving 40  $\mu$ gms. showed pronounced effects at both apices it was apparent that, when both apices were affected, the inhibition of growth and the morphological effects were relatively greater at the apex of

branch A. Thus it is probable that the relative amounts of 2,4-D translocated into each branch did not vary with the dosage applied but that the actual amount reaching the apex of branch B in those plants given only 10  $\mu$ gms. was insufficient to produce a visible response.

These observations are consistent with the widely held view that the transport of 2,4-D is associated with the movement of natural assimilates. The importance of the phyllotaxis in determining the pattern of assimilate translocation is well illustrated by the work of Jones et al. (1959) who showed that when  $C^{14}O_2$  was supplied to a single leaf of a tobacco plant labelled assimilates were preferentially transported into those leaves on the same side of the stem as the treated leaf. It was suggested that this restricted distribution could be attributed to the nature of the vascular connections. A similar correlation with the vascular supply is suggested by the present observations.

Of the various effects induced by the herbicide at the rhizome apices the induction of root formation was clearly the most characteristic. This root inducing property of 2,4-D was first demonstrated by the early work of Hitchcock and Zimmerman (1942) and since that time has been described by numerous investigators from experiments involving a wide range of flowering plants. Although the ferns have been relatively neglected Allsopp (1952) has studied the effects

2,4-D on the growth of Marsilea and was particularly impressed by the herbicides "remarkable capacity to induce root formation." In Allsopp's experiments the 2,4-D was added to the nutrient solution in which the roots and rhizomes were growing and could thus gain access directly to the responsive tissues. It was found that, under these conditions, an abundant proliferation of roots was induced in the rhizome and leaf bases when the 2,4-D was supplied at a concentration of only  $10^{-7}$  g/l. In the present investigation it was also apparent from a comparison of the plants of Experiment 3 that the number of roots induced increased with the dosage applied while their subsequent growth was correspondingly depressed. A similar relationship has previously been observed by other investigators in experiments with flowering plants (e.g. Hitchcock and Zimmerman, 1942; Audus, 1953).

It would seem, however, that the observed inhibition of rhizome development, although also showing a close correlation with the dosage applied, was not necessarily due directly to the action of the herbicide. The fact that this inhibition also showed a correlation with the rooting response suggests that it may have resulted, at least partly, from a failure of the apical meristem to compete successfully for food materials with the developing root primordia. A similar view was also expressed by Allsopp (loc. cit.) who observed

that, in Marsilea, the development in the rhizome of 2,4-D induced roots was associated with a marked reduction in the size and morphological complexity of the fronds. It was therefore suggested that the normal course of frond development at the rhizome apex may have been restricted by the "diversion of plastic materials from the developing leaves to the abnormally numerous root primordia."

Apart from the initiation of roots and the apparent proliferation of the cells in certain regions of the pericycle the several other histological abnormalities shown by the affected apices may perhaps be more correctly regarded as wound responses rather than as reactions induced directly by the action of the herbicide. For example, the abundant deposition of tannin in the remnants of the outer cortex, and in the endodermis and pericycle, and the marked radial elongation and thickened walls of the inner cortical cells, have all previously been described by Holden (1916) as responses typically induced by superficial injuries to the petiole of the bracken frond. Again, the sclerosis of the cells in the central region of the pith would seem to be a reaction of a similar nature to that observed by Bower (1911) in a specimen of Botrychium ternatum and also by Gwynne-Vaughan (1914) in Osmunda regalis. Both of the plants examined by these investigators were found to have thick-walled lignified elements of a tracheal nature scattered

irregularly throughout the parenchymatous tissue of the pith and, it is noteworthy, that in both instances there was evidence that these histological abnormalities had been induced by some form of traumatic injury which the plant had previously received.

Finally, with regard to the herbicidal effectiveness of the treatments applied, it may be noted that, in spite of the severe injuries produced in the rhizomes, all of the affected apices survived. Indeed, the apparently healthy condition of most of the inner tissues was a particularly remarkable feature in view of the almost complete destruction of the outer cortex and epidermis. Also noteworthy is the fact that, with the exception of a few of the most severely injured plants, the induced inhibition of rhizome development proved to be of only a temporary nature and was soon followed by a resumption of normal apical growth. On the other hand, the prolonged inhibition of growth and the pronounced and degenerative changes induced in the rhizome by 40  $\mu$ gms. of the herbicide suggest that, had the dosage been further increased, the effects may well have proved lethal. Clearly, however, no definite conclusion on this point can be reached without further investigations.

SECTION 2. THE EFFECT OF FOLIAR APPLICATIONS OF 2,4-D ON  
THE RHIZOME OF THE MATURE BRACKEN PLANT

Stephens (1953), in addition to the greenhouse experiments previously described, also reported on a preliminary field trial of the effects of various growth-regulating herbicides on mature bracken. The herbicides tested were 2,4-D, 2,4,5-T and MCPA. These compounds were used both as "pure chemicals" and in proprietary formulations and were applied "at the recommended rates and at twice these concentrations". The fronds were sprayed (a) when still at the crozier stage (late May) (b) when just fully expanded (late June) and (c) in late August when they had become fully mature. These treatments caused injury only to the fronds while "the subterranean parts of the plant, with the exception of an occasional frond bud near the soil surface, were apparently unaffected." In view of the results obtained in the greenhouse experiments described in the previous section it was considered desirable, however, that the effect of 2,4-D on mature bracken should receive further investigation. With this object a preliminary field experiment was carried out during the summer of 1957.

Observations on the seasonal activity of the rhizome apices

In discussing the factors which determine the effectiveness of 2,4-D when applied to the foliage of deep rooted

perennial weeds Crafts (1956b) pointed out that since this herbicide is considered to exert its toxic effects primarily on meristematic tissue it should be most effective if applied when the underground parts of the plant are in a state of active growth and thus in a physiologically responsive condition. This view was clearly consistent with the observed distribution of the morphological and anatomical effects induced by 2,4-D in the rhizome of the bracken sporeling for it was evident that these effects were largely confined to the meristematic and immature tissues at the rhizome apices. Thus, in order to ensure that the herbicide was applied at a time when the rhizome apices were active samples of rhizome were examined at intervals during the growing season and the onset and course of apical activity investigated.

The experimental site was at Shemore Farm, Luss, and was, in fact, the area from which samples were taken in the previous study of frond bud development (Part II, Sect. 3).

On each sampling occasion a number of fronds were selected at random and dug up together with the apical portion of the associated rhizome branch. Those apices completely covered in dark hairs were recorded as dormant while those on which any fresh white tomentum could be detected were taken as active. Where the frond was associated with a branching of the rhizome

the smaller of the two branches, usually represented by a small bud at the base of the frond, was ignored, only the condition of the main branch apex being recorded. To obtain a record of both the initial increase and subsequent decline of apical growth sampling was continued into the autumn, that is, after the period of maximum growth had been passed and after the herbicide had been applied.

The sampling dates and the proportion of active apices in each sample are shown in Table 7. In spite of the evident sampling variation these data demonstrate the general trend of apical activity within the rhizome system. Between early June and mid July there was apparently a progressive increase in the number of active apices and a high level of activity was then maintained at least until mid August; it then markedly declined and by mid September many of the apices had become dormant.

TABLE 7. Seasonal activity of rhizome apices

Date of sampling	29/5	7/6	23/6	27/6	13/7	19/7	16/8	27/8	2/9	12/9	20/9
Number of apices examined	15	20	20	20	20	30	40	40	40	35	30
Percentage active apices	0	30	25	75	75	93	82	50	48	14	13

While the accuracy of these observations is limited by

the somewhat subjective method employed in the assessment of apical growth and by the small number of apices examined on some of the sampling occasions it is of interest to note that they are in general agreement with the more precise quantitative data recorded by Watt (1940) in his Breckland studies.

#### Effect of herbicidal applications

When the rhizome samples collected on the 19th. July were examined and 93% of the apices were found to be in an active condition it was decided that the herbicide should be applied as soon as possible after that date. The onset of favourable weather conditions at the end of July enabled the applications to be made on the 1st. and 2nd. of August.

2,4-D solutions at concentrations of 500 p.p.m. and 1000 p.p.m. were formulated in the same manner as for the greenhouse experiments previously described except that the concentration of carbowax was increased to 2% (w/v).

Twenty-four fronds separated by several yards were carefully selected for uniformity in size and stage of development: they were approximately 5'6" in height and were just fully expanded. The apical 2-3 pairs of pinnae were still light green in colour and apparently immature. Separate lots of eight fronds each were treated with the 500 and the 1000 p.p.m. solutions while four others were sprayed with a 2% aqueous solution of carbowax, the remaining

four serving as controls. The solutions were applied by means of a small hand operated sprayer. To ensure that all parts of the frond were covered with the spray each pinna was sprayed separately. The total volume applied per frond was approximately 20 ml.

The herbicide was applied between 1.30 p.m. and 2 p.m. in bright sunshine. The shade temperature was 65°F and the relative humidity was 61%. During the four hours after application the temperature ranged from 63 - 69°F and the humidity from 56 - 62%. Examination of the fronds 30 minutes after treatment showed that the solution had evaporated leaving a shining film of carbowax on the leaf surface. The fine spell of weather continued for a further two weeks and no rain fell during this period.

Since there is considerable evidence in the literature that the penetration of 2,4-D into leaves is decreased if the solution dries rapidly on the leaf surface (Mitchell and Hamner, 1944; Hopp and Linder, 1946; Rice, 1948; Mitchell and Linder, 1957) it was decided to make a second application under conditions which would retard the rate of drying. Thus, on the following day a further twelve fronds were each sprayed with a 1000 p.p.m. solution but on this occasion the treatment was applied in the late evening. At the time of spraying the temperature was 65.5°F and the relative humidity was 86%. One hour after spraying the droplets of solution

had not diminished appreciably in volume. Heavy dew formation occurred at this time and it is probable that the herbicide remained in solution on the leaf surface throughout the night.

Five to six days after each application the fronds developed small necrotic areas at the tips of the pinnules and on the immature apical pinnae. The occurrence and extent of these injurious effects showed considerable variation from one frond to another and did not appear to be correlated with the dosage applied. Fronds treated with a carbowax solution only showed no injury.

Treated fronds were dug up at intervals of six, eight and ten weeks after treatment for examination. Irrespective of the sampling occasion all but two of the associated rhizome apices showed well marked though variable morphological responses. Those least affected showed only a slight swelling of the apical region. Hand sections revealed a zone of proliferating tissue around the outer edges of the vascular bundles and small clusters of root primordia in the inner cortex. The most severely affected apices (see figs. 38 and 39) were swollen and deeply fissured along the plane of the "lateral line". This longitudinal splitting of the epidermis and cortex was the most characteristic morphological effect and was accompanied in some instances by a necrosis of the cortical tissues in the vicinity of the wounds.

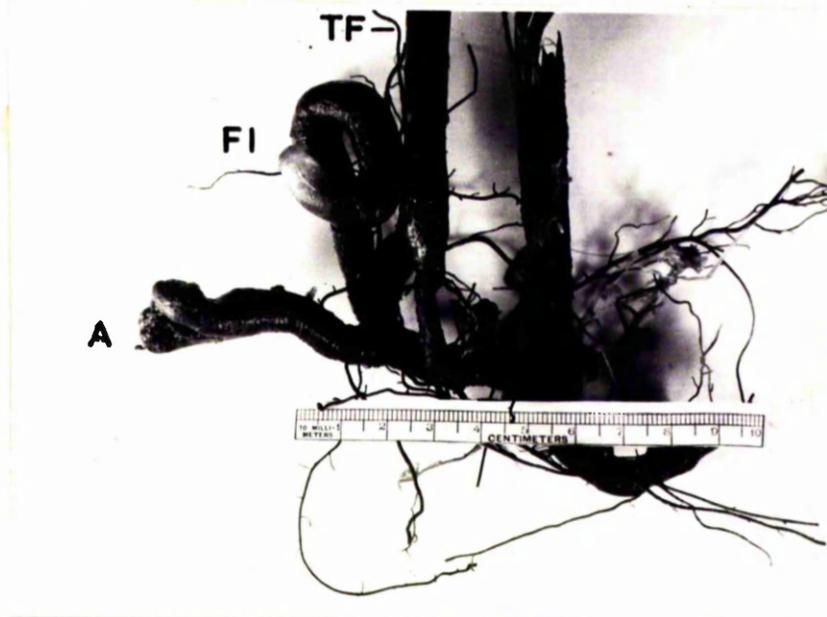


Fig. 38. The apical portion of a rhizome branch of the mature bracken plant showing the effects induced by the application of a 1000 p.p.m. solution of 2,4-D to the expanded frond (TF). The older frond bud (F1) is strongly contorted while the outer tissues of the rhizome apex (A) have been sloughed off exposing the tips of a large number of developing roots (these are just visible in the photograph).

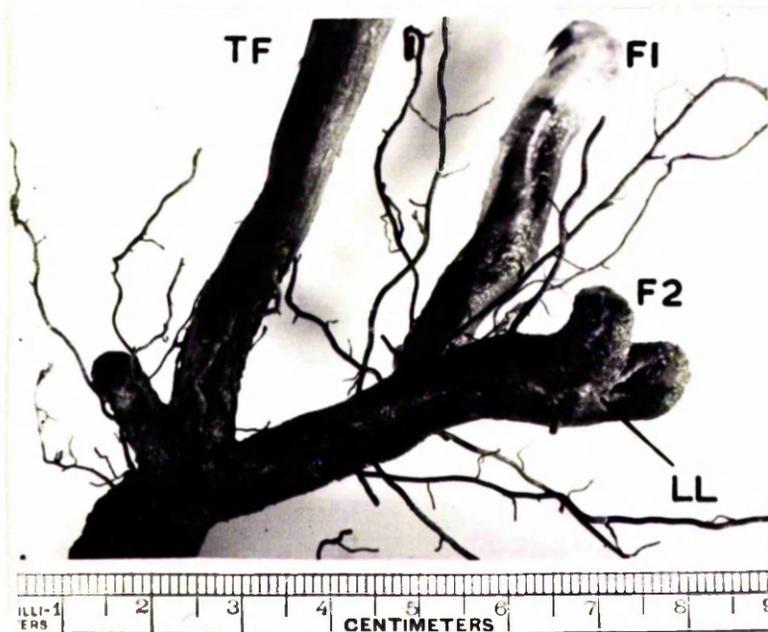


Fig. 39. The rhizome associated with a frond (TF) which received the same treatment as the one in fig. 38. The outer tissues at the apex have split open along the plane of the "lateral line" (LL). The frond buds (F1, F2) are apparently unaffected.

The extreme tips of three of the apices appeared to have been completely killed. Frond buds associated with the affected apices showed similar injurious effects and frequently were strongly contorted (fig. 38) but in some cases the rhizome apex was severely injured while the frond bud was of normal appearance (fig. 39). No evidence could be detected of any consistent difference in the degree of injury produced by different concentrations of the herbicide or by treatments applied under different environmental conditions.

An anatomical examination was made of the affected apex of the branch illustrated in fig. 39 together with the apex associated with one of the control fronds. The method employed in preparing the material for anatomical examination was the same as that previously described (p.55 ). The histological effects observed (compare figs. 40 and 41) were clearly of a similar nature to those produced in the rhizome of the young sporeling. Root primordia had been produced abundantly and much of the epidermis and outer cortex had disintegrated. There was also evidence that the presumptive xylem and xylem parenchyma cells had proliferated with a consequent enlargement of the vascular bundles. The large apical cell of the branch and its early derivatives had retained their characteristic structure and staining reaction but the meristematic tissue in the immediate

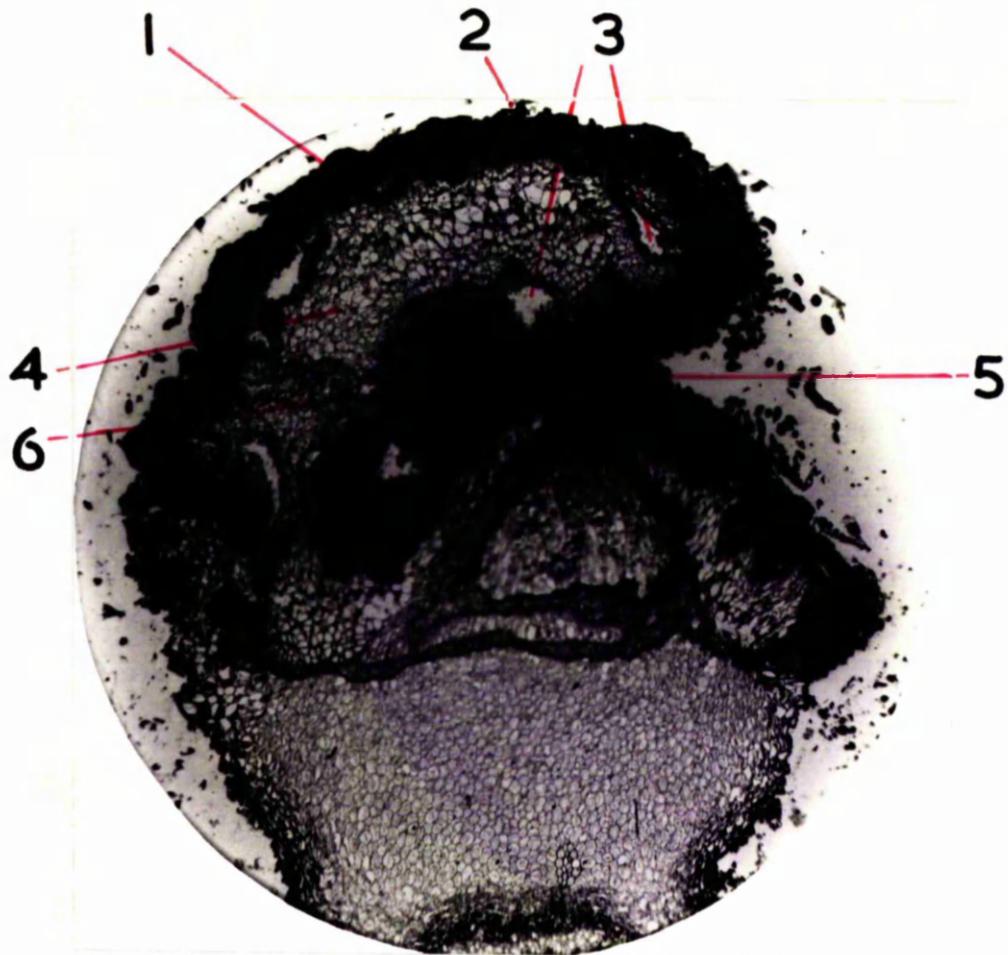


Fig. 40. A transverse section of the apex of the rhizome branch shown in fig. 39 illustrating the histological effects induced by the foliar application of a 1000 ppm solution of 2,4-D. 1. Root primordia produced around the outer edges of the vascular bundles and covered only by the persistent remnants of the outer cortical tissues (2). 3. Rupturing of the inner tissues at a number of points. 4. Large vascular bundle showing evidence of proliferation of the xylem and xylem parenchyma. 5. Rhizome apical meristem surrounded by proliferating tissue (6) containing a large number of root initials (these are not clearly visible in the photograph).

This section should be compared with the one shown in Fig. 41. (x 15)

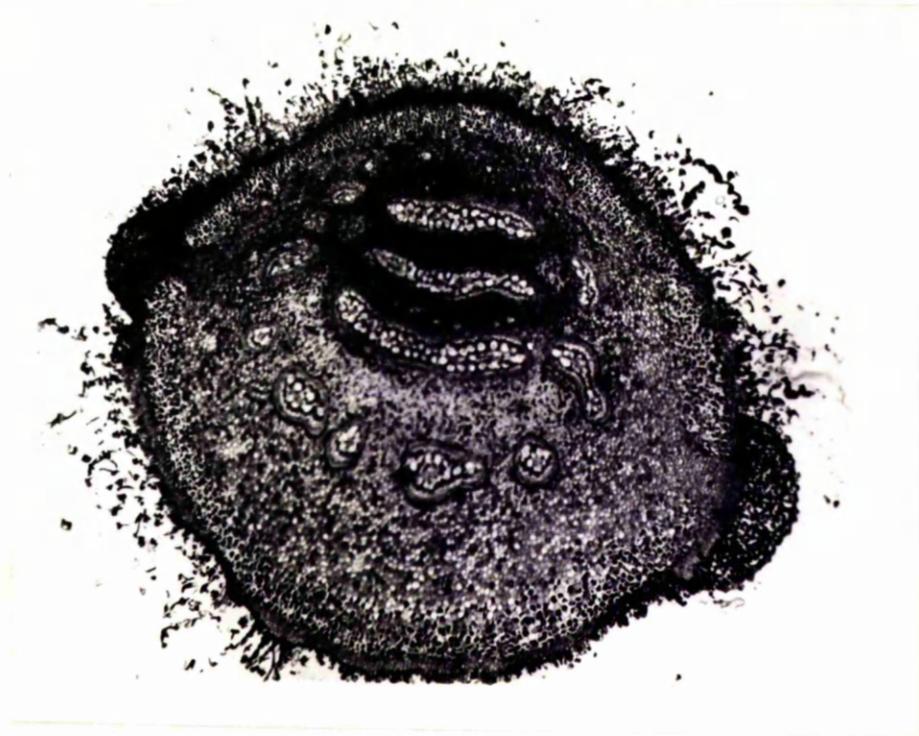


Fig. 41. A transverse section of a rhizome branch bearing an untreated frond for comparison with the section shown in fig. 40. (x 15)

vicinity of the apex had proliferated extensively and was found to contain large numbers of root initials (these initials are somewhat obscured in the illustrated section). A rupturing of the tissues was also apparent at a number of points but it is possible that this feature may be an artefact resulting from incomplete infiltration of the rather bulky material.

### Conclusion

There is evidence from the results of this investigation that 2,4-D is capable of being extensively translocated in the mature bracken plant and that, when applied to the fronds, may enter the rhizome in amounts sufficient to produce injurious effects on the meristematic and immature tissues at the rhizome apices.

SECTION 3. PRELIMINARY INVESTIGATIONS WITH 2,4-D LABELLED  
WITH RADIOACTIVE CARBON (C<sup>14</sup>)

The results described in the previous section suggested the need for further more extensive field trials with 2,4-D and other growth-regulating herbicides. It was also evident, however, that the potential value of these compounds as a means of bracken control could not be critically assessed without some knowledge of the various factors affecting their penetration into the fronds and their subsequent translocation within the plant. The availability of 2,4-D labelled in the carboxyl group with C<sup>14</sup> seemed to provide a promising experimental approach to a study of these problems and thus, in the autumn of 1958, some preliminary investigations with this labelled material were initiated.

The technique of autoradiography employed in this work was based on the methods outlined by Crafts (1956a) and recently described in greater detail by Yamaguchi and Crafts (1958). The nature of the bracken plant, however, and the fact that much of the work was carried out in the field, presented a number of difficulties and it was found necessary to devote a considerable time to problems of technique. Indeed, the main object of this preliminary work was to assess the technical problems which were obviously involved and to establish methods which could be used in later investigations.

Thus, in the following account of the experimental work a detailed description is given of the methods employed. Of the two experiments which are described, the first was carried out in the greenhouse and was concerned with a comparison of 2,4-D translocation in sporeling fronds of different stages of development. This was followed by a more extensive investigation in the field designed to determine the effect of the "stage of growth" on the translocation of the herbicide.

#### A. Translocation in the Sporeling

As previously remarked, there is considerable evidence in the literature that the translocation of 2,4-D occurs in association with the movement of food materials. It might therefore be expected that changes in the pattern of assimilate translocation in the developing bracken frond should be reflected in correlated changes in the direction of translocation within the frond of externally applied 2,4-D. A small greenhouse experiment was therefore designed to enable the translocation of the herbicide to be studied in relation to the stage of development of the frond.

#### Method

Eight young sporelings, approximately 4 months old, were selected for the experiment. Each plant had 6 fully expanded fronds and several younger fronds at various stages of

development. Eight fronds, each borne on a separate plant, were selected for treatment, and were arranged in pairs, each pair representing one of the following stages of development:-

- |          |      |  |
|----------|------|--|
| Stage 1. | 1st. | pair of pinnae fully expanded  |
| "        | 2.   | 2nd. " " " "   |
| "        | 3.   | 3rd. " " " "   |
| "        | 4.   | Frond almost fully expanded (several pairs of small pinnae at the frond apex were still only partly unfolded). |

2,4-D, with a specific activity of 15 mc./mM. was formulated as a 1000 p.p.m. solution in 50% alcohol containing 1% carbowax 1500. By means of an "Agla" micrometer pipette 10  $\mu$ l. (i.e. 10  $\mu$ gms. 2,4-D) of this solution was applied as two 5  $\mu$ l. drops to one frond of each of the four pairs, one drop being placed on each of the two basal pinnules of one of the 1st. pair of pinnae. The remaining four fronds were treated on the mid point of the petiole with a single 5  $\mu$ l. droplet of solution which was placed on a small V-shaped piece of aluminium foil retained in position against the petiole by means of a thick band of lanoline. When this application was made it was observed that some of the solution moved rapidly up the median groove which is present on the dorsal face of the petiole. Inspection with a hand lens showed, however, that the solution was carried upwards for

a distance no greater than 2 - 3 mm. No downward leakage could be detected.

During the experiment the plants were housed in a covered glass frame and were thus maintained under conditions of constantly high humidity. The temperature ranged from 61° - 80°F during the following 24 hours. Owing to the high humidity the droplets of solution evaporated very slowly and a film of moisture was still visible on the treated areas 5 hours after application. The treated pinnules of the fronds at the 1st. two stages of development showed signs of slight vein and marginal necrosis within three hours and were completely necrotic after 24 hours. The more mature pinnules of the two older fronds were relatively less affected and developed no visible symptoms of injury until 12 - 18 hours after treatment. Petiole applications resulted in only a slight discoloration of the treated parts.

After 24 hours the treated parts of the frond were covered with small pieces of linen masking tape and the plants were then removed from their pots for drying. To prevent the fronds from curling up during the drying process each frond, while still attached to the plant, was laid between two sheets of filter paper and then enclosed in a small press constructed from two sheets of perforated aluminium. Pressure was applied by means of large paper clips fastened around the edges of the press. As each frond was thus secured it was rapidly frozen

by covering the press with pulverised "dry ice" (solid  $\text{CO}_2$ ) and was then severed from the plant at its junction with the rhizome.

The eight presses, each containing a single frond, were immediately transferred to a pre-cooled steel vacuum chamber containing calcium hydride to act as a desiccant and the chamber was then placed in the freezing compartment of a refrigerator where a temperature of approximately  $-15^\circ\text{C}$  was maintained. A vacuum pump attached to the chamber was switched on and allowed to run continuously for 3 days. After 48 hours the pressure within the chamber had fallen to approximately 0.05 mm. of Hg. and remained close to this level for the remainder of the drying period.

The dried fronds were mounted on sheets of lithograph paper with rubber adhesive and were covered with a layer of plastic ("Terylene") film. This plastic covering, 6 microns in thickness, and known to absorb approximately 22% of the  $\text{C}^{14}$  radiation, ensured that the film to which the mounted specimens were subsequently exposed was effectively isolated from any residual moisture or plant constituents which might otherwise have produced a visible effect on the emulsion.

After the mounted fronds had been pressed in an ordinary plant press for 24 hours they were transferred to the dark-room where each was placed in contact with a sheet of Kodirex No-screen X-Ray film for exposure. In the resulting stack of

films and plants the films were each backed by a sheet of  $\frac{1}{4}$  inch plywood while each mounted specimen was backed by a sheet of polyurethane "foam". This arrangement ensured that when the bundle was then strapped up tightly each film made intimate contact with all parts of the superimposed frond. The bundle was wrapped in a sheet of black plastic and placed in a light proof box for an exposure period of three weeks. The films were developed in Ilford D. 19B developer and fixed, washed and dried according to standard procedure.

#### Experimental Results

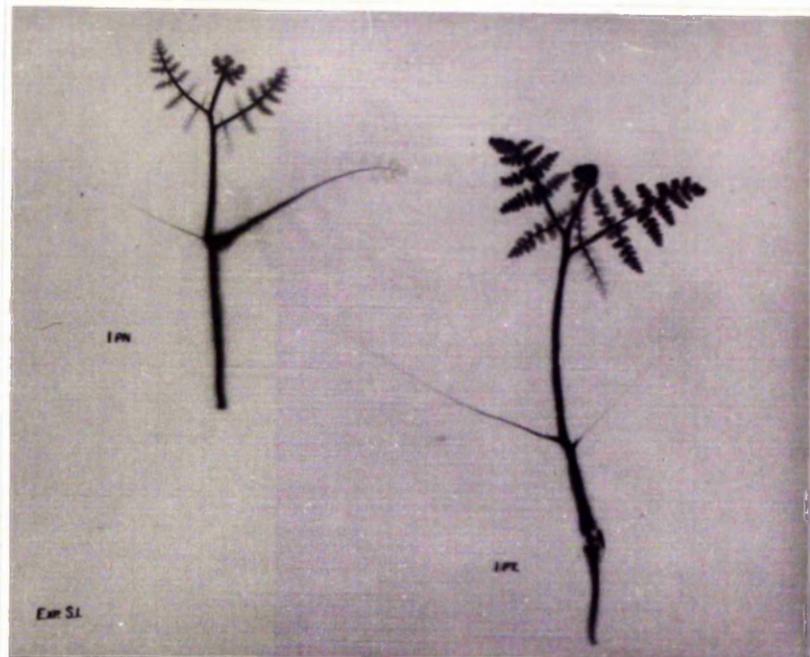
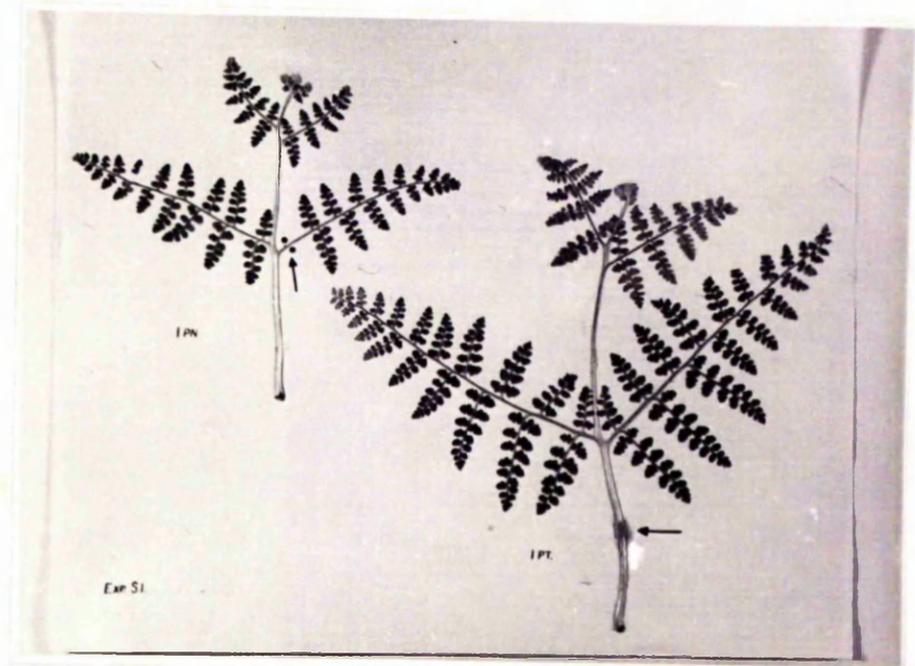
Inspection of the autoradiograms provided convincing evidence that the translocation of the 2,4-D had occurred in association with natural assimilates. The tracer had moved upwards from both sites of application into young expanding pinnae and downwards to the base of the petiole while the relative amounts which had been translocated in each direction showed a well defined correlation with the stage of development of the frond. From the pinnule applications considerable upward movement occurred at the earliest stage of development (fig. 42A) but basipetal transport had clearly become predominant by the time the 3rd. pair of pinnae were fully expanded (fig. 44A) and, finally, in the almost fully expanded frond (fig. 45A) upward translocation could be detected for a distance of only a few millimetres above the

level of insertion of the treated pinna.

When the 2,4-D was applied to the petiole it was transported upwards to the growing regions of the frond at all four stages of development and apparently in considerably larger amounts than from the pinnule applications. Nevertheless there was again evidence of a marked reduction in upward movement as the frond approached maturity (fig. 44B) and by the time expansion of the apical pinnae was almost complete transport had become predominantly basipetal (fig. 45B).

That the translocation of the herbicide from both sites of application had occurred mainly or entirely in the phloem was particularly evident from the almost complete absence of radioactivity in those parts of the frond which were fully expanded when the treatment was applied.

Also of interest is the observation that, at all four stages of development the 2,4-D was translocated down to the base of the petiole when applied to the pinna and upward into the apical growing regions when applied to the petiole. If it be assumed that translocation from both sites of application occurred in association with the transport of food materials then these observations suggest that assimilates produced in the basal expanded pinnae may be translocated out of the frond while the young developing pinnae are still



A

B

Fig. 42. Sporeling fronds treated with radioactive 2,4-D (concentration, 750 p.p.m.; specific activity, 15 mg./mM.) when they had only one pair of pinnae fully expanded.

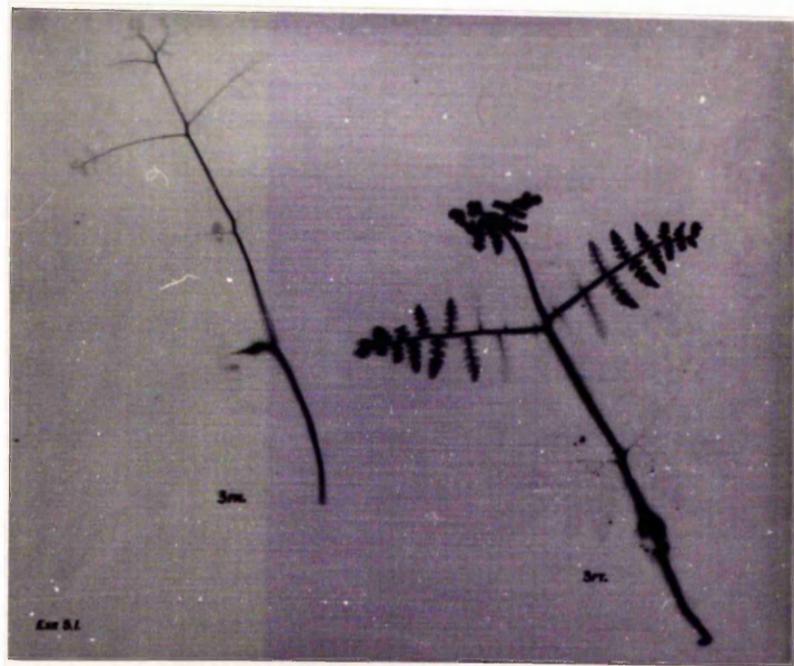
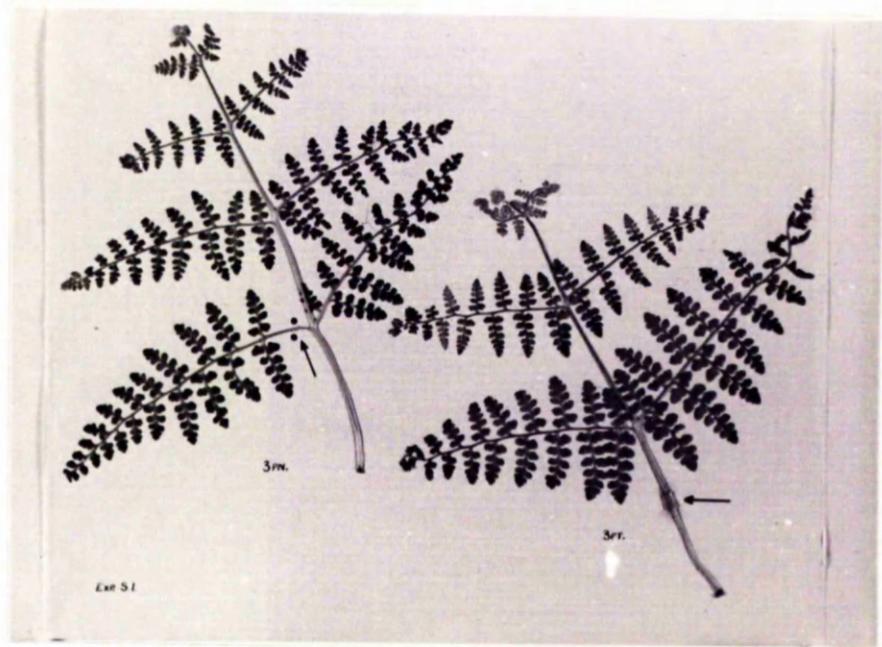
Above, treated fronds; below, autoradiograms.

A, pinnule application : Two 5  $\mu$ l. drops (i.e. 10  $\mu$ gm. 2,4-D)

B, petiole application : One 5  $\mu$ l. drop (i.e. 5  $\mu$ gm. 2,4-D)

The points of application are indicated by arrows.

(x  $\frac{1}{3}$ )



A

B

Fig. 43. Fronds treated as described under fig. 42 when they had two pairs of pinnae fully expanded. Above, treated fronds; below, autoradiograms. A, pinnule application; B, petiole application.  
 (x  $\frac{1}{3}$ )

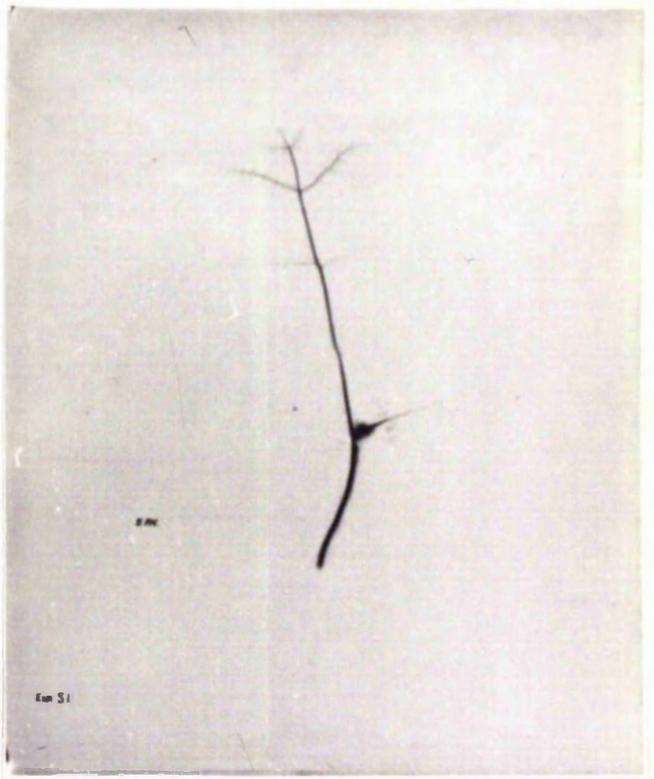
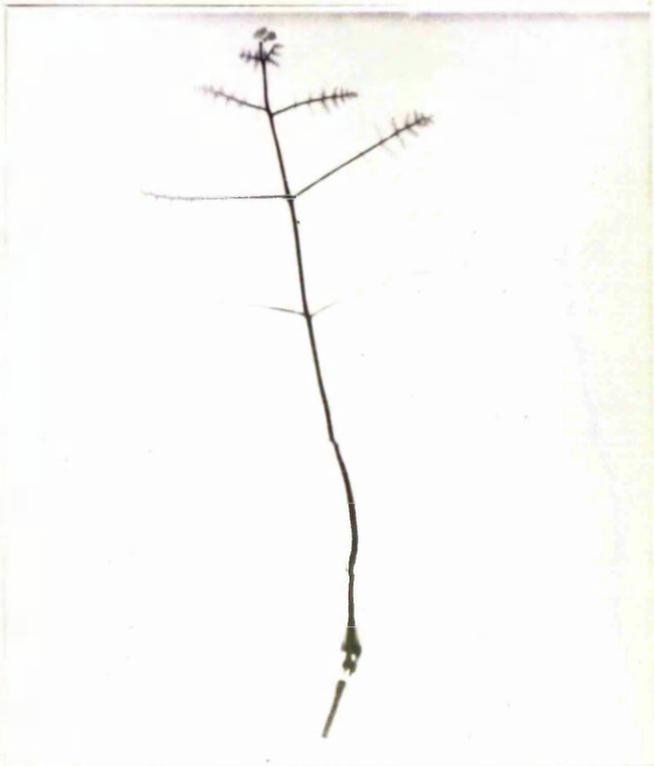
**A****B**

Fig. 44. Fronds treated as described under fig. 42 when they had three pairs of pinnae fully expanded. Left, treated fronds; right, autoradiograms. A, pinnule application; B, petiole application.

(x  $\frac{1}{3}$ )

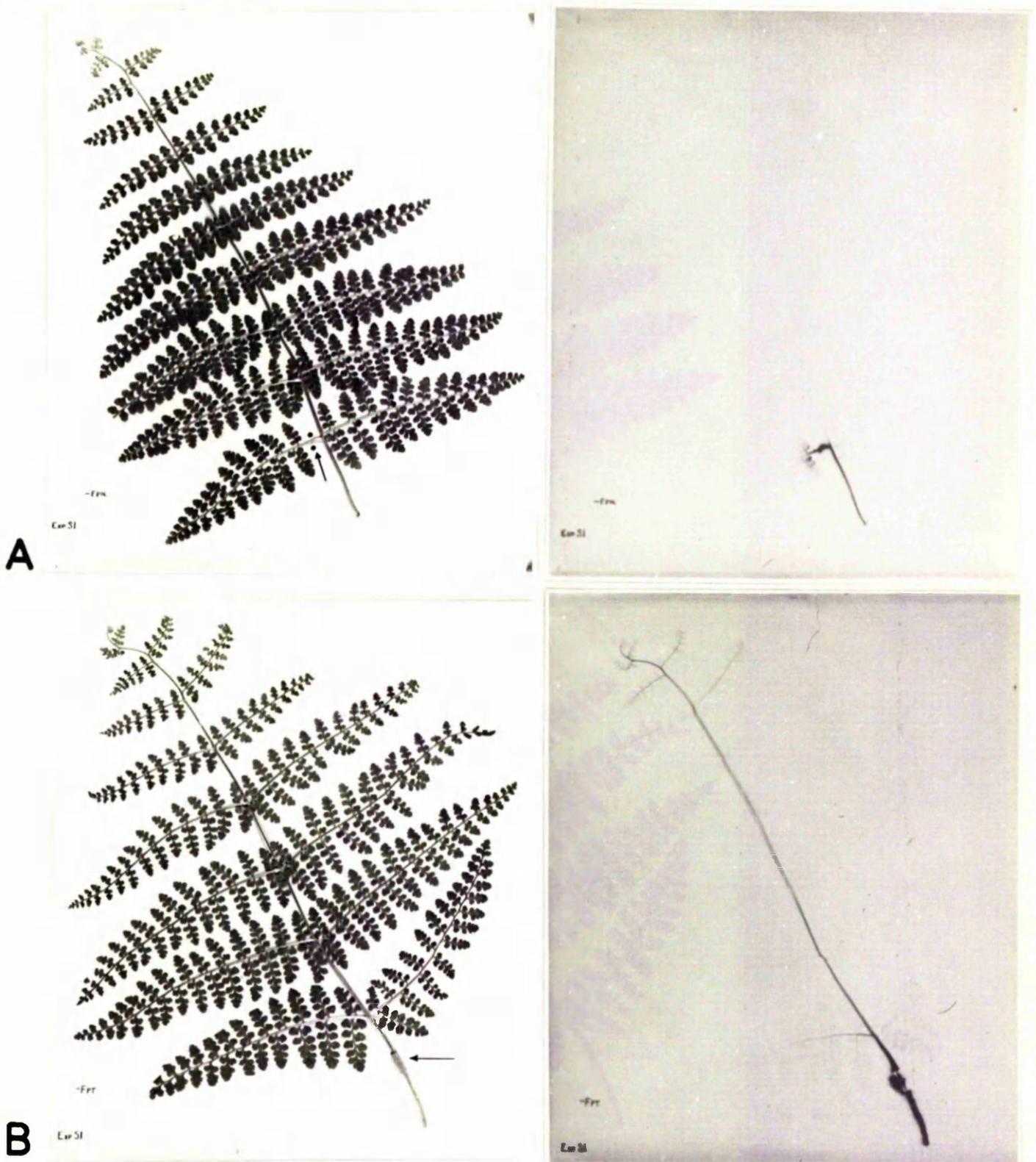


Fig. 45. Fronds treated as described under fig. 42 when they were almost fully expanded.  
 Left, treated frond; right, autoradiograms.  
 A, pinnule application; B, petiole application.

(x  $\frac{1}{3}$ )

receiving food materials from the rhizome and probably from older fully expanded fronds. This conclusion is well supported by the work of Jones, Martin and Porter (1959) who have shown, in experiments with tobacco plants, that the developing leaf passes through a stage at which the export and import of food materials proceed simultaneously.

Finally, it may be noted that since no attempt was made in this investigation to extract and identify the radioactive material present in the fronds it cannot be assumed that all of this material was present as unchanged 2,4-D. There is now considerable evidence in the literature (reviewed by Hay and Thimann, 1956) that 2,4-D is readily broken down in plants and that the carboxyl carbon may become incorporated in various plant constituents. Nevertheless, in view of the short duration of the present experiment, it seems unlikely that more than a small proportion of the 2,4-D was metabolised and it is therefore probable that the autoradiograms do, in fact, present a true picture of the pattern of translocation of the applied herbicide.

#### B. Translocation in the Mature Bracken Plant

Field studies by Crafts (1956b) and Leonard and Crafts (1956) on the translocation of  $C^{14}$ -labelled 2,4-D in a number of deep rooted perennial weeds showed that when the herbicide was applied to the leaves the extent of its

downward movement into the roots and rhizomes was determined largely by the stage of development of the plant at the time of application. The observed differences in the amount and direction of translocation were mainly attributed to differences in the age of the leaves and to the changing patterns of movement of food materials. Since it seemed probable that similar factors would be of importance in determining the effectiveness of 2,4-D when applied to the fronds of the mature bracken plant a preliminary investigation was carried out in the summer of 1959 with the object of studying the translocation of the labelled herbicide in relation to the stage of development of the plant.

Shortly after this work had been completed it was discovered that the stock solution of 2,4-D, purchased from the Amersham Radiochemical Centre, contained a considerable amount of a radio-active impurity. Chromatography of the solution showed that, in addition to the 2,4-D, with an Rf of 0.7, there was also present a substance which had an Rf of 0.95 and which was found to contribute almost 40% of the total radioactivity. A subsequent analysis by the suppliers of their own stock solution of the labelled material also revealed the presence of this impurity, but to the extent of only 10% of the activity of the solution. Thus it appeared that the reaction or decomposition resulting in the formation of the contaminating substance had been accelerated after the

sample had been received in June and that the relative amount of the contaminant had increased progressively during the period of the experiment (June - September).

Although the impurity has not yet been identified chemically preliminary investigations<sup>(1)</sup> have shown that when it is eluted from the chromatogram and hydrolysed with aqueous alcoholic sodium hydroxide a substance having the same Rf as 2,4-D is obtained. Thus, there is some evidence that the impurity is a closely related derivative of 2,4-D and it is therefore possible, particularly in view of the qualitative nature of the method employed in the present investigation, that the presence of this impurity may have had no appreciable effect on the results obtained. However, while it is proposed to make this assumption in describing the experiment and in the discussion of the results it is clear that the validity of these results must remain in doubt unless confirmed by further investigations with labelled material of known purity.

#### Experimental Methods

Some preliminary investigations were carried out during the autumn of 1958 with the object of determining (a) an

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(1) These investigations were conducted by R.G. Powell of the A.R.C. Unit of Experimental Agronomy, to whom I am indebted for this assistance.

efficient method of protecting from rain those parts of the frond to which the labelled herbicide had been applied (b) the approximate amount of the radioactive material (in micro curies) which would require to be applied to a single mature frond to produce a "positive" autoradiogram of sections taken from parts of the associated rhizome branch, and (c) a suitable method of sampling and of preparing autoradiograms. The results of this preliminary work are included in the account of the methods employed in the experiment of the following summer.

The main experiment and the preliminary investigations were carried out on a small area of bracken in Bagley Wood, near Oxford. Although restricted by surrounding trees to an area of rather less than an acre the bracken was very vigorous, the fronds ranging from 4 - 6 feet in height with an average density of 25 - 30 per square yard.

Preliminary investigations had shown that the dimensions of the petiole of the frond were closely proportional to those of the frond as a whole and since the procedure adopted in the sampling and autoradiography of treated fronds made uniformity in the size of the petiole particularly desirable this feature was used as the main criterion in the selection of suitable fronds. The diameter of the petioles of all selected fronds varied only from 7 - 7.5 mm. (the measurement being taken at the mid point of the petiole) and their length

(excluding the underground portion) ranged from 56 - 61 cms. The exact stage of development of the fronds was recorded in diagrammatic drawings prepared in the field. In these diagrams, one of which is illustrated in fig. 47, curved lines were used to denote those pinnae and pinnules which were only partly expanded. The degree of curvature of the lines was approximately proportional to the degree of expansion of the part represented. As will be described later, these drawings were also used to record those parts of the frond which were to be sampled for autoradiography. Fronds required for treatments to be applied later in the season were selected as soon as they had become fully expanded and were labelled with the date on which they had attained this stage of development. Thus their exact age could later be determined.

During the summer of 1959 many fronds were found to have been damaged by insects. Such damage (probably caused mainly by the larvae of the Ghost Moth, Hepialus sp.) was particularly common close to the base of the frond and it was therefore necessary to remove the soil from around the petiole of each selected frond to make certain that the basal region was uninjured. Despite this precaution several of the fronds used in the experiment were found, on subsequent examination in the laboratory, to have suffered a certain amount of damage close to their junction with the rhizome.

However, a comparison of the autoradiograms subsequently obtained from these fronds with those from undamaged fronds which had received the same treatment suggested that the injury had produced little or no effect on the translocation of the herbicide.

It was clearly essential that those parts of the frond to which the radioactive solution was to be applied should be well protected from rain. Although efficient protection could have been provided by enclosing either the whole frond or the treated part in a covering of a transparent material it was evident that any such arrangement would have had a very marked effect on the ambient condition of temperature and humidity. This difficulty was overcome by the method illustrated in fig. 46. A piece of glass 12 inches square was held at a distance of about one inch above the treated pinna by means of clamps attached to garden canes pushed firmly into the ground on either side of the frond. A loose fitting cork collar placed around the rachis of the frond was wired to a "bulldog" paper clip fastened to the edge of the glass to prevent the frond from being moved about by the wind and thereby displacing the treated pinna from its protected position. Additional support and stability was provided by a third cane fastened to the other side of the glass. Rain drops which tended to accumulate around the edges of the glass were prevented from running across the



Fig. 46. Illustrating the method employed in protecting those parts of the frond to which the radioactive herbicide was applied. A full description of the method is given in the text.

(x  $\frac{1}{6}$  approx.)

under surface and dripping on to the treated pinnules by the marginal application of a thin film of vaseline. The herbicide was always applied to the third and fourth pairs of pinnules and since these parts of the pinna lay directly under the centre of the glass they were afforded maximum protection. This protective device was thoroughly tested during the very wet and stormy autumn of 1958 and was found to be entirely satisfactory.

Preliminary investigations showed that when approximately 5.0  $\mu\text{c}$ . of radioactive 2,4-D were applied to one of the apical pinnae of a single fully mature frond the presence of the tracer could be detected in autoradiograms of sections taken from the associated rhizome branch at a distance of approximately 150 cms. from the treated part of the frond. Other tests in which quantities ranging from 0.1 - 0.5  $\mu\text{c}$ . were applied gave evidence of translocation down the mid-rib of the treated pinna for a distance of only 3 - 10 cms. With these relatively small applications none of the tracer could be detected in any part of the rachis, petiole or rhizome. On the basis of these preliminary results it was decided that in the main experiment a quantity somewhat in excess of 5.0  $\mu\text{c}$ . should be applied so that satisfactory autoradiograms would be obtained.

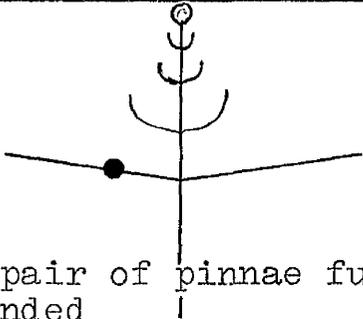
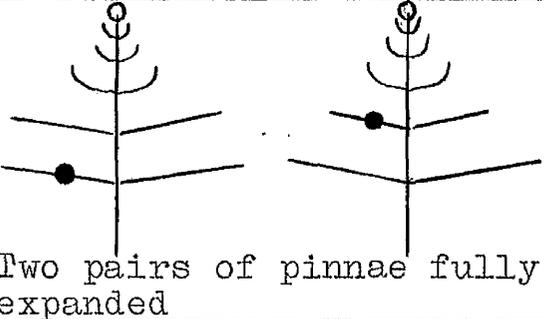
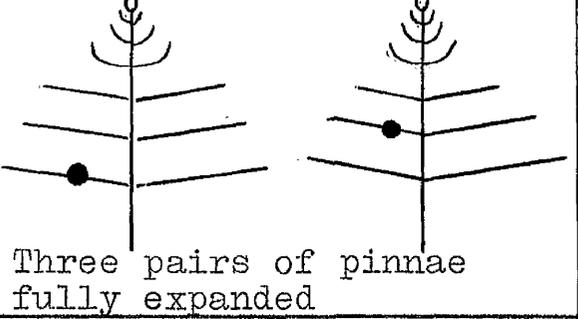
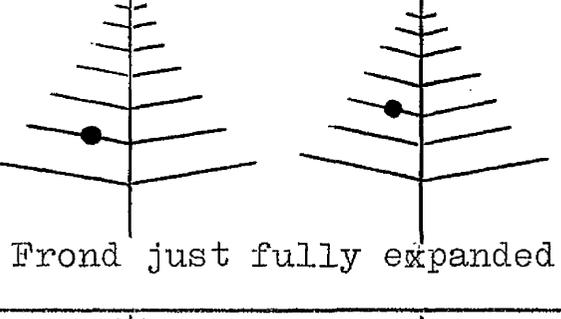
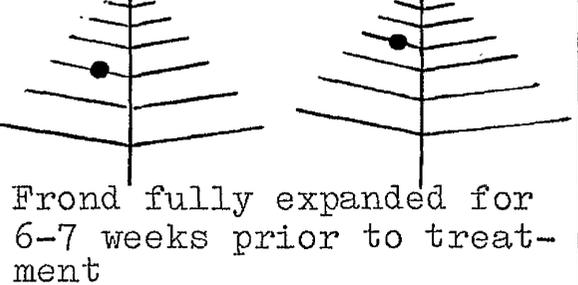
The 2,4-D used throughout the investigation had a specific activity of 15  $\text{mc.}/\mu\text{M}$ . and was formulated as a

750 p.p.m. solution in 50% alcohol plus 1% carbowax 1500. 160  $\mu$  litres of this solution (i.e. 120  $\mu$ gms. 2,4-D and 7.1  $\mu$ c.) was applied to each frond selected for treatment.

For convenient reference the general design of the experiment is summarised in Table 8. The first applications were made during the second week of June to fronds which had emerged from the soil some three weeks before and had only their first pair of pinnae fully expanded. Three fronds were treated on this occasion, the herbicide being applied to one of the first pair of pinnae, while a fourth frond was selected to serve as a control and was treated only with the carrier solution (i.e. alcohol plus carbowax). This control frond was also harvested, sampled and autoradiographed. Three pairs of fronds were selected for treatment at each of the next three stages of growth. These pairs served as replicates and were treated at 2-day intervals. All six fronds treated at each growth stage were very uniform in size and stage of development. The two members of each pair were treated on different pinnae and thus on parts which were at different stages of maturity. Since the fronds were at the same stage of development and were treated at the same time and thus under identical environmental conditions they provided a means of studying the effect on absorption and translocation of the degree of maturity of the treated part of the frond as distinct from effects resulting from the stage of development and general physiological condition of the plant

TABLE 8. Design of Field Experiment with C<sup>14</sup>-labelled 2,4-D

Dates on which pairs of fronds were treated (● = site of application) Stage of frond development Treated pinnae No. of fronds treated at each stage of development

9th) 13th) June		1st. pair	4 (including one "control")
19th) 23rd) June 25th)		1st. and 2nd. pairs	6
11th) 13th) July 15th)		1st. and 2nd. pairs	6
21st) 23rd) August		2nd. and 3rd. pairs	6
21st) 23rd) September		3rd. and 4th. pairs	4

as a whole.

The actual pinnae to which treatments were applied are indicated in Table 8. At the fourth growth stage the first pair of pinnae were completely withered and the herbicide was therefore applied to the second and third pairs. With the death of the second pair early in September the third and fourth pairs were treated on the final occasion.

The solutions were applied by means of an "Agl" micrometer pipette to the upper surface of the third and fourth pairs of pinnules of a single pinna on each selected frond. A small quantity of anhydrous lanoline was first applied to the base of each pinnule so as to prevent the solution from running down on to the mid-rib of the pinna. A further lanoline application was made to the mid-rib of each pinnule at a point 4 cms. from the base and the solution was then distributed evenly along the short length of mid-rib thus delimited. Although a considerable volume of the solution remained over the mid-rib some of it invariably spread outwards over the lateral lobes of the pinnules. The time required for drying of the solution was rarely more than 5 minutes and did not exceed 15 minutes in any of the treatments. No visible injury was produced at the site of application. The treatment period for all plants had a duration of 3 days.

At the end of the treatment period the treated pinnules were removed from the frond and the latter was then dug up

together with a short portion of the associated rhizome branch. For convenience this sample will hereafter be referred to as the "plant" although in fact it consisted only of a single frond and a small apical portion of one branch of the extensive rhizome system. A typical rhizome sample is illustrated in fig. 48. To avoid injuring the growing point of the rhizome or the slender and frequently rather attenuated basal regions of the frond considerable care in the excavation of the plants was essential. The soil was first removed from around the base of the petiole either entirely by hand or with the aid of a small trowel until the associated branch of the rhizome had been sufficiently exposed. The branch was then severed from the rhizome system at a distance of 6 - 12 inches behind the treated frond. The plant was then immediately placed in a large polythene bag and brought back to the laboratory.

#### The preparation of autoradiograms

The method employed in preparing the plants for autoradiography is described with reference to the plant illustrated in figs. 47-49. The general procedure was as follows:-

The rhizome of each plant was washed free from soil, severed from the frond at the base of the petiole and stored in a small polythene bag in the refrigerator (+ 5°C) until sampling of the frond had been completed. The frond (fig. 47)

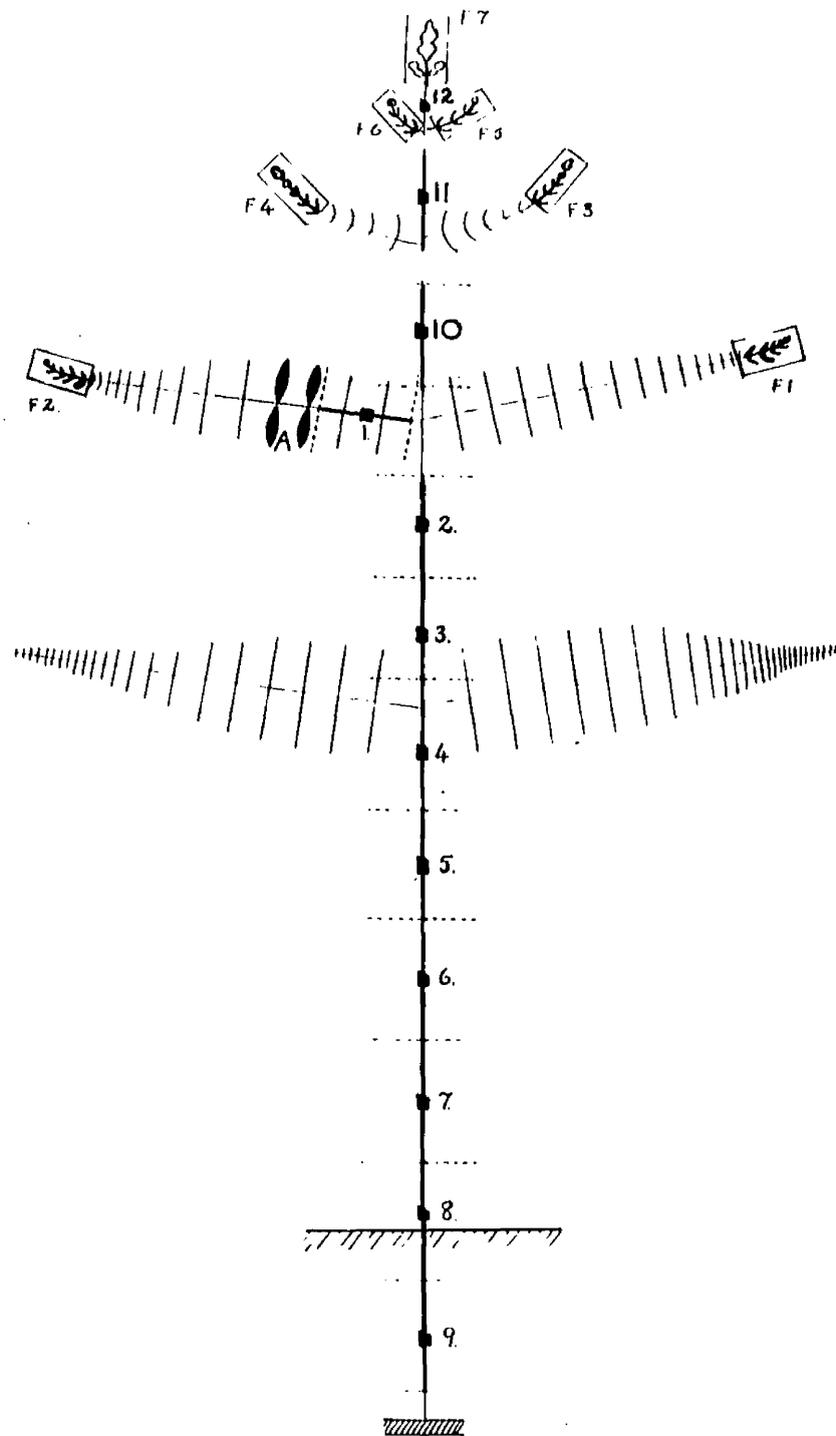


Fig. 47. Diagram of frond providing a record of (i) location of treated pinnules (A); (ii) exact stage of development of frond; (iii) parts sampled for autoradiography (F1-F7, samples of frond meristems; 1-12, sub-samples for sectioning from the pinna, rachis and petiole). Pinnae and pinnules not fully expanded are represented by curved lines. Dotted lines indicate points at which frond was cut in the initial sub-division and sampling.

(approx.  $\times \frac{1}{10}$ )

was rapidly subdivided by means of razor blades into a number of segments. In every case the first segment to be removed consisted of the basal region of the mid-rib of the treated pinna. The main axis of the frond, from the level of insertion of the treated pinna to the base of the petiole, was then cut into short portions each approximately 10 cms. in length. Similar segments were taken from the middle of each "internode" of the rachis above the treated pinna. As each segment was cut from the plant it was labelled with a small strip of masking tape bearing the number assigned to it in the diagram and was then placed in a covered dish lined with wet filter paper. Samples were also taken from the apical meristematic regions of partly expanded pinnae (F1 - F6) and from the apex of the frond itself (F7).

A small segment approximately one centimetre in length was cut from the middle of each sampled portion of the rachis and petiole (fig. 47, 1-12). Two such segments were usually taken from the mid-rib of the treated pinna, although only one was taken from this region in the plant in fig. 47. The morphologically upper end of each of these small segments was cut obliquely in order that the longitudinal sections into which each segment was divided could be correctly orientated when mounted for exposure to film. This oblique cut was always made in such a way that the longer side of the segment was the one which, on the intact plant, had been located on the same

side of the rachis (or petiole) as the treated pinna.

Each of the segments was cut by hand into longitudinal sections which varied from approximately 0.75 - 1.0 mm. in thickness. This sectioning was done with razor blades. To avoid the possible transfer of radioactive material a different blade was used in the sectioning of each segment. The blades were later washed thoroughly in several changes of absolute alcohol and dried with absorbent tissue before being used again.

The sections were laid out in rows in small perforated zinc trays, each tray being just large enough to accommodate all the sections (usually 5 - 9) comprising a single segment. As soon as a tray had been filled it was pressed into a layer of powdered "dry ice" contained in a large vacuum flask. After the sections were frozen the tray was transferred to a known position in a larger tray constructed of the same material and kept in the freezing compartment of the refrigerator ( $-5^{\circ}\text{C}$ ) while sectioning was in progress. Each of these larger trays was designed to hold all the sections (including rhizome sections) from a single plant.

The sampling technique applied to the rhizome is illustrated in fig. 48. In all plants the entire apical portion of the rhizome i.e. that part between the apex (A) and the base of the treated frond (TF), was retained for sectioning. Normally, late in the season, the apical portion bore a small frond bud

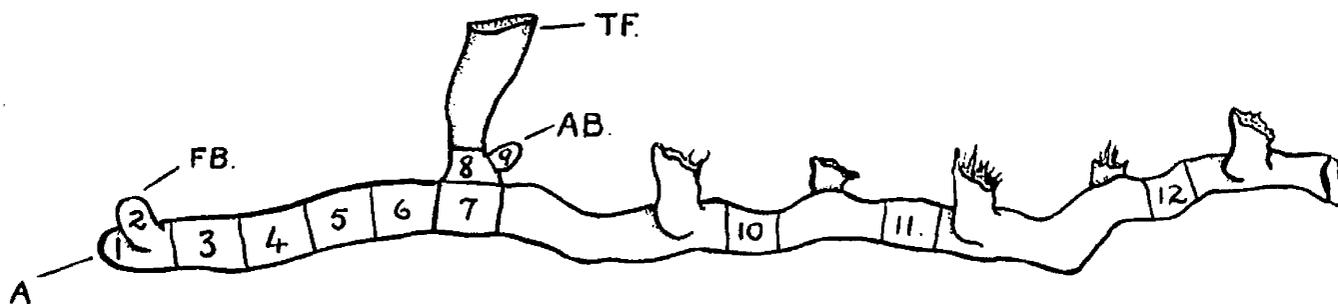


Fig. 48. Diagram of a typical rhizome sample dug up while still attached to the treated frond (TF).  
A, rhizome apex; FB, young frond bud;  
AB, abaxial rhizome bud; 1-12, parts removed for sectioning and autoradiography.

(x  $\frac{3}{4}$ )

(F8) a short distance behind the apex. If a rhizome bud (AB) was present at the base of the treated frond (such buds occurred in approximately 50% of the plants) it was also sectioned together with the adjacent portion of the petiole (8)

Samples were also taken from several positions on the older portion of the rhizome branch (10, 11 and 12). Since segments which included an old frond base were found difficult to section and were thus avoided, the positions from which these latter samples were taken were dependent to some extent on the internode length of the rhizome - a feature which showed considerable variation. In general, however, samples were taken at distances of approximately 3, 6, 9 and 12 cms. behind the treated frond.

All sampled parts, each of which, with the exception of the frond and abaxial buds, were approximately one centimetre in length were also sectioned longitudinally. After they had been frozen the sections were arranged in the prepared trays in the refrigerator.

When all plants (a maximum of three were harvested on each occasion) had been sampled the trays of sections were transferred to the vacuum chamber in the refrigerator and the plant material freeze dried for 48 hours.

All sections and sampled parts from one plant were mounted with rubber adhesive on a single sheet of paper as shown in

fig. 49, care being taken to ensure that the rows of sections from each sample were arranged in the same relative positions as they had occupied on the intact plant. The reference number assigned to each plant and the date of treatment were written on each specimen sheet with radioactive ink and were thus transmitted to the film during exposure and permanently recorded on the autoradiograms. Cross lines, drawn at opposite corners of each sheet, also with radioactive ink, served as a guide when the autoradiograms were later superimposed on the specimen sheet and enabled the distribution of the tracer within the individual sections to be studied more accurately than would otherwise have been possible. Ordinary (inactive) ink was used to label all rows of sections and other sampled parts with the numbers assigned to them in the diagrams of each plant.

Standard samples of known activity were attached to each sheet to serve as a check on the uniformity of the procedure (i.e. exposure and development) by which the autoradiograms were prepared on each occasion. These samples, which unfortunately were not included on all specimens, but which are shown in figs. 52 and 53, were prepared by applying a small known quantity of radioactive 2,4-D to pieces of filter paper by means of an Agla pipette.

Each sheet of specimens was covered with a layer of Terylene film (as previously described), placed in a plant press for 36 hours, and then exposed to X-ray film for a

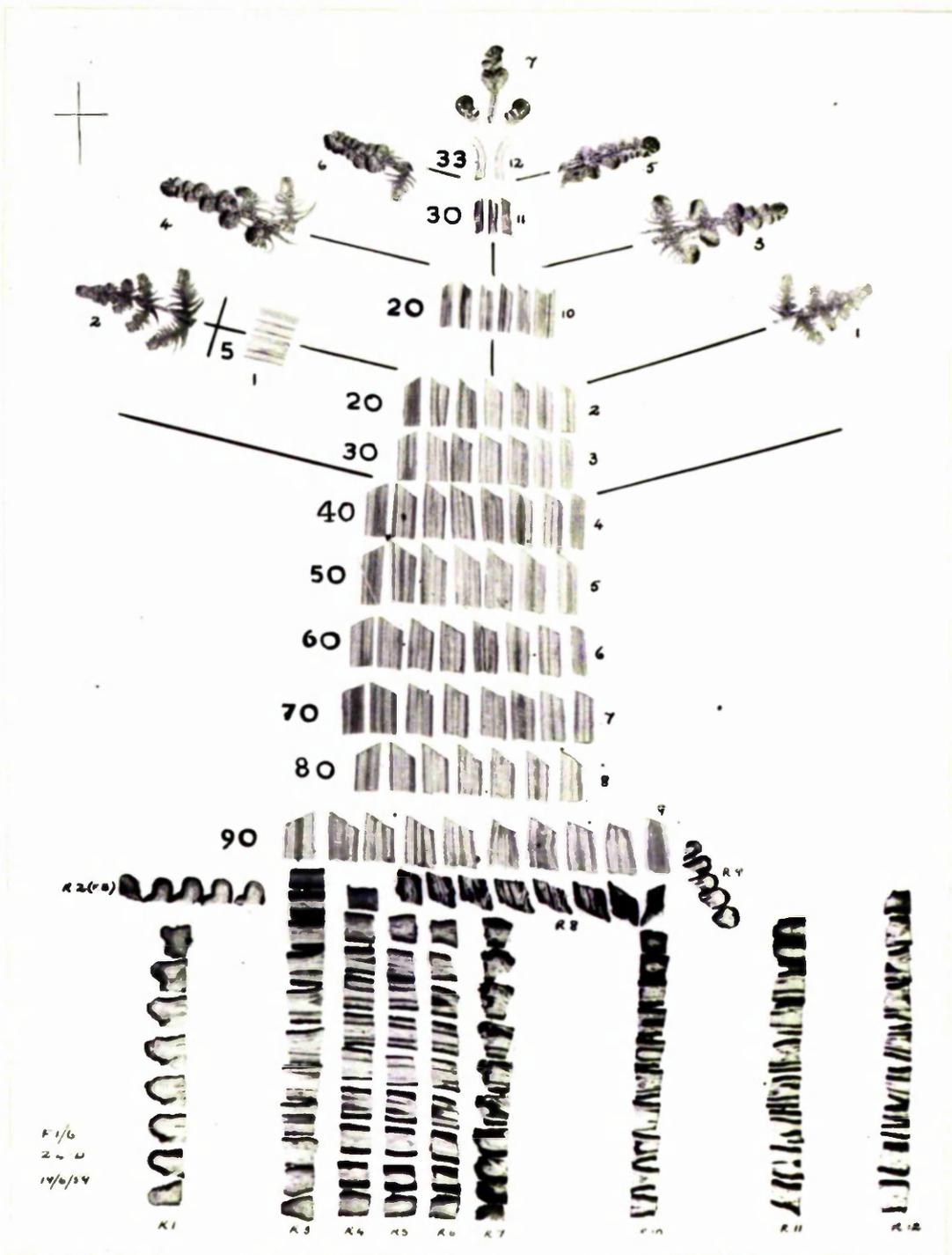


Fig. 49. Samples taken from the frond and rhizome shown in figs. 47 and 48 prepared for exposure to film. The number on the left of each row of sections indicates the distance (in cm.) of the sample from the treated pinnules (A). The reference number of each sample is given on the right.

(approx.  $\times \frac{2}{5}$ )

period of 3 weeks.

### Experimental Results

The most significant feature of the results was the well defined correlation between the degree of maturity of the treated part of the frond and the amount of the tracer present in the frond and its associated rhizome branch. This correlation is well illustrated by the autoradiograms which are shown in figs. 50-56. On the first two sampling occasions the 2,4-D was applied to pinnae which had either just completely uncurled or which had been fully expanded for a period of 10 - 15 days but which were evidently not yet fully mature. The autoradiograms obtained from these plants (figs. 50-52) showed the  $C^{14}$  to be present in a relatively high concentration in all samples taken from the fronds below the site of application and to be well distributed throughout the associated rhizome branch. On the other hand, the autoradiograms from plants which were treated 3 weeks later (figs. 53, 54) were of a considerably lower intensity. It was evident that translocation from these later applications had been considerably reduced. That this reduction was causally related to a change in the state of maturity of the treated part of the frond is clearly indicated by a comparison of those plants which had been treated on different pinnae. Application to the fully mature first pair of pinnae (fig. 53) resulted in autoradiograms

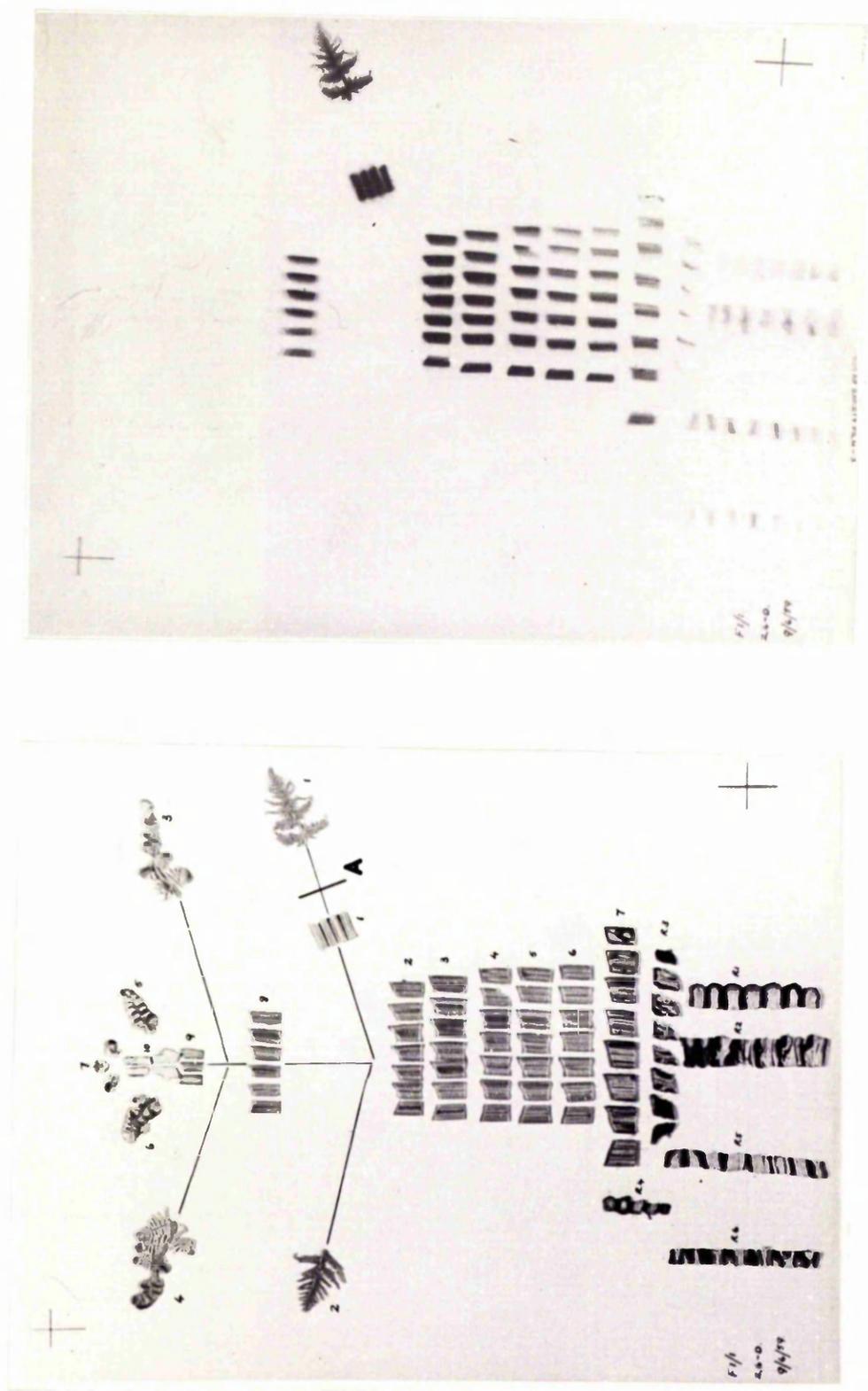


Fig. 50. Samples (left) and autoradiogram (right) from a plant treated with radioactive 2,4-D on the 9th. June. Dosage: 160  $\mu$ L. of a 750 p.p.m. solution (i.e. 120  $\mu$ g. 2,4-D and 8.14  $\mu$ c.) The treatment was applied to pinnules (A) of one of the 1st. pair of pinnae when only this pair were fully expanded. ( $\times \frac{1}{3}$ )

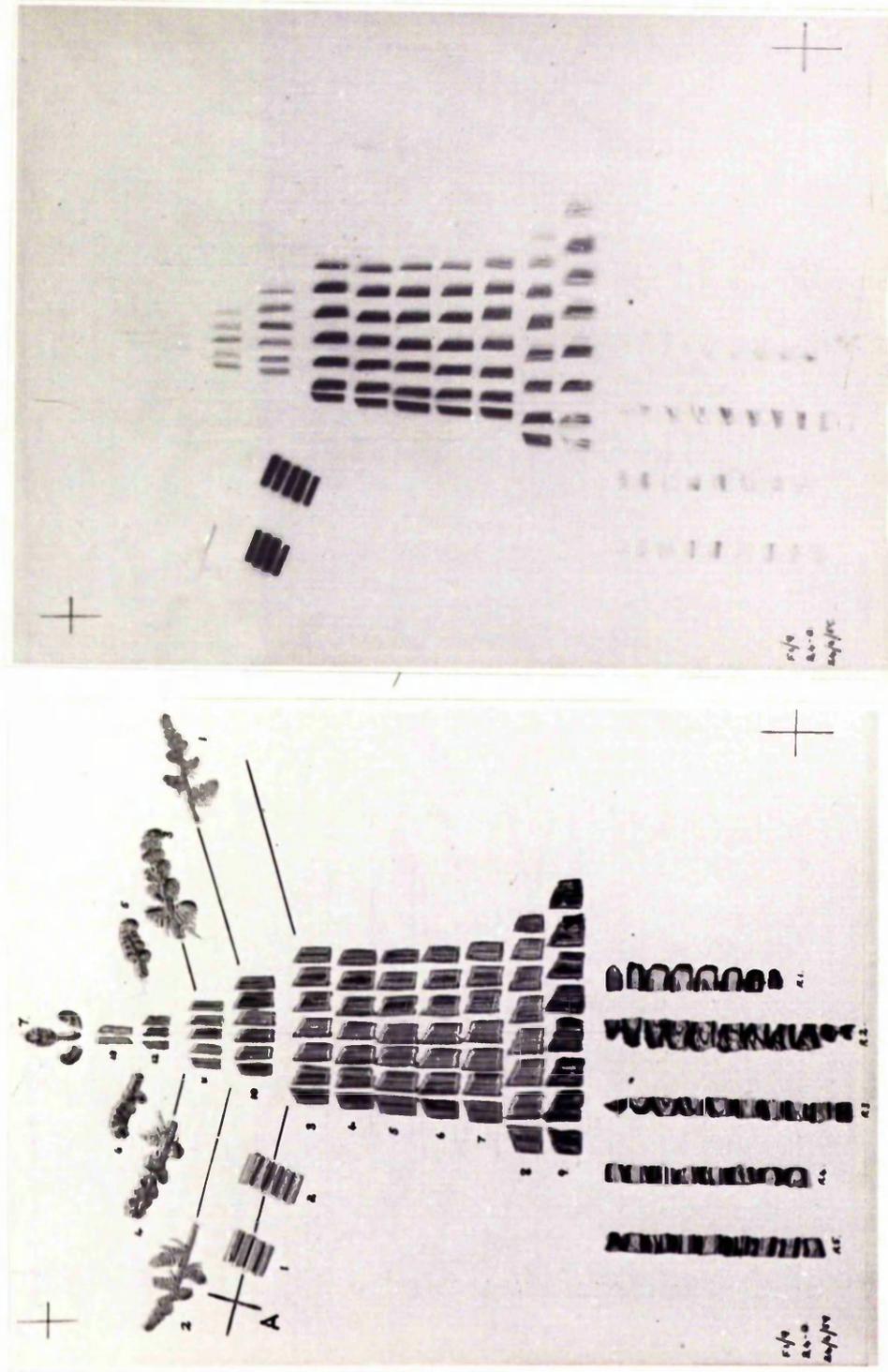


Fig. 51. A plant treated as described under fig. 50 on the 24th. June. At the time of application the frond had two pairs of pinnae fully expanded and the treatment was applied to pinnules (A) of one of the 1st. pair of pinnae. Left, samples; right, autoradiogram.

( x  $\frac{1}{3}$  )

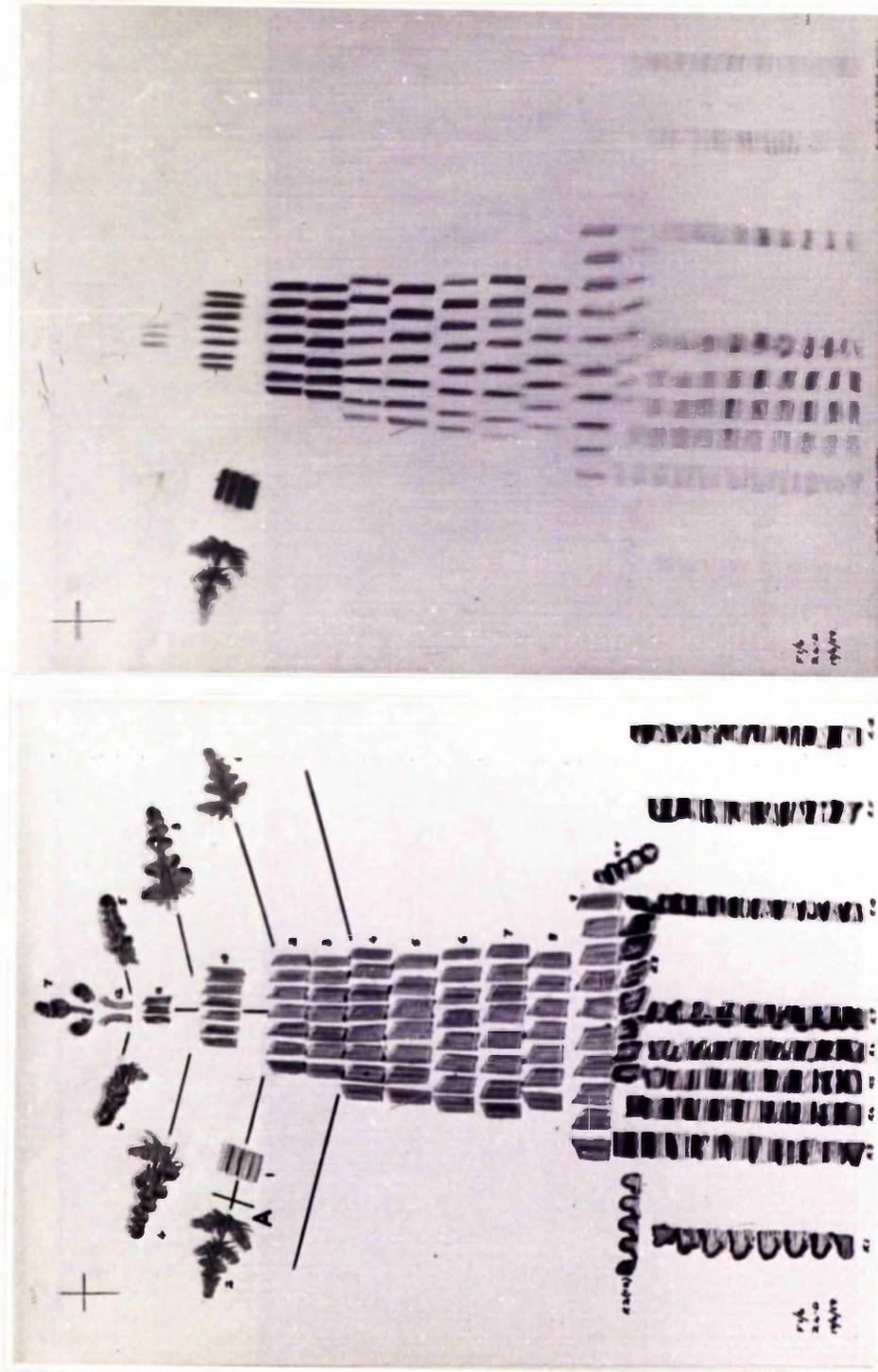


Fig. 52. A plant treated as described under fig. 50 on the 19th. June. The frond was at the same stage of development as the one shown in fig. 51, but the treatment was applied to pinnules (A) of one of the 2nd. pair of pinnae. Left, samples; right, autoradiogram.  $(\times \frac{1}{3})$

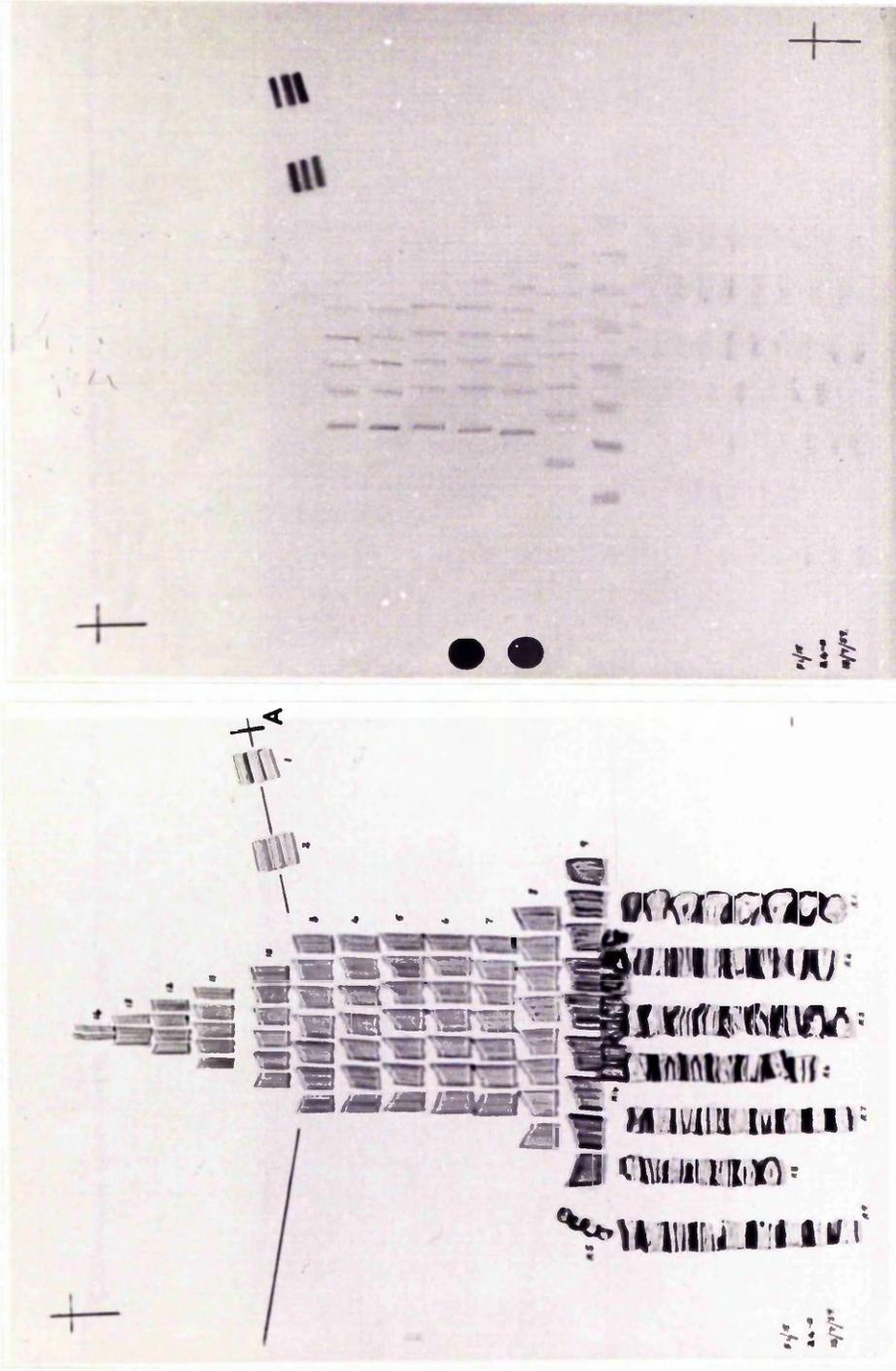


Fig. 53. A plant treated as described under fig. 50 on the 15th. July. The frond had its 4th. pair of pinnae fully expanded and was treated on pinnules (A) of one of the 1st. pair of pinnae. Left, samples; right, autoradiogram.

(x  $\frac{1}{3}$ )

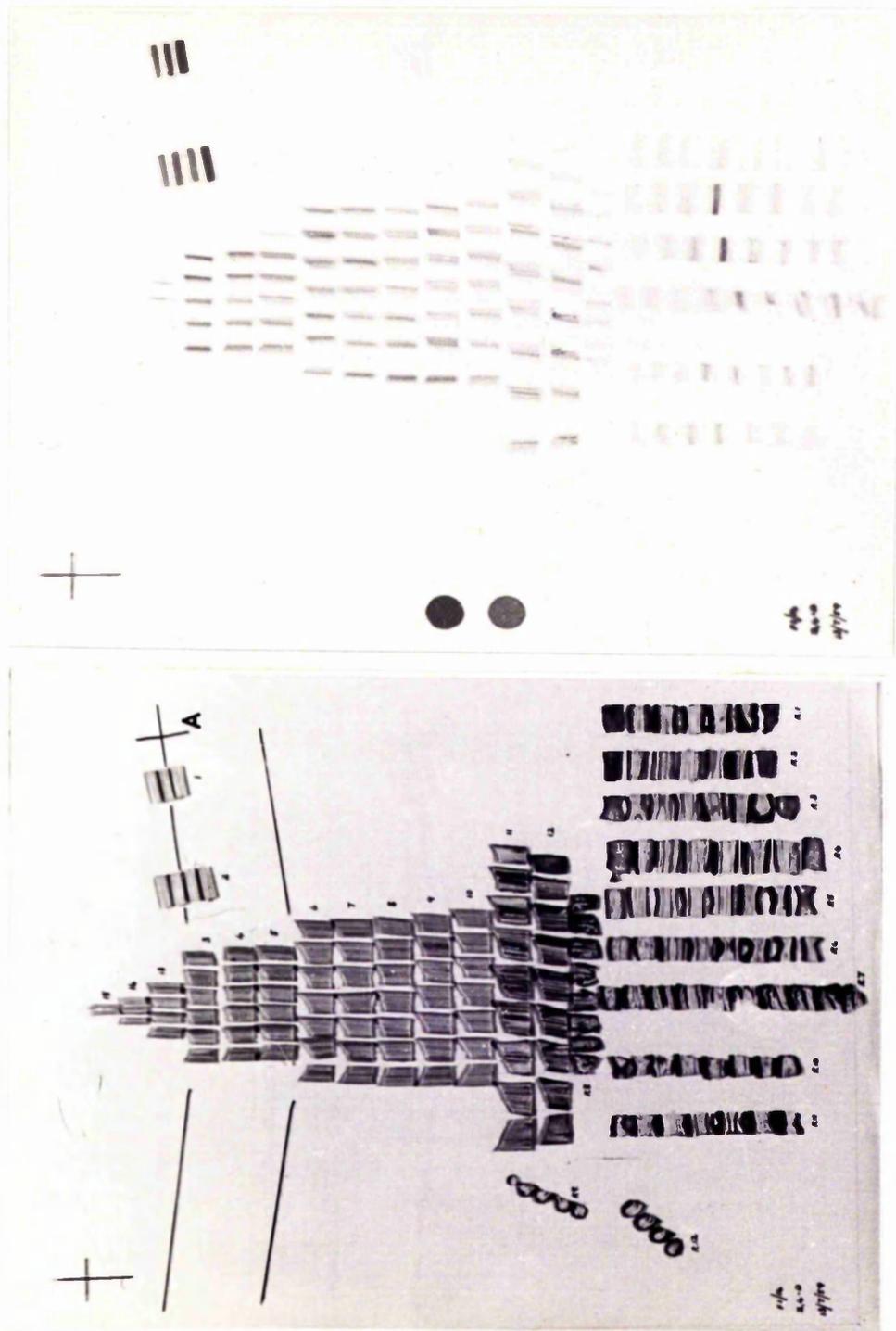


Fig. 54. A plant treated as described under fig. 50 on the 13th. July. The frond was at the same stage of development as the one shown in fig. 53 but was treated on pinnules (A) of one of the 2nd. pair of pinnae. Left, samples; right, autoradiogram.

(x  $\frac{1}{2}$ )

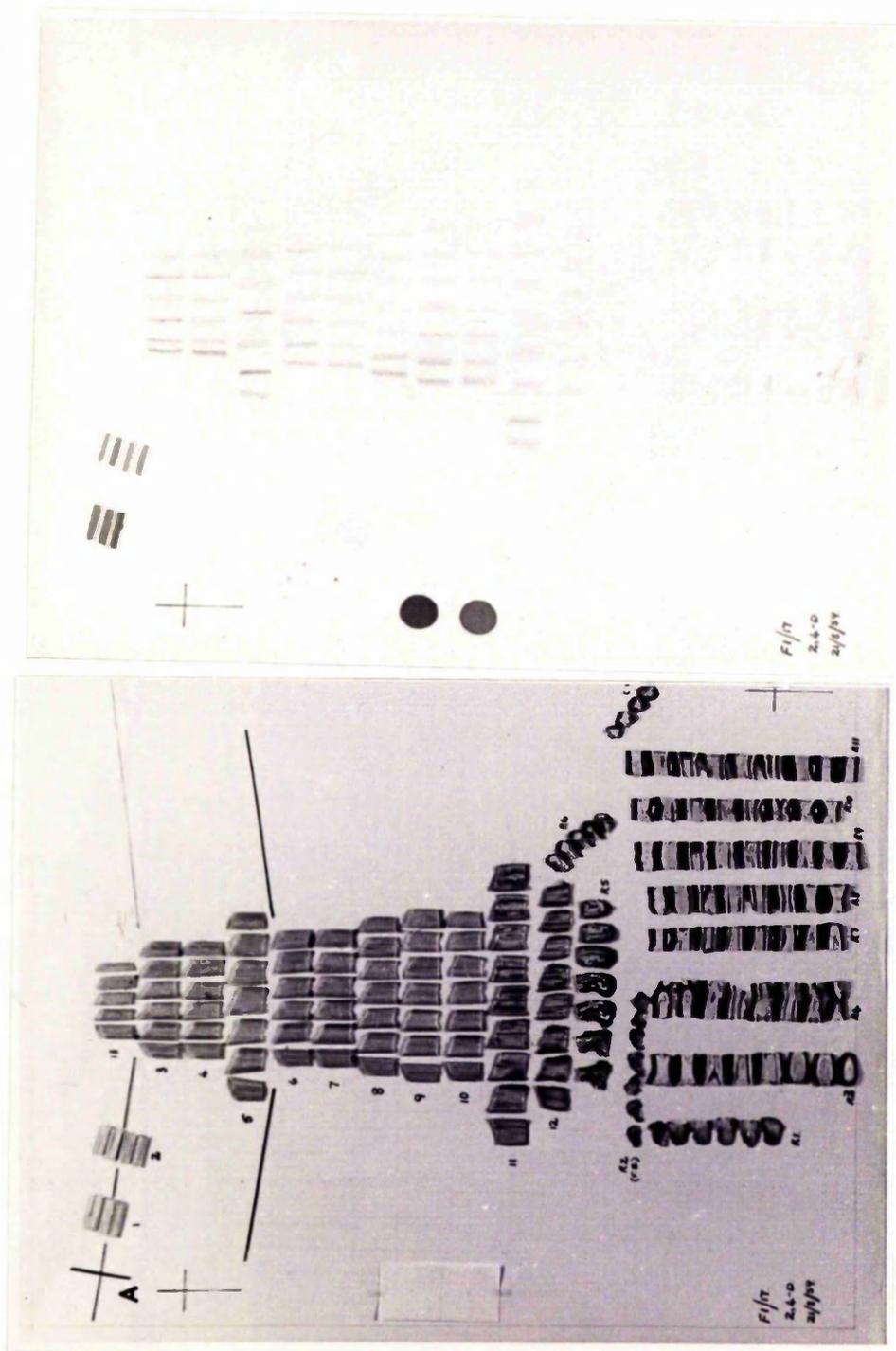


Fig. 55. A plant treated as described under fig. 50 on the 21st. August. The frond was just fully expanded and was treated on pinnales (A) of one of the 2nd. pair of pinnae. Left, samples; right, autoradiogram.  $(\times \frac{1}{3})$

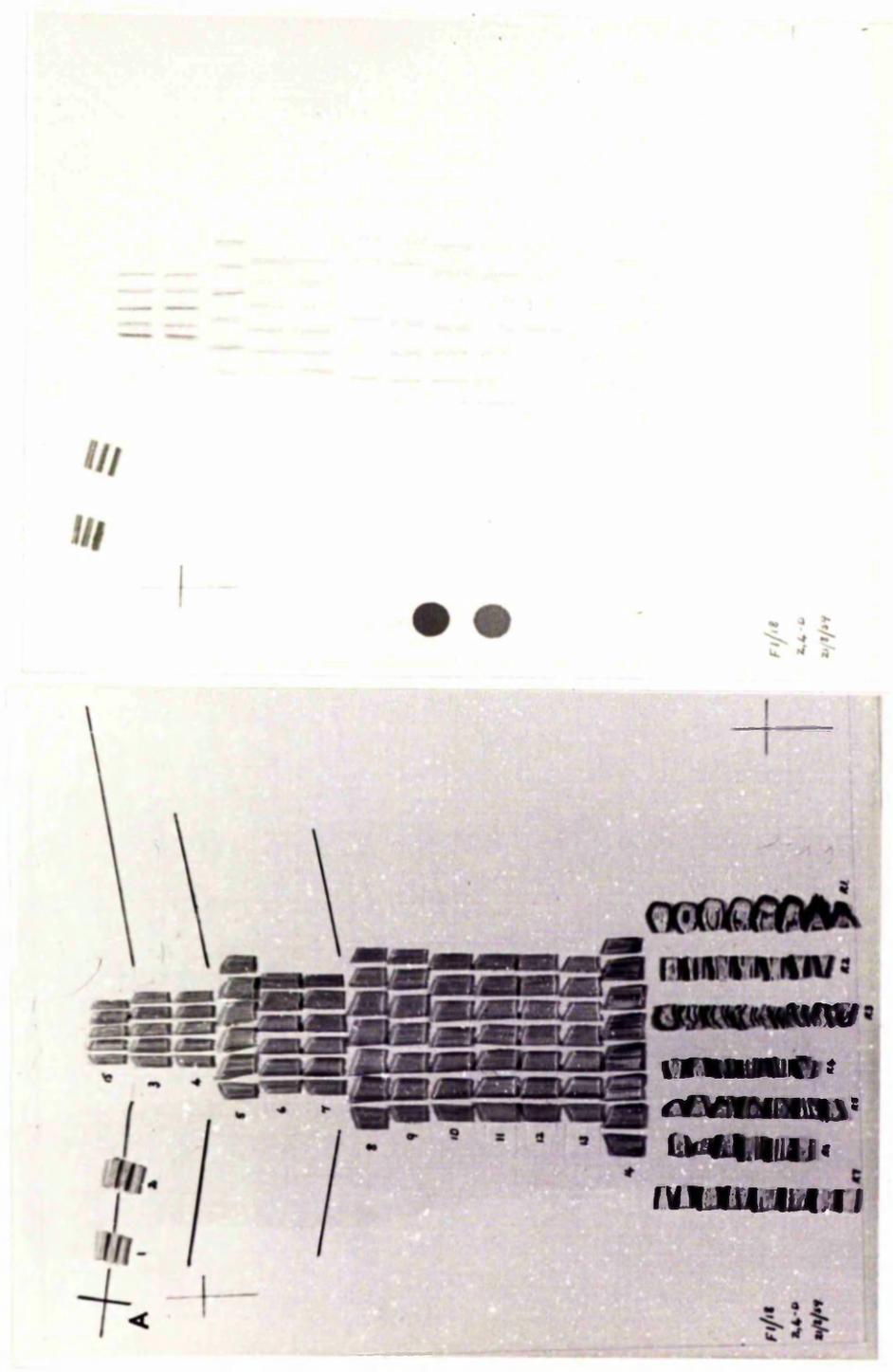


Fig. 56. A plant treated as described under fig. 50. The frond was at the same stage of development and was treated on the same date as the one shown in fig. 55, but the application was made to pinnales (A) of one of the 3rd. pair of pinnae.

Left, samples; right, autoradiogram.

(x  $\frac{1}{3}$ )

of very low intensity in which the presence of the  $C^{14}$  in the rhizome could only just be detected. On the other hand, autoradiograms from plants which were treated on the same occasion but on the younger second pair of pinnae (fig. 54) showed the presence of the tracer in distinctly higher concentrations not only in the petiole but in all samples taken from the rhizome. It must be emphasized that this difference was similar in each of the three pairs of fronds treated on this occasion. Indeed, so close was the agreement between replicates that they could be distinguished from one another only by careful inspection.

When the 2,4-D was applied six weeks later to fronds which had just fully expanded translocation into the rhizome from the second pair of pinnae (fig. 55) was clearly much reduced as compared with the corresponding application on the previous occasion (fig. 54). It seems probable that this reduction can be attributed to the greater maturity of the treated pinna. On the other hand, there was no appreciable difference between autoradiograms from plants treated on the second and third pairs of pinnae of the fully expanded frond (figs. 55, 56), possibly because these pinnae, when the herbicide was applied, were both fully mature.

It is evident from these results that the amount of 2,4-D translocated throughout the plant is greatest when the application is made to pinnae which are still immature and

declines markedly as the pinnae approach maturity. A similar effect of leaf maturity has been reported from experiments with bean plants which showed that more 2,4-D is exported from leaves which are only partly expanded (Mitchell and Linder, 1950) or just fully expanded (Fang et al., 1951) than from those which are fully mature. That this can be attributed to a difference in the amount of 2,4-D which penetrates into the leaf and which is thus available for translocation is suggested by the work of Weintraub et al. (1954) whose study of the entry of 2,4-D into bean leaves led then to conclude that "each leaf appears to pass during its expansion through a relatively brief stage of high absorbability which then falls markedly and remains relatively constant as long as the leaf remains green." Clearly this conclusion agrees well with the present observations and suggests that in bracken the penetration of the herbicide may be greatly reduced as the frond matures. Although the reason for this postulated reduction in penetration requires further more critical investigation a thickening of the leaf cuticle associated with the maturation of the frond is suggested as a possible explanation.

There was also some indication of a difference in the pattern of distribution of the herbicide within the plant which appeared to be associated with the age of the frond rather than with the degree of maturity of the treated pinnae.

This difference in distribution is apparent from a comparison of plants treated on the first two occasions with those at later stages of development. In the early treatments (figs. 50-52) the tracer appeared to be present in relatively high concentrations in those samples from the petiole or rachis immediately below the level of insertion of the treated pinnae and showed a well marked gradient of decreasing concentration down to the base of the petiole. In plants treated at later stages of development this gradient was either absent or much less pronounced (figs. 53-56). A possible explanation of this difference is suggested by the work of Yamaguchi and Crafts (1957) who also found evidence from autoradiograms of a marked concentration gradient of 2,4-D along the path of transport. It was postulated that this gradient resulted from a tendency for 2,4-D to be absorbed and thus immobilised by parenchymatous cells bordering the conducting elements. Since experiments showed that a more uniform distribution of the tracer was obtained by growing the plants under more favourable conditions it was also suggested that the absorption of the herbicide into non-conducting tissues may be reduced by factors which tend to increase the rate of growth of the plant and thus to promote rapid translocation. It might be postulated on the basis of these observations that the difference in the distribution of the tracer noted in the present experiment is a reflection of differences in the rate at which it was translocated down into

the rhizome in plants at different stages of development and that the rate of transport was greater in the more mature plants. Clearly, however, this can be regarded merely as a working hypothesis for later investigations.

Finally, certain variations in the direction of translocation within the fronds and rhizomes are worthy of comment. At the two earliest stages of development application to either the first or second pair of pinnae resulted in some upward movement into the immature parts of the frond, there being high concentrations of the tracer in samples taken from the rachis at distances of 20 - 30 cms. above the insertion of the treated pinna. However, even in these young rapidly growing fronds transport of the herbicide from the lower fully expanded pinnae was predominantly basipetal and, in fronds at later stages of development (e.g. figs. 53, 54) upward translocation occurred to but a negligible extent.

Within the rhizome the direction of translocation showed a consistent correlation with the position of the treated pinna relative to the rhizome apex. When the treated pinna was on the abaxial side of the rachis (e.g. fig. 51) the tracer failed to be transported in the direction of the rhizome apex. On the other hand, when the pinna was borne on the adaxial side of the frond translocation within the rhizome occurred in both directions (fig. 52). This relationship, which was a consistent feature of all the autoradiograms, may be attributed to the nature of the vascular connections between the frond and rhizome.

SECTION 4. DISCUSSION OF PART III

Although at the time when the present investigations were initiated the evidence available seemed to indicate that growth regulating herbicides were unlikely to be effective for bracken control the results which have been reported from more recent investigations have considerably increased the prospects of success. The present observations on the toxic effects of 2,4-D on the rhizome of the young sporeling are in agreement with the work of Conway and Forrest (1956) who have shown that these effects are produced, not only by 2,4-D, but also by a number of other substituted phenoxyacetic acids and by their butyric homologues. Later investigations (Conway, 1959) showed that certain of these compounds are also translocated in mature bracken in the field with the production of injurious effects at the rhizome apices associated with the treated fronds. Again the effects described are similar to those observed in the present investigation. The results of a more extensive field trial with a number of commercially formulated "hormone-type" herbicides have been summarised by Fletcher (1959) who reported that most of the rhizomes dug up from the treated plots had been killed and that the density and height of the fronds which subsequently emerged were substantially reduced. Finally, Conway and Forest (1959) have described the effects on both young sporelings and mature bracken of a wide range of growth regulating substances. Of the various herbicides used

in these investigations 4-chlorophenoxyacetic acid (4 CPA) appeared to be particularly effective. The results of a preliminary field trial with this compound showed that a high proportion of the rhizome apices associated with the sprayed fronds had either been killed or were in a moribund condition and, furthermore, that many branches without an emergent frond had been similarly affected.

In spite of the promising results of these recent investigations, however, it has not yet been established that any of the compounds which have been tested will provide an effective and economical method of bracken control. The work so far reported has been mainly of an observational nature and it is generally recognised (e.g. Conway and Forrest, loc. cit.) that further more critical investigations will be required before the potential value of these herbicides can be fully assessed.

One problem demanding particular attention and which may be briefly considered in relation to the results of the present investigation is the linkage between the effectiveness of the treatment and the stage of development of the plant. That this relationship is of considerable importance is clearly indicated from the results of the field investigation with labelled material described in Section 3. It appeared from this experiment that the amount of 2,4-D translocated into the rhizome was much greater when the application was

made to parts of the frond which were still immature and it was postulated that the penetration of the herbicide may be progressively restricted as the frond approaches maturity. When considering the practical implications of this hypothesis, however, it must be borne in mind that the 2,4-D was used at a concentration of only 750 p.p.m. and that none of the applications caused any visible injury to the treated parts of the frond. On the other hand, there is some evidence from the recent investigations by Fletcher (loc. cit.) and Conway and Forrest (loc. cit.) that the much higher concentrations normally employed in commercial formulations may cause rapid and severe injury to immature fronds and that translocation into the rhizome may consequently be seriously impeded. The efficiency of penetration, however, need not be entirely dependent upon the degree of maturity of the frond; the nature of the formulation is also a factor of considerable importance (discussed by Crafts, (1956b)). Thus, by the use of a suitable formulation, it may prove possible to obtain adequate penetration into the fully mature frond while, at the same time, advantage may be taken of the lessened sensitivity to injury which is apparently associated with the attainment of maturity.

So far as the translocation of the herbicide is concerned, however, the physiological condition of the plant as determined by its stage of development is undoubtedly the dominant

factor, The present observations were found to be in good agreement with the generally accepted conclusion that 2,4-D and other phenoxyacetic acids are translocated in the phloem in association with carbohydrates, and are thus preferentially transported to regions of active growth. The existence of this relationship was suggested by the distribution of the morphological responses induced in the rhizomes of the young sporophyte (Section 1) and was particularly evident in the changing pattern of movement of  $C^{14}$  in the developing frond (Section 3). In view of this evidence it might be postulated that conditions should be especially favourable for the translocation of the herbicide in the mature bracken plant during that stage of growth when the movement of assimilates into the rhizome is proceeding most actively from all parts of the frond. It may further be expected that this period will extend from the time that the frond first becomes fully expanded until the ageing of the lower pinnae leads to a reduction in their photosynthetic activity. Clearly, however, this assumption requires to be tested experimentally. In particular there is an obvious need for a study of the translocation of assimilates in the bracken plant in relation to the stage of growth and to the environmental conditions.

Finally, attention must also be given to the seasonal changes which occur in the activity and rate of growth of

the rhizome apices. Since there is some evidence that 2,4-D and other related compounds are most toxic to tissues which are in an active, meristematic condition (Van Overbeek, 1947) it seems probable that the increasing apical activity within the rhizome system observed to occur during the early part of the growing season (Section 2) will be associated with an increasing sensitivity of the rhizome apices to the effect of the herbicide. At the same time, it may be expected that the flow of assimilates to the rhizome apex from the expanded frond will be greatest during the period of most active apical growth and that this period will therefore be most favourable for the translocation into the apical region of the applied herbicide. According to the present observations the number of actively growing apices approaches a maximum between late June and mid August and thus during the period when the fronds are fully expanded but before any very marked reduction in their photosynthetic activity is likely to have occurred. As previously suggested, it is also during this stage of growth that the translocation of the herbicide into the rhizome may be expected to attain its greatest efficiency.

Thus, on theoretical grounds and from the results of the present investigation it may be concluded that the application of a growth regulating herbicide to the fronds of the mature bracken plant is likely to be most effective

when, or shortly after, the fronds have become fully expanded. There is evidence, however, that at this stage the penetration of the herbicide may be a limiting factor. The effectiveness of the treatment may thus be determined largely by the nature of the formulation which should be designed with the object of achieving adequate penetration but, at the same time, of excluding or minimising the production of toxic foliar effects which would reduce the efficiency of translocation.

SUMMARY OF PART III

The application of 2,4-D' to the frond of the bracken sporeling induces an abundant proliferation of roots at the apices of the rhizome and a temporary inhibition of rhizome development. The failure of these effects to be produced when a segment of the treated frond had been killed indicated that the basipetal transport of the herbicide occurs in living tissues. The number of roots induced and the inhibition of rhizome development increases with the dosage applied while the elongation of the roots is correspondingly depressed. There is evidence that the direction of translocation within the rhizome is dependent upon the position of the treated frond, movement being greater into that branch arising on the same side of the stem as that on which the treated frond is inserted. This relationship is attributed to the nature of the vascular connections. The induction of large numbers of root primordia at the rhizome apices was found to be the most characteristic of the histological effects.

It has also been shown that the application of 2,4-D to fully expanded fronds of mature bracken produces injurious effects at the apices of the rhizomes. The effects were similar to those induced by this treatment in the young sporeling.

Autoradiograms from sporeling fronds treated with 2,4-D

containing radioactive carbon ( $C^{14}$ ) showed that the direction of translocation is largely determined by the stage of development of the frond and provided good evidence that the herbicide is translocated in association with food materials. Employing the same technique in a field experiment it was found that the amount of the tracer which moved into the rhizome was considerably greater from parts of the frond which were still immature. It is suggested, as a possible explanation of this result, that the penetration of the herbicide may be restricted by changes associated with the maturation of the frond.

ACKNOWLEDGEMENTS

The work presented in all but the final section of this thesis was carried out in the Botany Department of the West of Scotland Agricultural College with the aid of a Colin Thomson Research Scholarship. I wish to thank the Governors of the College for the award of this grant and also to record my gratitude to Professor W.W. Fletcher for his unfailing interest and encouragement throughout the course of the work. I am indebted also to Professor J. Walton, who acted as supervisor, and to Dr. E. Conway for her stimulating interest and for some helpful discussions.

The investigation described in the third section of Part III was supported by a grant from the Agricultural Research Council and was carried out at the A.R.C. Unit of Experimental Agronomy in the Department of Agriculture of Oxford University. I would like to express my thanks to Professor G.E. Blackman for the provision of facilities in his department and for his critical reading of the manuscript. I am also grateful to Dr. E.K. Woodford for his supervision of the investigation and to other members of the Unit for their interest and advice. In particular, I am indebted to Mr. R.G. Powell, who formulated the radioactive solutions and offered many helpful suggestions. My thanks are due

also to Mr. P. Selwood for his assistance with the illustrations.

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## APPENDIX

Part II. Section 1.The Effect of Removing the Primary Frond of the Bracken  
Sporeling on the Rate of Growth of the Second FrondA. Experiments 1-3

Stage of development of primary frond when removed	Time after removal of primary frond (hrs.)	Mean length of Second Frond (mm.)		Sig. of diff. of means (P)
		Control plants	Defoliated plants	
<u>Experiment 1</u> Lamina only partly expanded	0	0.73±0.039	0.76±0.043	N.S.
	12	0.86±0.030	0.77±0.053	"
	24	1.03±0.038	1.02±0.058	"
	36	1.20±0.063	1.29±0.064	"
	48	1.38±0.081	1.52±0.078	"
	60	1.81±0.127	1.88±0.111	"
	72	2.34±0.175	2.54±0.155	"
	84	3.08±0.271	3.35±0.231	"
<u>Experiment 2</u> Lamina just fully expanded	0	0.78±0.044	0.76±0.032	N.S.
	24	0.94±0.053	0.92±0.041	"
	48	1.24±0.080	1.29±0.074	"
	72	1.74±0.149	2.00±0.144	"
	96	2.81±0.288	3.31±0.317	"
<u>Experiment 3</u> Lamina fully expanded for 24 hours	0	0.88±0.028	0.77±0.025	0.01
	12	1.03±0.032	0.88±0.034	0.01
	24	1.26±0.054	1.15±0.066	N.S.
	36	1.46±0.070	1.32±0.061	"
	48	1.88±0.119	1.71±0.103	"
	60	2.40±0.160	2.18±0.146	"

B. Experiments 4-6

Mean length of primary frond when removed (mm.)	Time after removal of primary frond (Hrs.)	Mean length of second frond (mm.)		Sig. of diff. of means (P)
		Control plants	Defoliated plants	
<u>Experiment 4</u> 1.3 - 2.0*	108	1.08±0.083	1.27±0.087	N.S.
	120	1.22±0.110	1.60±0.134	0.05
	132	1.44±0.128	2.05±0.174	0.01
	156	2.32±0.246	3.50±0.293	0.01
<u>Experiment 5</u> Controls: 1.46 ±0.062 Defoliated: 1.40 ±0.062	96	1.26±0.081	1.40±0.118	N.S.
	108	1.47±0.122	1.72±0.149	"
	120	1.85±0.184	2.27±0.213	"
	132	2.26±0.251	2.82±0.278	"
	144	2.83±0.368	3.32±0.405	"
<u>Experiment 6</u> Controls: 1.16 ±0.101 Defoliated: 1.15 ±0.031	105	1.08±0.060	1.37±0.080	0.05
	117	1.30±0.070	1.77±0.134	0.01
	129	1.60±0.105	2.30±0.171	"
	141	1.97±0.150	2.95±0.248	"
	153	2.46±0.253	3.66±0.314	"

\* Mean not recorded. Range given includes both sets of plants.