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Senescence studies of festuca pratensis

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Senescence studies of Festuca pratensis

by

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Declaration

The work described in this thesis has been carried out by the undersigned at the Department of Botany of Durham University and has not been previously submitted for any other Diploma or degree.

Signed *Michael J. Lee*

Date *26/9/79*

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ABSTRACT

The first part of this investigation deals with an anatomical study of the senescence of Festuca pratensis. Since there were few observable structural changes at the anatomical level the progressing senescence was followed by using chlorophyll loss as a parameter of senescence along the Festuca leaf. As the ageing process was found to occur from the tip to the base of the leaf, as if it were excised, the possibility of an 'abscission zone' near the base of the leaf was investigated. Feeding the plants with dyes and radiochemicals showed in older leaves that there was a definite retardation of the entry of these nutrients into the leaf at the ligule region i.e. it appears to act as a controlling mechanism for senescence.

In the second part of this study the ligule region was examined closely to try and ascertain whether there were any structural changes in this region as the leaf ages which could account for the retardation of the entry of materials into the leaf. By using sectioning techniques and scanning Electron Microscopy evidence was found for the presence of possible tyloses in the xylem vessels of the ligule region in older leaves. These could indicate a possible mechanism at the ligule which may control or even induce senescence.

Acknowledgments

INTRODUCTION

Plant senescence is an area of study which has until recent years, received very limited, and in many cases, indirect attention. The phenomenon can be regarded as the ordered change which occurs within a plant, or part of that plant, prior to death.

In contrast to animals the process has some positive function (Leopold 1961) as it releases materials for the growth of new leaves and the formation of fruits (Thrower 1967). Senescence of the complete plant also ensures a rapid turnover of generations resulting in ecological adaptations and evolution of the species.

Several different kinds of senescence have been described in plants

1. Overall senescence - this is where the whole plant may die following flowering and fruiting

2. Synchronous or simultaneous senescence - this is the annual senescence of leaves which occurs in deciduous trees.

3. Sequential or progressive senescence - this occurs when particular leaves of a plant senesce in response to the formation of new leaves or fruits.

Whilst the three kinds of senescence give similar results, Simon (1967) has pointed out that there appears

to be different patterns of metabolic activity during senescence in the three cases with regard to protein synthesis and transportation of proteins from the leaves and so senescence must not be regarded as an identical process in all plants. In most of the work to date the process of senescence has been studied in excised leaves but Lewington & Simon (1967) have pointed out that this also should be considered as a unique form of ageing and it is not necessarily the same as that which occurs in the intact plant. It is also recognised that in at least the early stages that senescence is reversible and in many monocarpic species the onset of senescence can be retarded for example by the removal of flower buds (Murneek 1926, Leopold et al., 1959) or the apical meristem (Davies & Wareing 1963) or the application of plant growth regulators (R. Wollgiehn 1961). Senescence therefore appears to result from a physiological imbalance rather than irreversible damage due to the mutation of the cells genetic information (Szilard 1959).

It is generally accepted that an outward sign of the onset of senescence is the yellowing of the leaves due to the breakdown of the chlorophylls which renders the xanthophylls and carotenoids visible although there are exceptional cases. Stoddart & Thomas (1975) have shown

that Festuca pratensis BF 986 does not show yellowing during senescence which does cast some doubt on the case of yellowing as a parameter for senescence. However the normal decline in the chlorophyll content does not appear to correlate directly with a changing photosynthetic rate but there is a decline in photosynthesis following full leaf expansion (Smillie 1962, Hardwick et al., 1968, Peat 1970, Ludlow & Wilson 1971). The chlorophyll degradation is accompanied by the breakdown of the membranes of the grana of the chloroplasts which yield lipid globules in which the carotenoids readily dissolve (J.S. Dodge 1970). At the same time the endoplasmic reticulum disintegrates, the nucleus degenerates and one of the last cell components to disappear is the plasma lemma. Butler & Simon 1971 suggest that the degenerative changes may become irreversible once the membrane system and in particular the tonoplast has ruptured releasing lysosomal enzymes.

The structural changes mentioned above are accompanied by many biochemical changes which include the breakdown of protein and a decline in the RNA and DNA content of the leaf. Barker & Hollinshead (1967) demonstrated the existence of a ribonuclease enzyme which breaks down RNA into its constituent nucleotides. There is also some evidence (Carr & Pate 1967) that at least at the onset of

senescence there is a rise in the protein content of the leaf which is probably due to an increase in the formation of degradative enzymes such as ribonuclease. However with increasing age the total soluble protein content declines and this is accompanied by a decline in the ribosomal population and in most species a reduced respiratory rate (Yemm 1956, Smillie 1962) although this in some species is delayed to late in the life cycle of the leaf (Woolhouse 1967). Along with these changes there is a reduction in the photosynthetic rate as Callow in 1969 demonstrated using sequentially ageing leaves of Perilla fructescens— he showed a definite decline in the cyclic photophosphorylation process.

It appears that the process of senescence can be initiated by either environmental or internal factors. The environmental factors which have been found to induce senescence include temperature (Mothes & Baudisch 1958), Nutrient deficiency (Williams 1936), Drought (Gates 1955), Light intensity (Brougham 1958), Darkness (Vickery et al., 1937) and the length of the photoperiod (Lochart & Gottschall, 1961, Krizekef et al., 1966).

It is apparent that the environmental factors are linked in some way to the plant's growth hormone system which has been shown to internally regulate senescence.

Chibnall in 1954 observed that if Phaseolus leaves are allowed

to root then their life span is extended, presumably due to

a factor (kinetin) produced by the roots. Subsequently

Richmond & Lang (1957) showed that kinetin (a synthetic

cytokinin) delayed the onset of senescence in detached

leaves of X. pensylvanicum. Gibberellic acid has also been

shown to retard senescence on Taraxacum officinale and Rumex

(Fletcher & Osborne 1965, Whyte & Luckwill 1966, Goldthwaite

& Laetsch 1967). There is thus direct evidence that kinetins

and to a lesser extent Gibberellins retard the senescence

rate in excised leaves and recently it has been shown that

possibly kinetin effects DNA dependant RNA synthesis so

preventing the formation of degradative enzymes and arresting

protein breakdown (Stoddart et al., 1974).

It has also been found in many plants that abscisic

acid and ethylene are promotors of senescence (Addicott 1969,

Aspinall et al., 1967, El-Antably et al., Waring et al.,

1968 and Burg 1968) but their mode of action is somewhat

conjecture.

In dicots it is well established that there is a

distinct region in the petiole where cells loosen and the

conductive tissue is gradually blocked known as the Abscission

zone and after leaf senescence leaf fall occurs at this

point. The gradual blockage of the xylem and phloem seems

to occur as part of the sequential process of senescence rather than acting as a trigger for the process. The petiole itself has been shown by Misha, Surga, Deo and Bhagma (1977) to have some influence or control over the senescence process. They have shown in normal petiolated leaves that protein degradation is less than in depetiolated leaves left in the same conditions for the same time.

However in monocots the situation has appeared to be different. Most monocots can be seen to senesce from the tip of their leaves to the base with no apparent abscission zone. It is known that few of the leaves act as photosynthetic organs and there is a need for fine control of transport systems and functions of the leaves. In the following investigation some aspects of the ageing process of Festuca pratensis were studied. An anatomical survey of excised leaves of varying ages was made and there was an attempt to relate these anatomical studies with functional changes in the leaves. The possibility of the presence of an "abscission zone" or cut off zone which could initiate or control senescence was also investigated. This would most probably not be an abscission zone as in dicots where the leaf falls off, but a zone of restriction of some kind.

MATERIALS AND METHODS

1. Plant Materials

Seeds of meadow grass Festuca pratensis "Perdita" were kindly supplied by the Welsh Plant Breeding Station at Aberystwyth. The seeds were sown individually in pots containing Levington's potting compost and following germination the seedlings were kept in a greenhouse at a mean temperature of $22^{o}C$ under natural light. Mature $(5th)$ leaves judged to be so when there was full emergence of the ligule, approximately 2 months old, were used for the investigations indicated.

2. Senescence studies

a) Anatomy

Hand sections were cut from leaf tissues mounted in pith using a razor blade. Selected sections were mounted in water on a slide and were examined by light microscopy using a Watson binocular microscope. A record of anatomical detail was made using a Kodak camera mounted on the microscope and fitted with an Ilford Pan F film.

b) Incubations

Sections of various lengths were cut from mature excised leaves and were incubated in petri dishes on filter paper moistened with water. The dishes were kept in the light or dark at approx. $20^{o}C$ for different times

as indicated in the results.

c) <u>Chlorophyll extractions</u>

To extract chloroplast pigments segments of leaf tissue were boiled in 80% ethyl alcohol for 10 minutes (approx. 5 cm^3/30 mg tissue) followed by washing in cold ethyl alcohol. After such treatment total extraction of the pigment was obtained. Alternatively sections were incubated in 80% acetone overnight followed by grinding of the tissue in the acetone in a pestle and mortar and then centrifugation at approx. 1500 xg to remove cell debris.

In both cases the extracts were made up to a standard volume using the respective solvents. Pigment content was analysed using a sample of the extract in a Perkin-Elmer spectrophotometer. The level of chlorophyll was taken as the absorption value at 660 n.m. A typical absorption spectrum for chloroplast pigments dissolved in ethanol is shown in Fig. 1.

d) <u>Plant Growth Regulator studies</u>

Solutions of kinetin were made by boiling the growth regulators in aqueous solution and then by making up to a standard concentration (5 mg/cm^3). Leaf segments were incubated by floating them lower surface down, on the kinetin solutions as required.

Fig. 1   Graph showing an absorption spectrum of chloroplast pigments extracted from Festuca Pratensis

From the graph the Optical Density

for chlorophyll = O.D. at 660 nm = 0.68
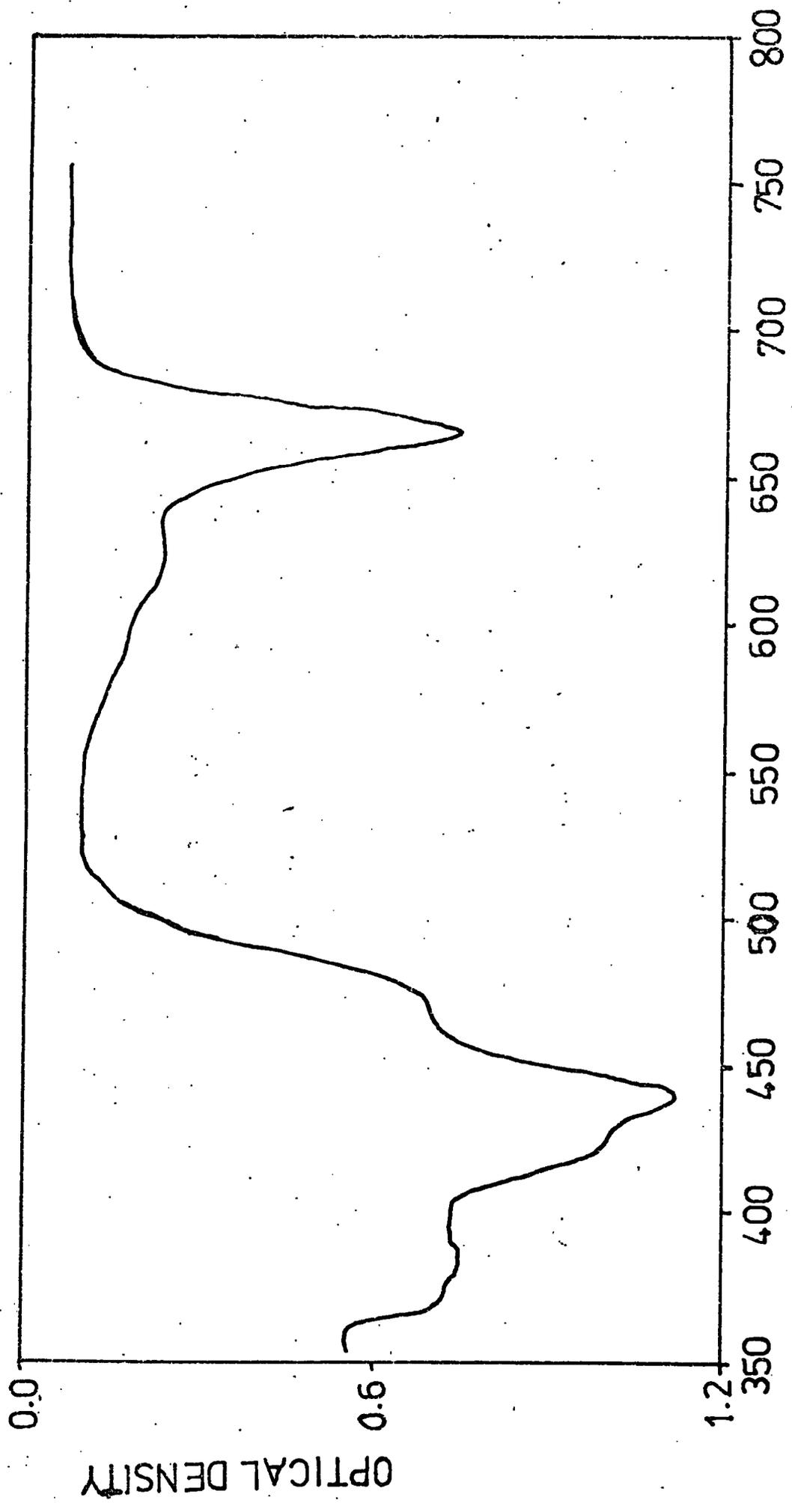
FIG 1

3. Abscission zone studies

a) Anatomy

Portions of senescing and non senescing leaves were embedded in paraffin wax by immersing them in molten wax at 80°C contained in a watch glass. The watch glass and contents was then plunged into cold water to solidify the wax as quickly as possible. The resulting blocks were cut to size with a razor blade and then sectioned on a Cambridge Rocker Microtome at 20 μm thickness.

Alternatively the sections were embedded in molten gelatin at 60°C which was allowed to cool naturally. The gelatin blocks were then cut to shape and frozen solid on a manual freezing microtome using liquid nitrogen before sectioning - sections were cut 20 μm thickness.

All of the above sections were stained for the presence of callose tissue by mounting them in 1% Aniline blue in 0.1N K_3PO_4 and examined at a wavelength of 257 nm under a Leitz fluorescence microscope. Photographs for later detailed examination were taken using a Kodak coloursnap camera mounted on a Watson microscope. Kodak Tri X film was used with Kodak D76 developer, Kodafix and printed on Kodak Veribrom paper with Ilfospeed developer.

b) <u>Dye experiments</u>

Whole plants with excised roots were incubated vertically in glass specimen tubes with the cut surface at the base of the plant approximately 4 mm below the surface of a 0.02% aqueous solution of either Eosin or Toluidine blue. The plants were held in place with cotton wool plugs and the majority of the length of their leaves protruded from the specimen tubes. At the end of the experiments leaves and their sheaths were carefully removed from the plants and the extent of dye movement was observed. Permanent record of this was made by taking photographs with a Kodak 35 coloursnap camera mounted on a microscope using a Kodak Kodacolour II film.

c) <u>Radiochemical experiments</u>

Confirmation of the dye results were sought by the use of radiochemicals. Aqueous solutions of [4-5 ^{3}H]Adenine or a mixture of ^{14}C amino acids were used having specific activities of 17.8 Ci/nmol and 56 m Ci/m atom carbon respectively.

In the undiluted form 1 µl of 3H adenine was injected into plants at one of the injection points shown in Fig. 2. while the plants were supported in vials containing water and incubated for 2 hrs in still air.

Fig. 2   Diagrammatic representation of a single
                Festuca leaf

                1 and 2 = points of injection with
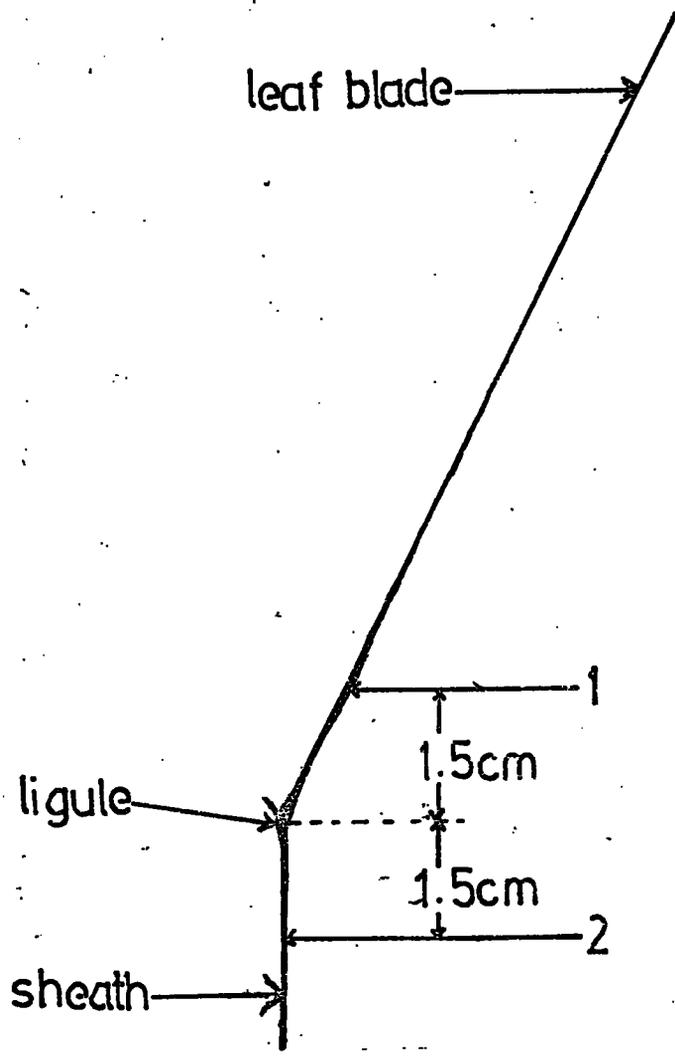
                                        radiochemical

leaf blade

ligule

sheath

1

1.5cm

1.5cm

2

FIG 2

Alternatively plants with excised roots were inoculated for 2 hrs vertically in still air in vials containing 3 µCi 3H Adenine or ^{14}C amino acids in 5 cm^3 of water.

After the required incubation period the distribution of the radiochemical was determined by the following methods.

(i) Portions of individual leaves above and below the ligule region were excised and after weighing were ground up in a pestle and mortar with 0.5 cm^3 of trichloroacetic acid so denaturing the protein. The ground tissue was then transferred to a scintillation vial and 10 cm^3 of scintillation fluid (2 vols toluene : 1 vol Triton X-100 4 gm Diphenyloxazole), was added. The grinding technique used gave a very fine homogenate which became finely dispersed in the scintillation fluid. The level of radio-activity was recorded in a Beckman LS 200B liquid scintillation system with the appropriate channel selection.

(ii) Following the incubation of the intact plant leaves were peeled off in succession and to prevent any further migration of the radiochemical they were subjected to freeze drying overnight. This was carried out by clamping the leaves between two pieces of filter paper and wire gauze, flooding them with liquid air and transferring them to a dessicator and moisture trap. Following this treatment

sections of tissue 1 cm above and below the ligule were taken and placed in age sequence on a Kodak x-ray plate and the leaves and plate were clamped between two pieces of perspex for 6 days in a dark cupboard to allow adequate exposure time. After this period the plates were developed using Phenisol developer and Kodafix fixer mounted as shown in the results.

d) Scanning Electron Microscopy

Sections of the ligule region of senescing and non senescing leaves were cut using a razor blade and mounted in silver D.A.G. and examined on a Cambridge SEM 600 scanning EM at an acceleration voltage of 7.5 KV at the magnifications shown on the prints. Photographs of the whole ligule in transverse section were taken at the lower magnification and of individual vascular bundles at the higher magnification using a Practika camera and Ilford F.P. 4 filter for later detailed examination.

RESULTS

a) <u>Senescence studies</u>

Using light microscopy and a pith and razor blade method of sectioning an anatomical study of the whole length of mature Festuca leaves was carried out. This investigation was carried out to determine the distribution of the tissues within the leaf and in particular the arrangement of the vascular tissue as well as to assess any readily observable differences in leaf structure during the ageing process remembering that the leaves are sequentially older from the tip to the base.

Representative sections at points along the leaf were photographed as shown in Fig. 3.

The leaf is typical of monocots and has well pronounced and supported vascular bundles. Around each vascular bundle can be found a sheath of cells which suggests Kranz anatomy, however there is no evidence that this particular species shows the Hatch-Slack photosynthetic pathway (H. Thomas - personal communication).

During the ageing of the tissue no distinct loss of cell integrity could be observed along the leaf age sequence. Even when the tissues were yellowing there was no evidence of cellular collapse - Fig. 3a. However there

Fig. 3.   Sections of Festuca Leaf taken at different

regions along the leaf.

a) from tip of leaf (yellowing) - oldest tissue
    ct - thickened walls

b) from mid-region of leaf - medium age

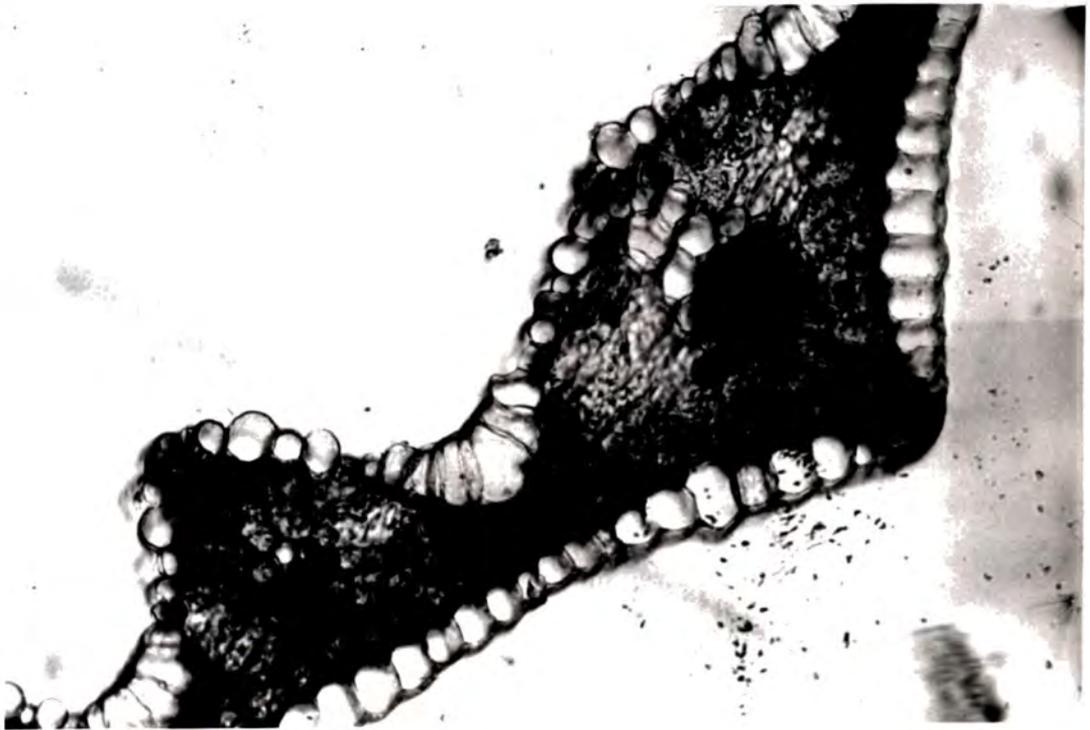c) from base of leaf - youngest tissue

a.

ct
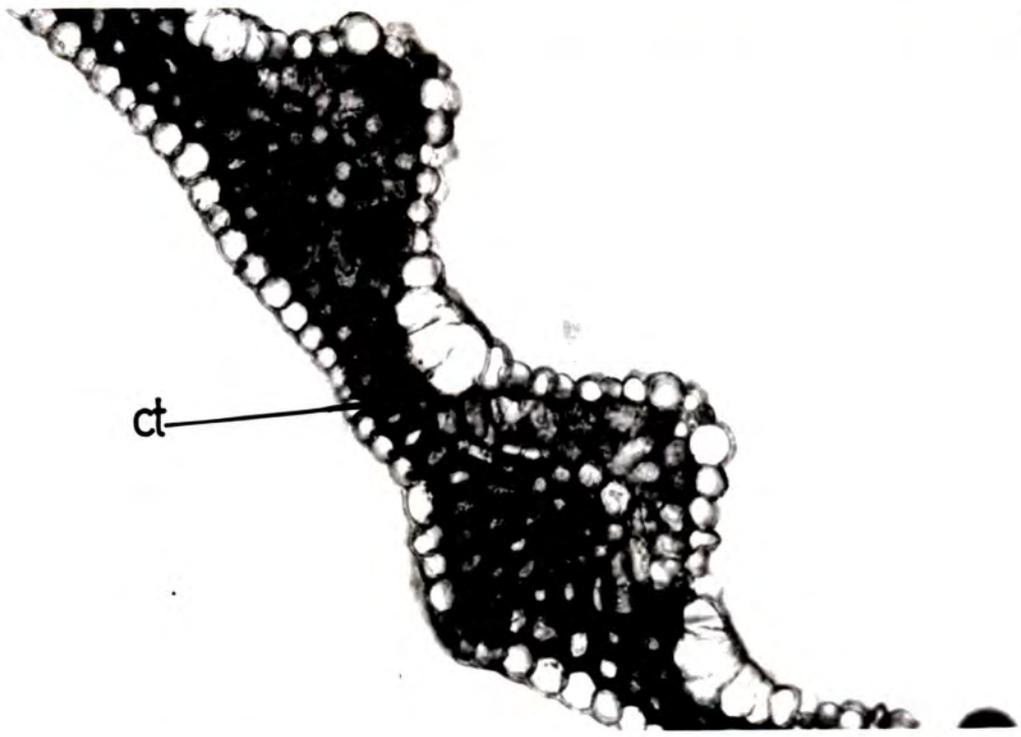
b.

FIG 3.

c.

FIG 3

was an apparent thickening due to deposits of some kind in the walls of some of the mesophyll cells in the older tissue (ct). This may represent a deposition of waste materials from within the plant as a whole or the result of metabolic changes causing changes in cell wall structure which could restrict the flow of compounds in or out of the cells.

Since there were few structural changes observable at the anatomical level during the ageing process the progress of senescence was followed as a change in the chlorophyll content of the leaves as this parameter would not be evident from anatomical studies.

To determine if there is any significant change in the senescence rate along the age sequence of the leaf mature excised leaves from equally aged different plants were cut into 1 cm length sections using a razor blade. Each individual section was then cut longitudinally along the midrib, which was possible due to the bilateral symmetry of the leaf, so providing two essentially the same sets of leaf sections.

One set of leaf section halves had their chlorophyll extracted immediately following excision whilst the replicate batch was incubated for 5 days in the dark on water prior to their chlorophyll determination - results
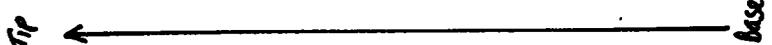
shown in Figs. 4 and 5.

The table shows that there is a gradual increase in the chlorophyll content of the leaf from the tip to two thirds down the leaf which relates to the age sequence down the leaf.

The graph shows that the rate of senescence along the leaf is not uniform and in fact it indicates three main areas of higher chlorophyll degradation. These results are similar to those reported by Hedley 1972 for the incorporation of labelled precursors into protein. There is apparently a peak of activity which occurs in young tissue and possibly corresponds to metabolic activities in building up new tissues, a static peak corresponding to general metabolic activities and a third peak in old tissues corresponding to degradation activities. Whilst the results here may not exactly correspond to those of Hedley the implication is that there are zones along the Festuca leaf where enhanced senescence activity takes place. This probably indicates differences in metabolic activity.

In many studies the indications are that the rate of senescence of excised leaves is accelerated by allowing them to senesce in complete darkness

This was investigated in Festuca as follows:-

Fig. 4 - Table showing the % loss of Chlorophyll in differently aged

regions of leaf

Region of Leaf	Approx. age of leaf sections in weeks	Optical Density of initial chlorophyll content	Optical Density of chlorophyll content after 5 days	% loss of Chlorophyll
1	14	0.045	0.005	88.8
2	13	0.10	0.015	85.0
3	12	0.14	0.03	78.0
4	11	0.19	0.05	73.0
5	16	0.22	0.05	77.0
6	9	0.21	0.06	71.0
7	8	0.21	0.06	71.0
8	7	0.22	0.06	72.0
9	6	0.25	0.07	72.0
10	5	0.25	0.06	76.0
11	4	0.23	0.08	65.0
12	3	0.24	0.07	70.0
13	2	0.22	0.07	68.0
14	1	0.19	0.06	68.0

Tip

base

Fig. 5   Graph showing the % chlorophyll loss with
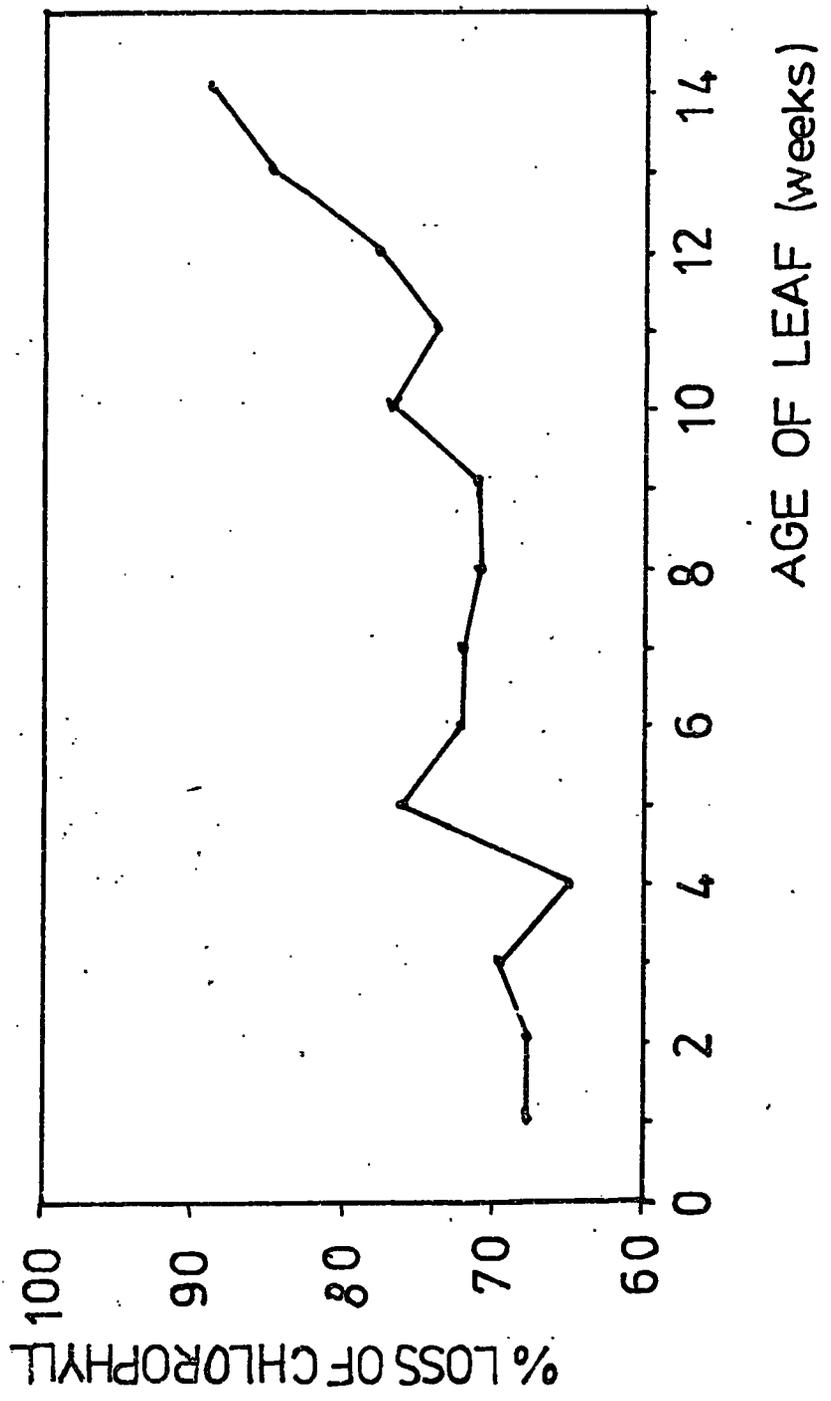   regard to the age of the Leaf sections (Festuca)

FIG 5

Mature excised leaves from equally aged different plants were cut into 3 cm length sections and each section was cut longitudinally. The resulting leaf sections were then divided into three groups. One set of leaf section halves had their chlorophyll content determined immediately, a second set was allowed to senesce in the dark for 6 days before determination and a third set in a light/dark environment (12 hrs light 12 hrs dark) before chlorophyll determination. All of the leaf sections were kept at a constant temperature throughout the experiment and the results are shown in Figs. 6 and 7.

With reference to Figs. 6 and 7 the rate of senescence at all points along the leaf is slightly greater in a light/dark environment than in complete darkness. This experiment demonstrates that complete darkness in these senescence studies was not required to accelerate senescence in Festuca.

The rate of senescence was also studied to determine if the levels of senescence so far were really a function of the time when the sections were examined. Mature excised leaves of a similar age were taken and cut into 3 cm length sections from the tip of the leaf to the base and each section was cut longitudinally as before. The resulting leaf section halves were divided into four sets

Table showing the % loss of chlorophyll in senescing leaves exposed to complete darkness

and a light/dark environment

Region of Leaf	Approx. Age of leaf section (weeks)	Original chl. content (O.D.)	Chl. content in dark after 5 days	% loss in dark	Chl. content in light/dark O.D.	% loss in light/dark
1	10	0.40	0.19	52%	0.19	52%
2	8	0.88	0.39	55%	0.33	62%
3	6	0.94	0.50	46%	0.44	53%
4	4	0.91	0.49	46%	0.44	51%
5	2	0.62	0.55	11%	0.45	27%

TIP

BASE

FIG. 6

Fig. 7   Diagram showing the % chlorophyll loss with
          regard to the age of the leaf sections (Festuca)

          Broken line   Section allowed to senesce in
                           light/dark conditions

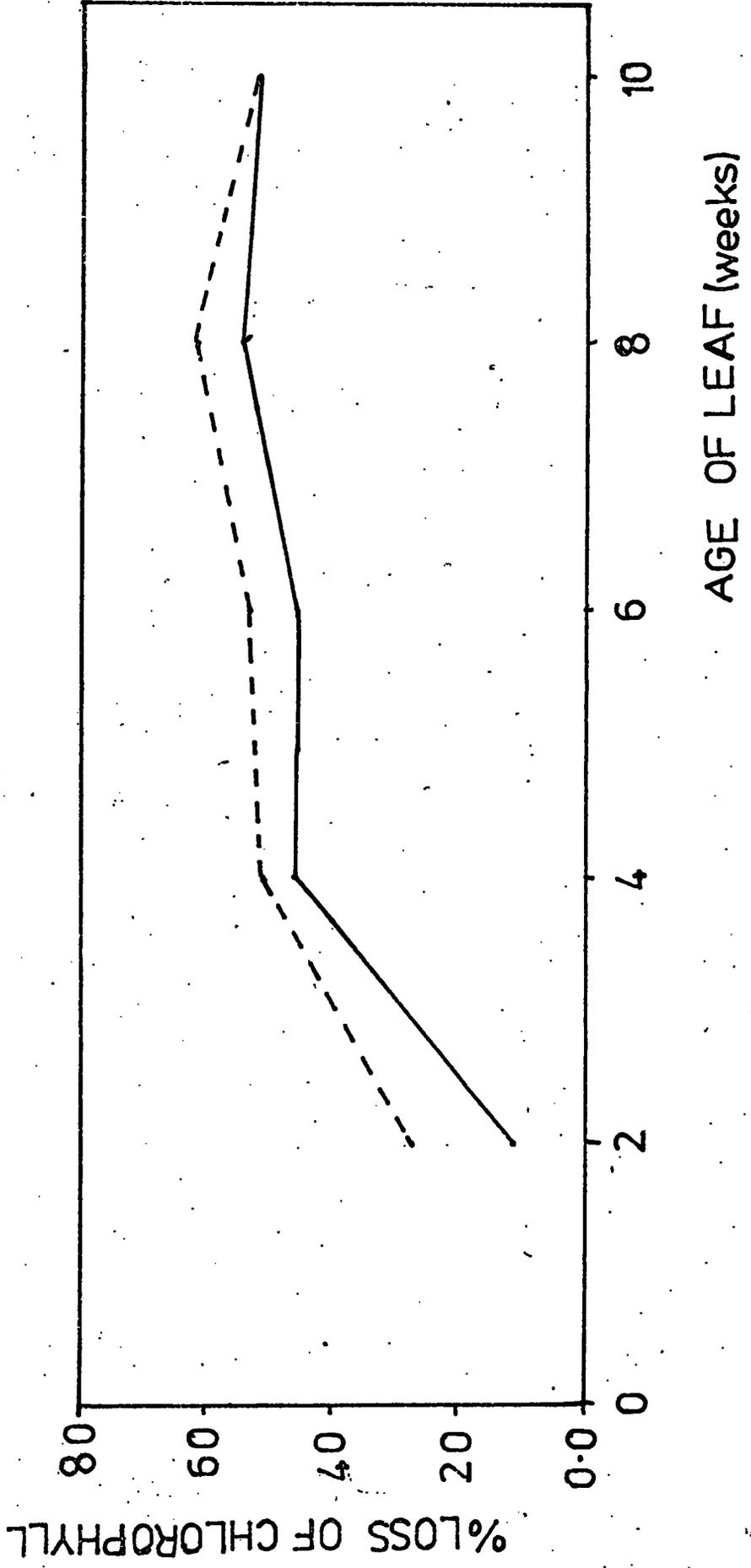   Unbroken line   Sections allowed to senesce in
                           the dark.

FIG 7

AGE OF LEAF (weeks)

%LOSS OF CHLOROPHYLL

and treated as indicated below:-

Set 1. Used for initial chlorophyll estimation

Set 2. Allowed to senesce for 2 days   )
) in light/dark
Set 3. Allowed to senesce for 4 days   ) conditions at
) constant temp.
Set 4. Allowed to senesce for 6 days   )

Using both the acetone and alcohol extraction method
very similar results were obtained. The table and graph
(Figs. 8 and 9) show the results using an acetone extraction
for chlorophyll and a weight correction factor for any weight
discrepancies in the amount of tissue used.

The results again indicate the presence of three peaks
of metabolic activity associated with senescence (Hedley) as
described earlier. However this pattern is not established
until the leaves have reached a late stage of senescence and
may reflect a gradual accumulative change in the metabolic
rate rather than an abrupt one.

The results so far have indicated that there appears to
be little change in the gross anatomy of the leaf during the
senescence process but chlorophyll degradation does take place
along the leaf. The rate of senescence is not constant along
the length of the leaf and three distinct peaks are evident,
the second peak lagging behind the others in the early stages
of senescence.

Fig. 8. Table showing % chlorophyll loss after 2,4 and 6 days of senescence in Light/Dark conditions

Chlorophyll Degradation

SECTIONS	Approx. age of leaf Sections	Wt. of original sample (mg)	Original Chlorophyll level	Wt. of 2 day senescence sample (mg)	Chlorophyll level of 2 day senescence sample	% loss of chlorophyll	Wt. of 4 day senescence sample (mg)	Chlorophyll level of 4 day senescence	% loss of chlorophyll	Wt. of 6 day senescence sample (mg)	Chlorophyll level of 6 day senescence sample	% loss of chlorophyll
1	14	18	16	14	4	90.8%	17	2	96.2%	10	0	100%
2	12	20	14	20	6	87.8%	30	8	89%	12	2	93%
3	14	30	15	27	24	66%	32	7	88%	22	0	100%
4	8	35	24	45	26	76%	35	6	92%	40	4	96%
5	6	35	51	45	13	94%	38	8	95%	40	3	96%
6	4	35	44	42	18	90.4%	32	9	94%	40	7	96%
7	2	45	33	35	12	86.6%	35	10	89%	50	17	87%

Tip ←

Base →

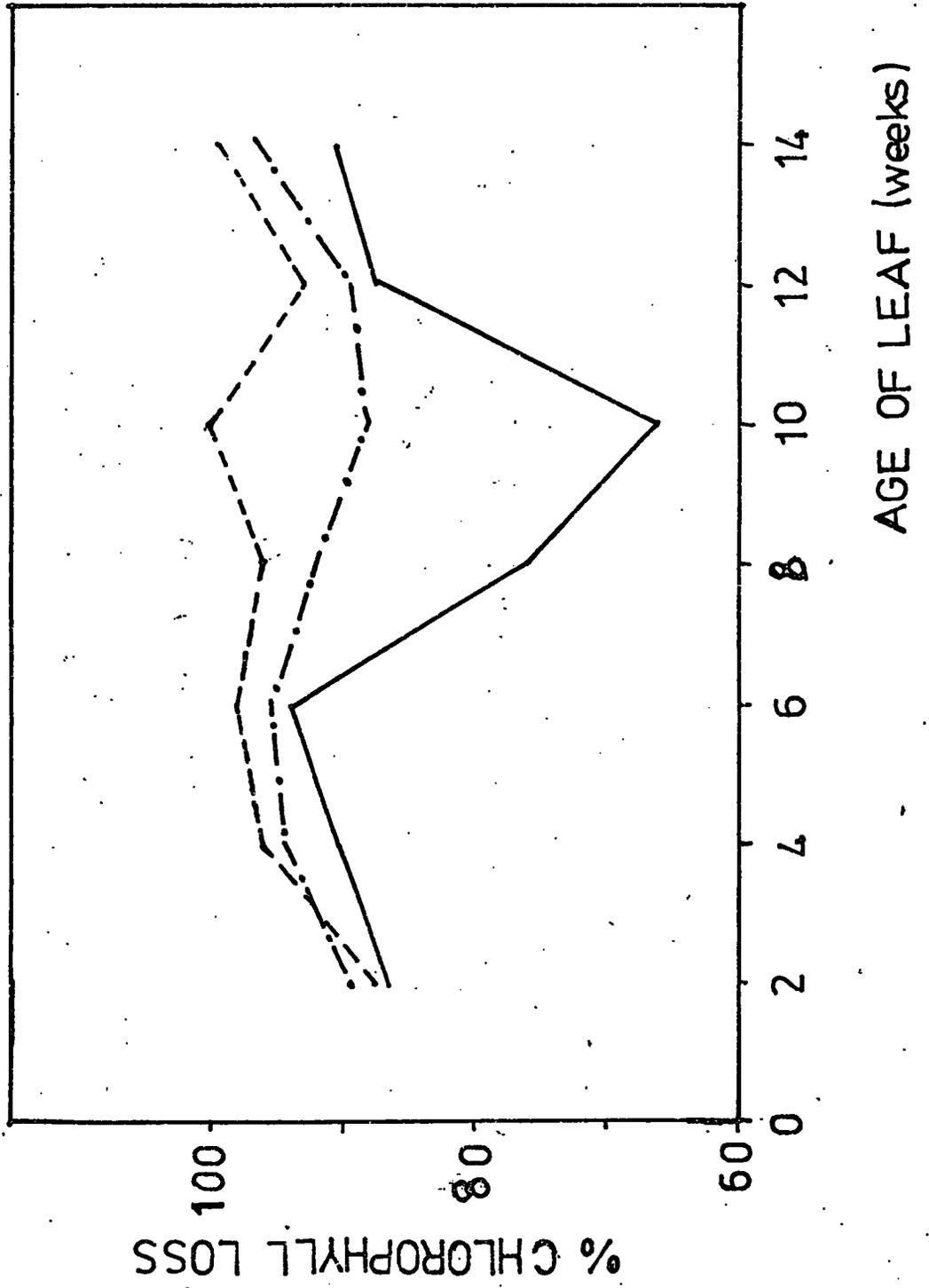Fig. 9   Diagram showing the % chlorophyll loss

           with regard to the age of leaf sections (Festuca)

           continuous line = 2 day senescence samples

           ——·——·——.   = 4 day senescence samples

           —— —— — — — —   = 6 day senescence samples

FIG 9

Once a leaf reaches maturity it begins to decline in activity. This suggests that there is a point at which the leaf's usefulness to the plant ceases and that it then becomes isolated from the rest of the plant. As the ageing is from the tip it suggests that there is a loss of function in this region and a removal of cellular components from this zone (yellowing). This being so then there may be a prevention of the movement of materials into the leaf and a possible "cut off" or abscission zone in the leaf structure initiating or controlling senescence.

b) "Abscission zone" studies

As the leaf blade senesces along its whole length it seemed reasonable to assume that if an abscission zone did exist then it would occur towards the base of the leaf and possibly below the ligule. To examine for the presence of an abscission zone an anatomical investigation was made of the leaf sheath below the ligule.

This region in senescing leaves was initially investigated by cutting it into cm portions and sectioning them as detailed in the method. Examples of the sections obtained are shown in Fig. 10.

No area of general tissue collapse (particularly in the vascular bundles) was noted even in the oldest tissue examined. However the photographs show that from the base

Fig. 10.   Photographs showing sections taken from the

leaf sheath of Festuca Leaves

from (1) the tip of the sheath (nearest ligule)
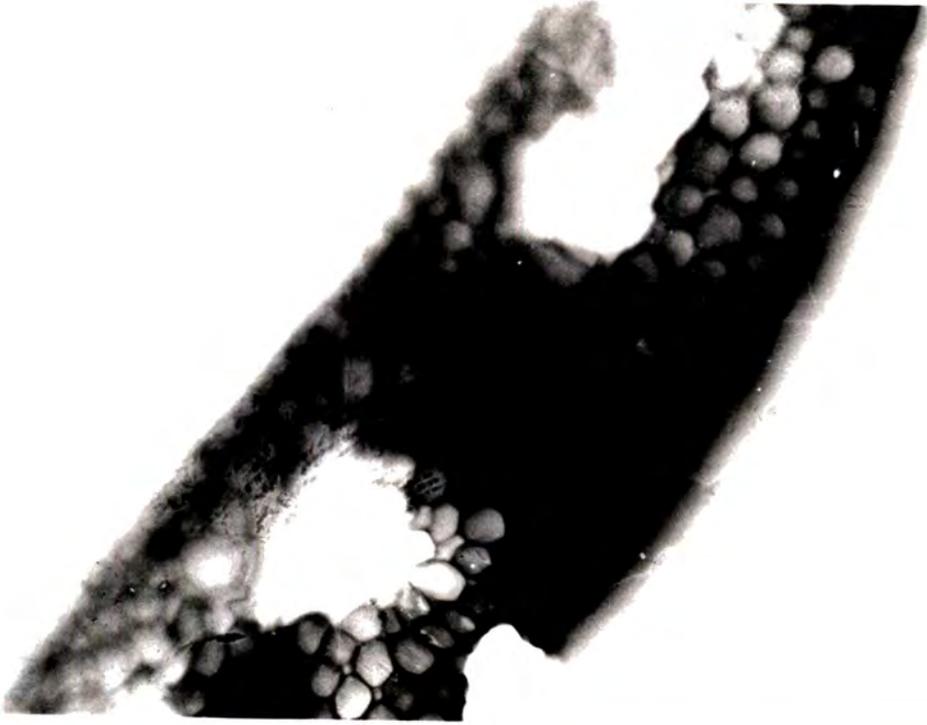
to (5) the base of the sheath.

1.
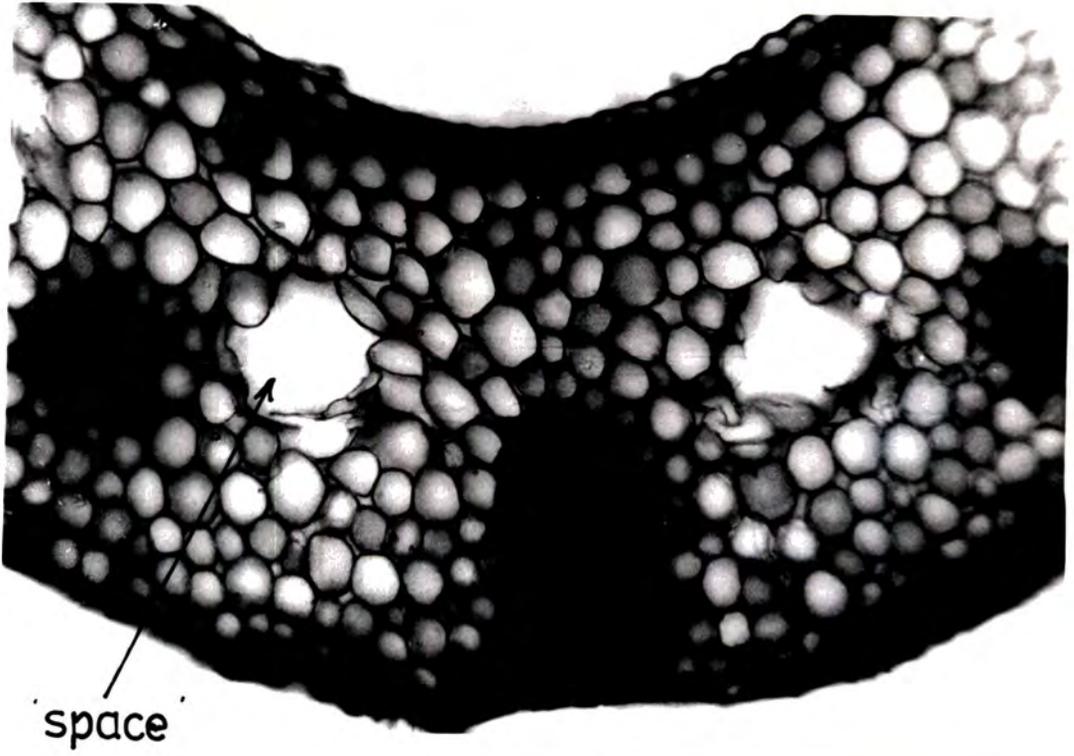
2.

FIG 10.

3.

4.

FIG 10.

5.

FIG 10

of the leaf sheath to the ligule that there is an increase

in size of spaces in the parenchyma tissue. This could have

been due to the rupture of the cells during sectioning but

there is little evidence to support this theory as the

cells around the edges of the spaces appear intact. It

seems more probable that the need for photosynthetic tissue

towards the ligule is reduced and so this tissue is broken

down leaving the vascular tissue intact and also creating

a lighter sheath. Also at the base of the leaf there are

collecting tissues that feed material from the plant into

the leaf.

On examining the vascular tissue more closely there is

little evidence in the sheath of an "abscission zone" or

any form of blockage in the conductive tissue. To

supplement the anatomical investigation and to try and

locate more precisely the position of a possible

abscission zone an investigation was made of the movement

of materials into the leaf. For this study use was made

of various vital dyes.

Intact plants were taken and the roots were excised

by a transverse cut under water. The cut end was then

immersed in dye solution in an incubation vial and the

plants placed in a growth cabinet under fluorescent light

with an air flow to induce transpiration.

In all leaves the vital dyes were taken up in varying
degrees depending upon the age and surface area of the
leaves as well as their rate of transpiration. In young
leaves the dye could be clearly seen in the leaf veins
of the leaf blade but in the senescing and fully senesced
leaves (totally yellow/brown) the dye appeared to travel
in the vascular strands as far as the ligule region
(see Fig. 1i) and a well defined band of stain could be
seen at the point in the leaf sheath where the ligule
emerged. (See Appendix II).

Longer incubation periods of three days or more
again showed that there was some movement of dye passed
the ligule particularly so in the younger leaves but there
was always a clearly defined build up of dye at the ligule
suggesting that the ligule may be acting as a control point
for the entry of compounds into the leaf. In the
older leaves as virtually no movement took place beyond the
ligule suggests that if an "abscission zone" does exist
then it most probably is in this region.

Whilst the dyes indicated that there was a restrictive
zone at the ligule there was the possibility that the dyes
were actually staining the ligule tissues rather than their
passage being retarded giving the impression that a blockage

Fig. 11(a)   Photograph showing the ligule region of a
              young leaf - red/brown coloration is the
              dye in the leaf veins.

     11(b)   Photograph showing the ligule region of
              an old leaf - red/brown coloration is the
              dye in the leaf veins.

ligule region →

FIG 11 a

ligule region →

FIG 11 b

existed. Further investigations were thus carried out which involved the use of radiochemical tracers and in particular compounds which were natural metabolites of the leaves - amino acids and adenine.

Exploratory experiments were carried out using whole Festuca plants with excised roots. The plants were incubated for 2 hrs in radioactive adenine (5 μCi/ml) and after the incubation period suitably aged leaves (old, medium age and young) were cut into four sections as indicated by the following Fig. 12.

Each section was weighed ground in a pestle and mortar and the radiochemical content was determined. The results obtained are shown in Figs. 13 and 14.

Fig. 15 indicates that in all of the leaves the ligule had retarded the transport of ^{14}C adenine to some extent but this is particularly noticeable in the medium age and oldest leaves. Fig. 14 shows again that the ligule has acted as a barrier for adenine transport. In the medium age and more so in the oldest leaf it seems to be preventing adenine transport as there is a decreasing level of radioactivity as the distance above the ligule increases. In comparison with the young leaf there was a gradual build up of radioactivity as the distance above the ligule increases indicating transport across the ligule.

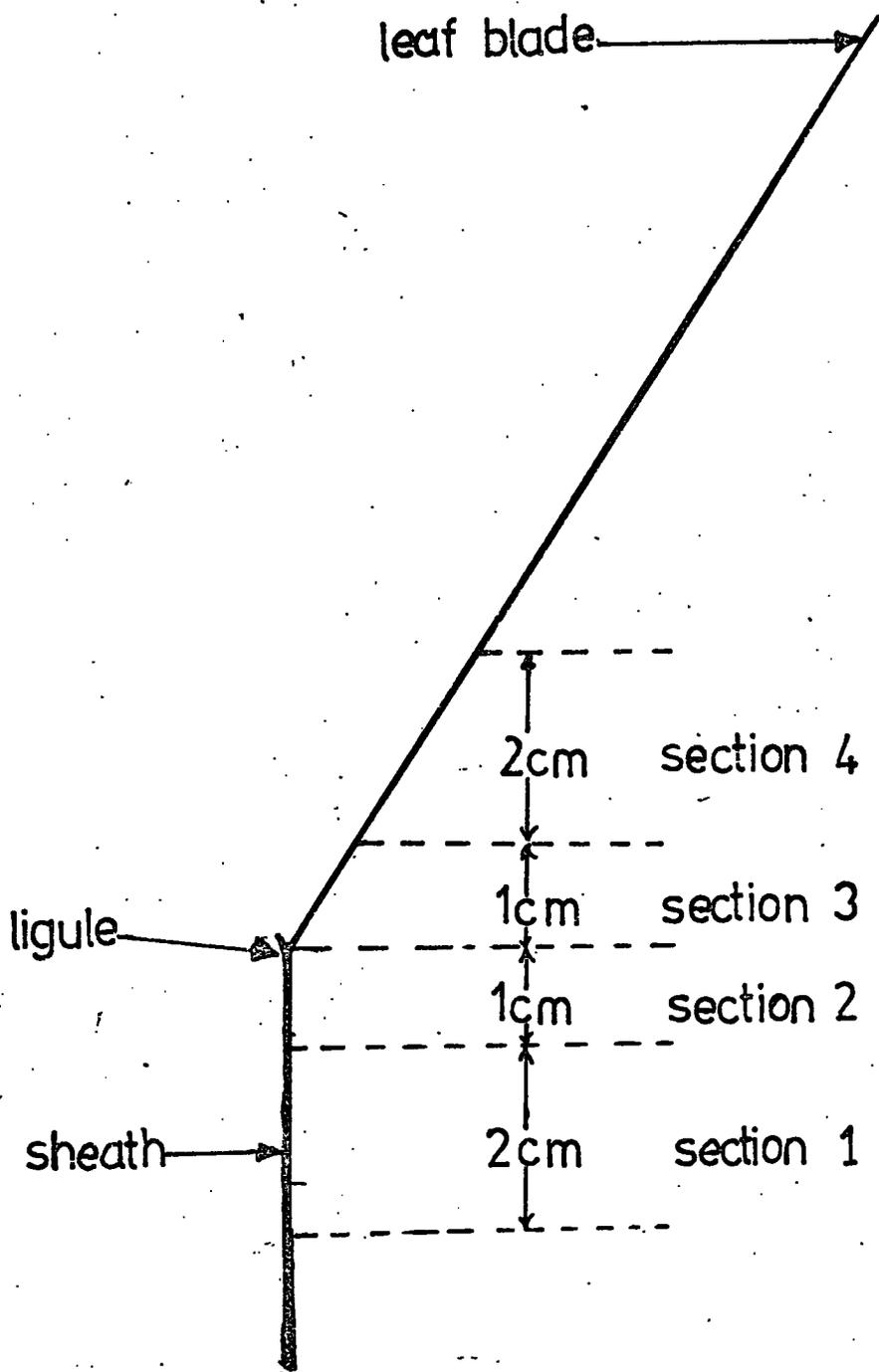Fig. 12   Diagrammatic representation of a Festuca pratensis leaf showing the leaf section taken.

leaf blade

2cm section 4

1cm section 3

ligule

1cm section 2

sheath

2cm section 1

FIG. 12

Fig. 13   Graph showing the % radioactivity above
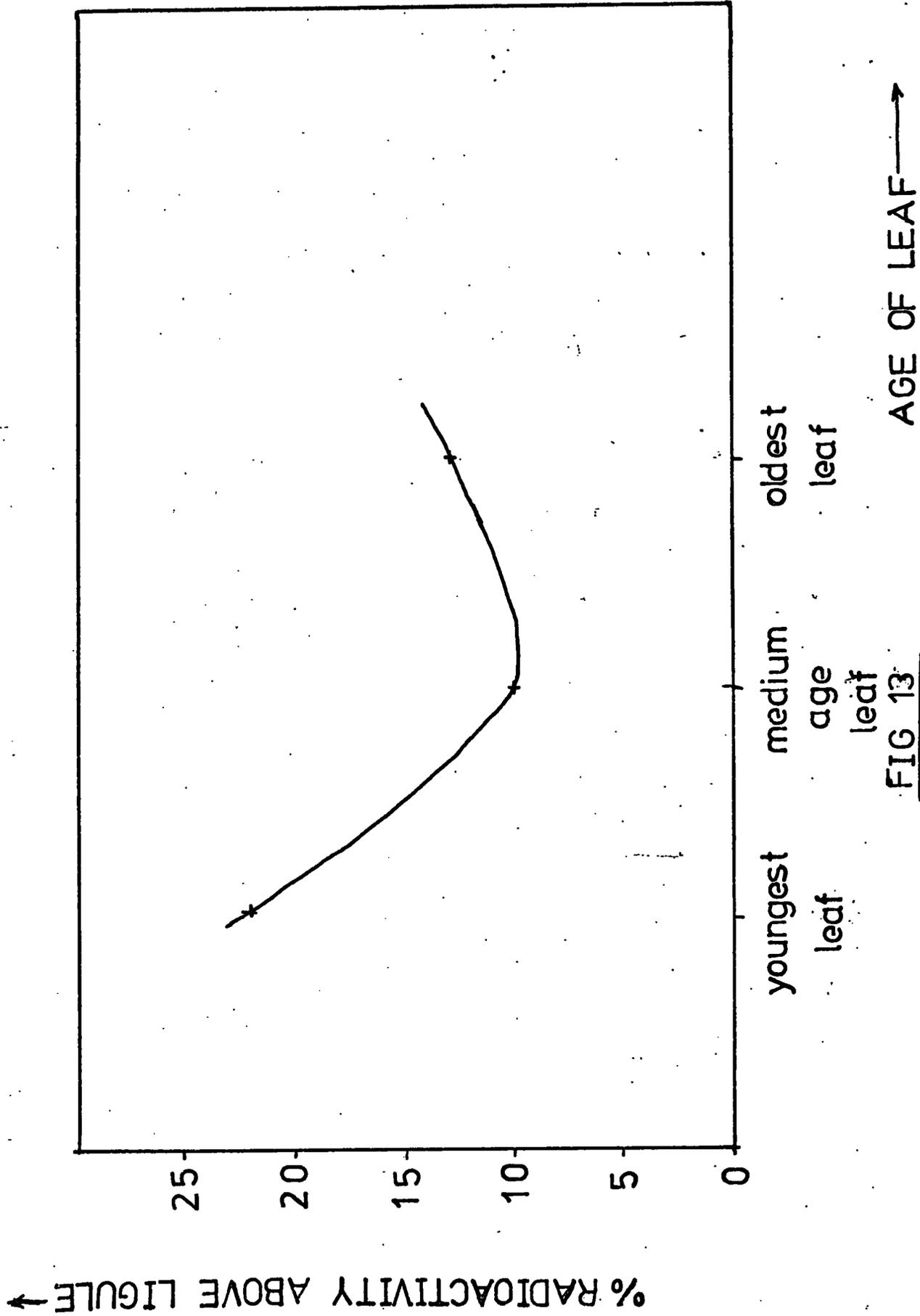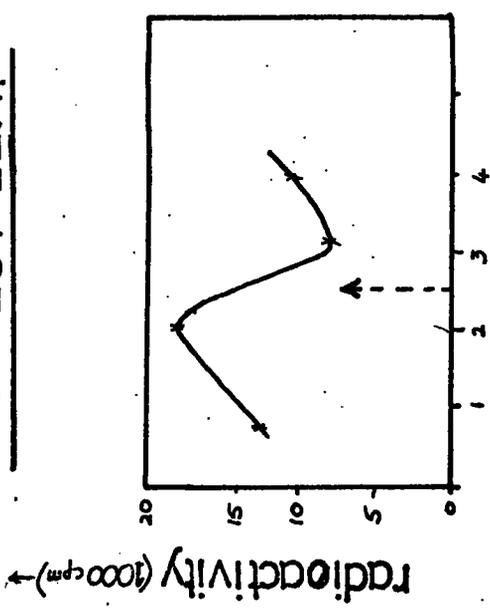   the ligule with regard to the age of the
            leaves (Festuca)

FIG 13

AGE OF LEAF →

% RADIOACTIVITY ABOVE LIGULE →

youngest leaf   medium age leaf   oldest leaf

Fig. 14   Graphs showing the % radioactivity along
            the length of differently aged leaves

            N.B   The arrow ---> indicates the position
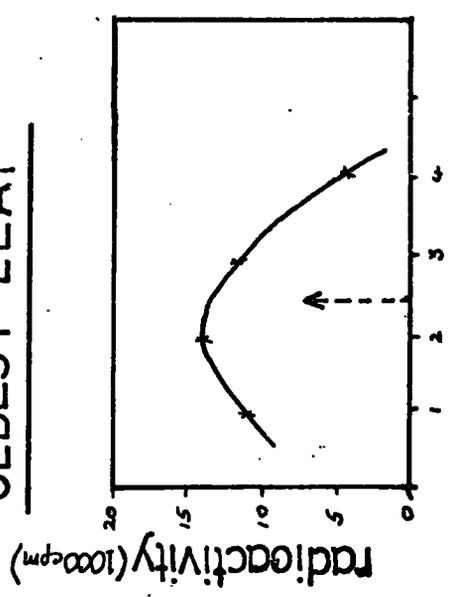   of the ligule.

YOUNGEST LEAF

radioactivity (1000 cpm) →

leaf section →

MEDIUM AGE LEAF

radioactivity (1000 cpm) →
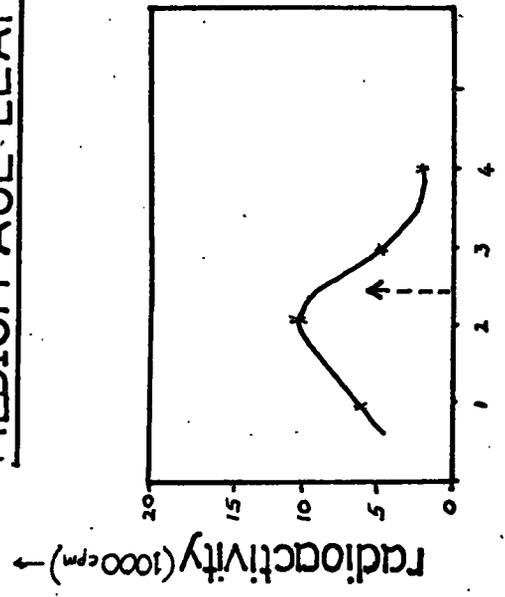
leaf section →

OLDEST LEAF

radioactivity (1000 cpm)

leaf section →

FIG 14.

Although the results indicated a differential transport across the ligule this may have been a function of the distance travelled through the leaf sheath i.e. in older sheaths transport may be slower.

In order to compensate for this a further experimental design was employed where 1 μl of ^{14}C amino acid mixture was applied by microsyringe to the leaf sheath 1.5 cms below the ligule in order to reduce the distance travelled into the leaf blade. Similarly 1 μl of the amino acid mixture was applied to the leaf blade 1.5 cms above the ligule.

After an incubation period of 2¼ hrs 0.5 cm portions from either side of the ligule were taken. The samples were weighed, denatured by immersing in 0.5 ml of trichloro-acetic acid, ground in a pestle and mortar and their radio-activity was recorded as detailed in the method. The results are presented diagrammatically in Fig. 15 (see Appendix I for full table of results).
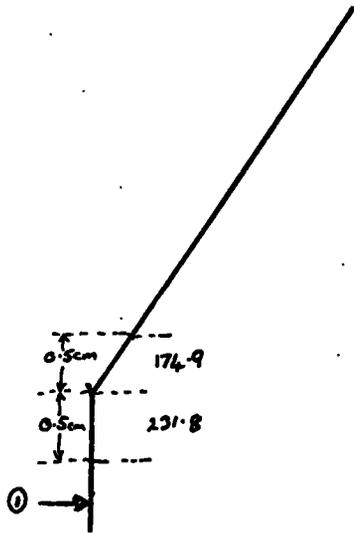
A) shows the movement of amino acids up the leaf. The ligule in all cases is offering a barrier to transport and this again is most noticeable in the older leaves.

B) shows the downward movement of amino acids. In the older leaf the ligule does appear to have some retarding effect on transport but the results do suggest that the ligule is acting as a one way controlling mechanism i.e.
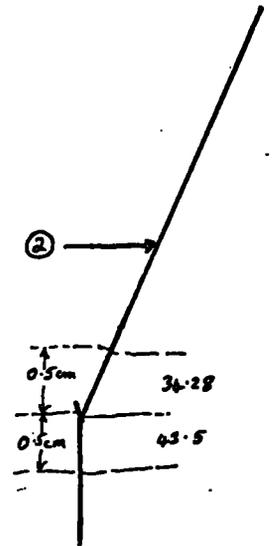
Fig. 15   Diagrammatic representation of Festuca Leaves

(a) Figures are the amounts of radioactivity in
c.p.m. in the 0.5 cm section above and below
the ligule.
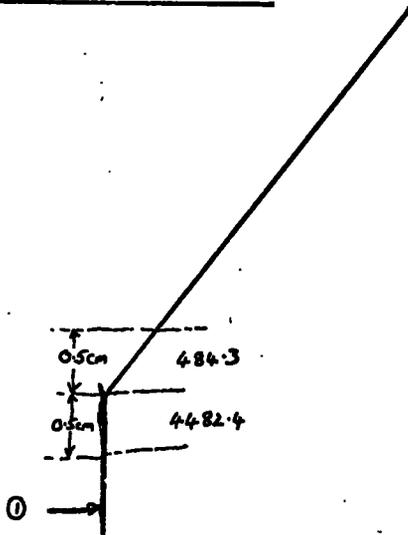
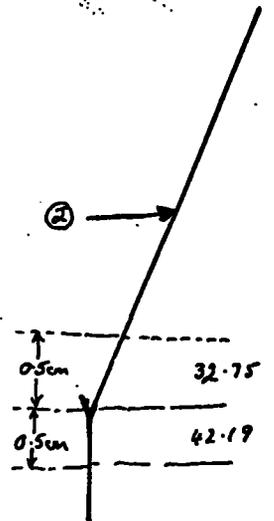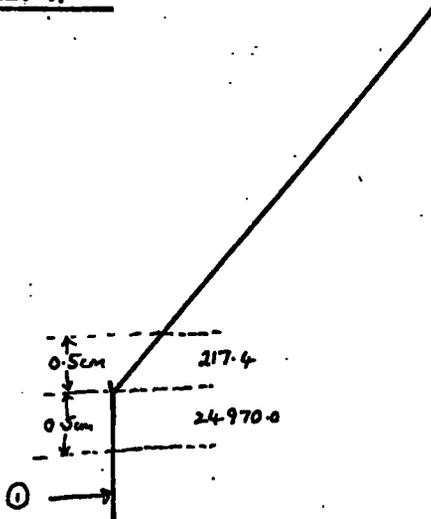(b) 1 and 2 are the pts of injection with radio-
chemical.

A

YOUNG LEAF

0·5cm — 174·9
0·5cm — 231·8
0 →

MEDIUM AGE LEAF

0·5cm — 484·3
0·5cm — 4482·4
0 →

OLD LEAF

0·5cm — 217·4
0·5cm — 24970·0
0 →

B

YOUNG LEAF

② →
0·5cm — 34·28
0·5cm — 43·5

MEDIUM AGE LEAF

② →
0·5cm — 32·75
0·5cm — 42·19
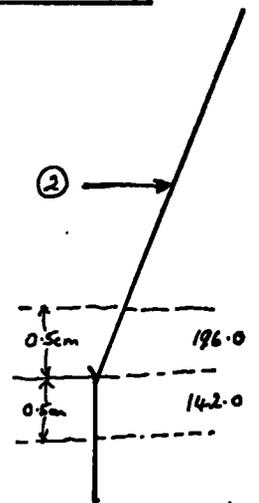
OLD LEAF

② →
0·5cm — 196·0
0·5cm — 142·0

FIG 15

from the base to the tip of the leaf. When the amino

acids are placed above the ligule there is no accumulation

of material and the radioactive material could have moved

up or down the leaf as there is no direct evidence from

this experiment as to where it ends up.

Further similar results were obtained by incubating

whole plants in ^{14}C amino acids for 1½ hrs (5 µCi/ 5 ml)

and again determing the radioactivity level in the 0.5 cm

section above or below the ligule - Figs. 16 and 17.

Key leaves   1 and 2   -   completely senesced

   leaf   3   -   yellow/green

   leaf   4   -   yellow tip (1 cm) - rest green

   leaf   5   -   complete by green

The graph - Fig. 17 show clearly that the proportions

of radioactivity above the ligule is reduced in older

tissue.

It is possible that the extraction methods used so

far could have been giving a distorted representation of

results and therefore an *in situ* analysis of the distribution

of the label was made using autoradiography.

Whole plants with excised roots were incubated for

3 hrs in a mixture of ^{14}C amino acids (2.5 µCi/ml). The

separated leaves after freeze drying to prevent any further

		Total Radioactivity in leaf section (cpm)	Radioactivity above ligule (cpm)	% Radioactivity above ligule
	1 (12 weeks)	5442.6	89.6	1.6%
	2 (8 weeks)	1330	232	17.4%
Age of leaf	3 (6 weeks)	684	104.5	15.2%
	4 (4 weeks)	248.5	133.6	54.2%
	5 (2 weeks)	251.9	137.8	54.58

FIG. 16

Fig. 17.   Graph showing the % radioactivity above the
             ligule with regard to the age of the leaves.

FIG 17

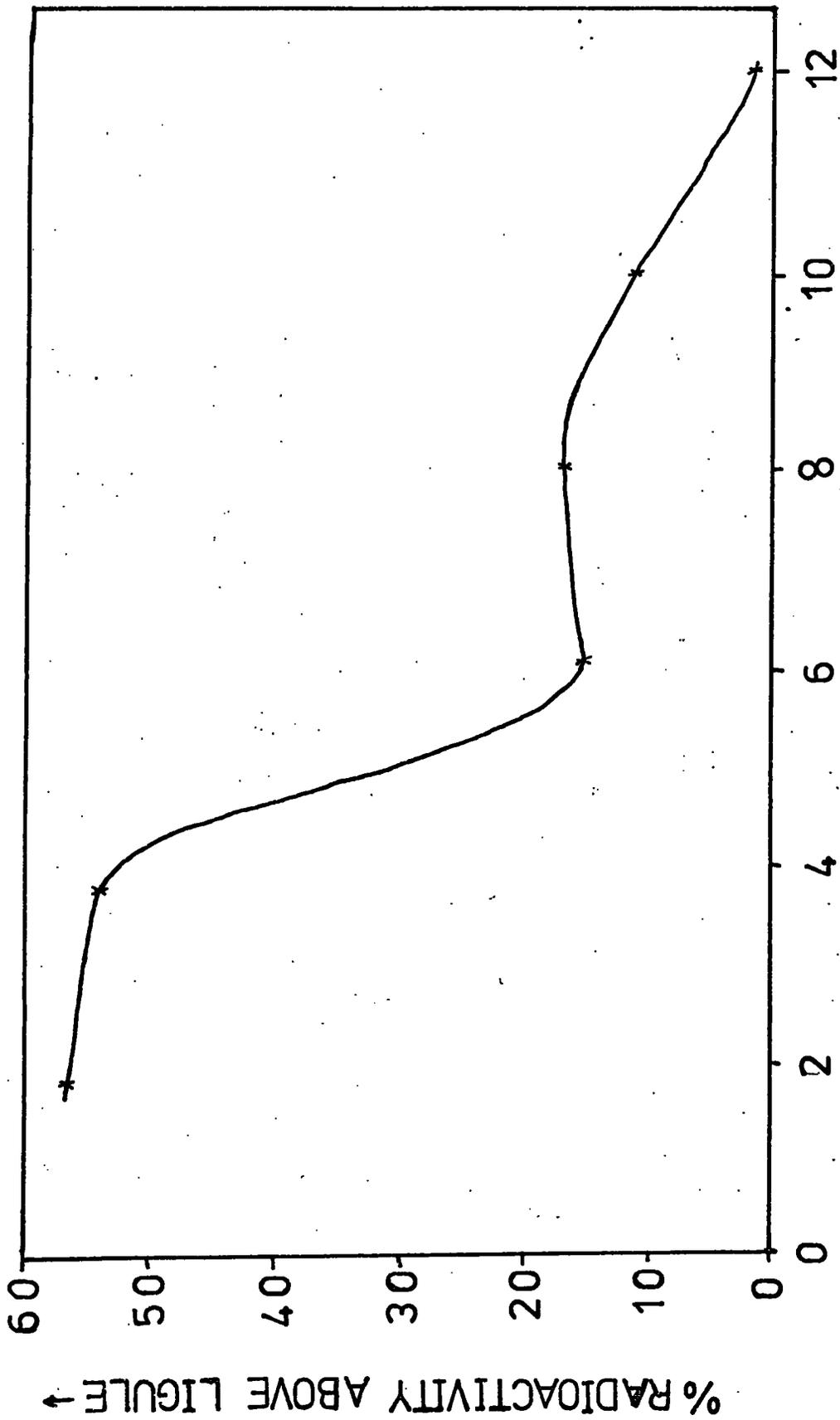Y-axis: % RADIOACTIVITY ABOVE LIGULE →

X-axis: AGE OF LEAVES (weeks) →

migration of radiochemical were exposed to x-ray plates
and the developed plate is shown over page - Fig. 18.

The plate shows autoradiographs of the ligule region
and a portion either side of the ligule from the oldest
leaf on the plant (1) to the youngest (8). The results
show in the youngest leaves (5 to 8) that the radiochemical
is present on both sides of the ligule but in the older
leaves which were yellow/brown in colour (4 to 1)
that the transport of radiochemical in detectable amounts
has been prevented in an upward direction. In conclusion
this experiment has confirmed previous findings and there
is indeed a real differential distribution of material
across the ligule and a variation with age.

Since there is an apparent blockage at this zone in
older leaves, it was investigated to see if this was a
physical blockage which could be detected.

Sections of the ligule region of young and senescing
leaves were cut using a freezing microtome. On staining
these for the presence of callose with a fluorescent dye
and examining them under a fluorescent microscope the
photographs - Fig. 19 were obtained.

The tissue used for sectioning was extremely fragile
and great difficulty was experienced in cutting the sections.

Fig. 18   Photograph of autoradiograph of Ligule region of

Festuca leaves in age sequence

<u>N.B.</u>   The outline of the leaves have been drawn on

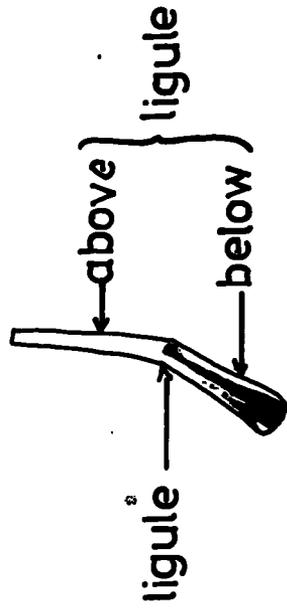the autoradiograph for clarrification.

FIG18

1. Oldest leaf.

above
below
ligule
ligule

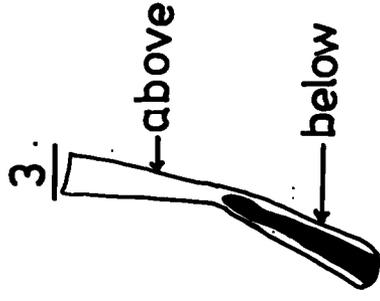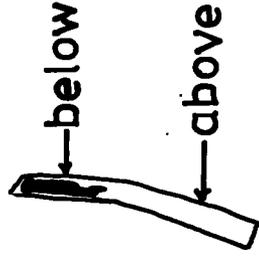2. below above

3. above below
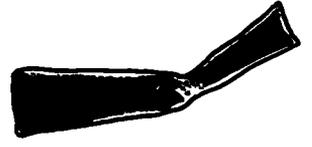
4.

5.

6.

7.

8. youngest leaf.

Fig. 19 (a)   LS of ligule region of young Festuca
              leaves

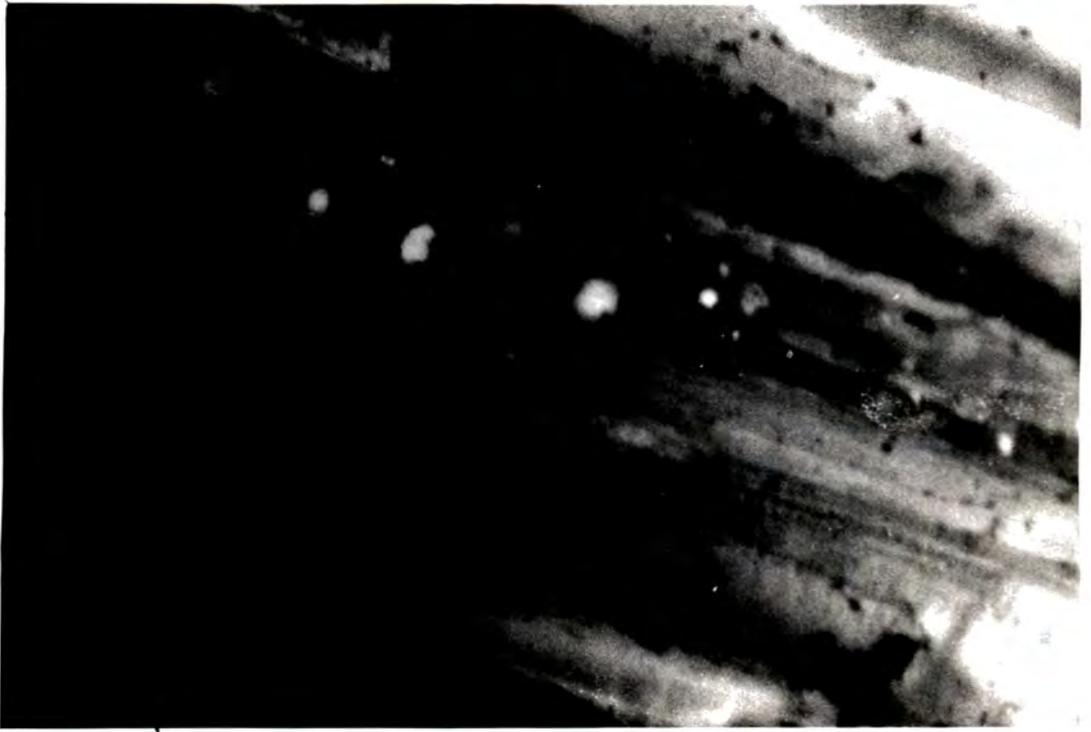   19 (b)   LS of Ligule region of Old Festuca
              leaves (senescing)

FIG 19 a

FIG 19 b

However the sections do show that there is no apparent collapse of tissue. Both the sections from the young ligules and old show the presence of callose particularly at the sieve plates of the phloem but there is no discernible difference between old and young tissue.

A scanning electron microscope was then used to look directly at the cells of the ligule region. Sections of the ligule region were cut using a razor blade, mounted, examined and photographed. A selection of the photographs is shown in Fig. 20.

In the green, yellowing and fully senesced tissue the vascular bundles are clearly defined and there is no obvious collapse of tissue. Detailed examination of the bundles from the green tissue (young) show that all of the cells are intact and there are no obvious blockages. However in the yellowing tissue and even more noticeably in the fully senesced tissue the development of closures and possible tyloses indicate a possible means of control of materials into the leaves. In none of the sections was there a marked area of blockage so it may be possible that the ligule may offer a functional control rather than a structural control selecting movement of materials into the leaf.

vascular bundle 1.   x 50

FIG 20 a
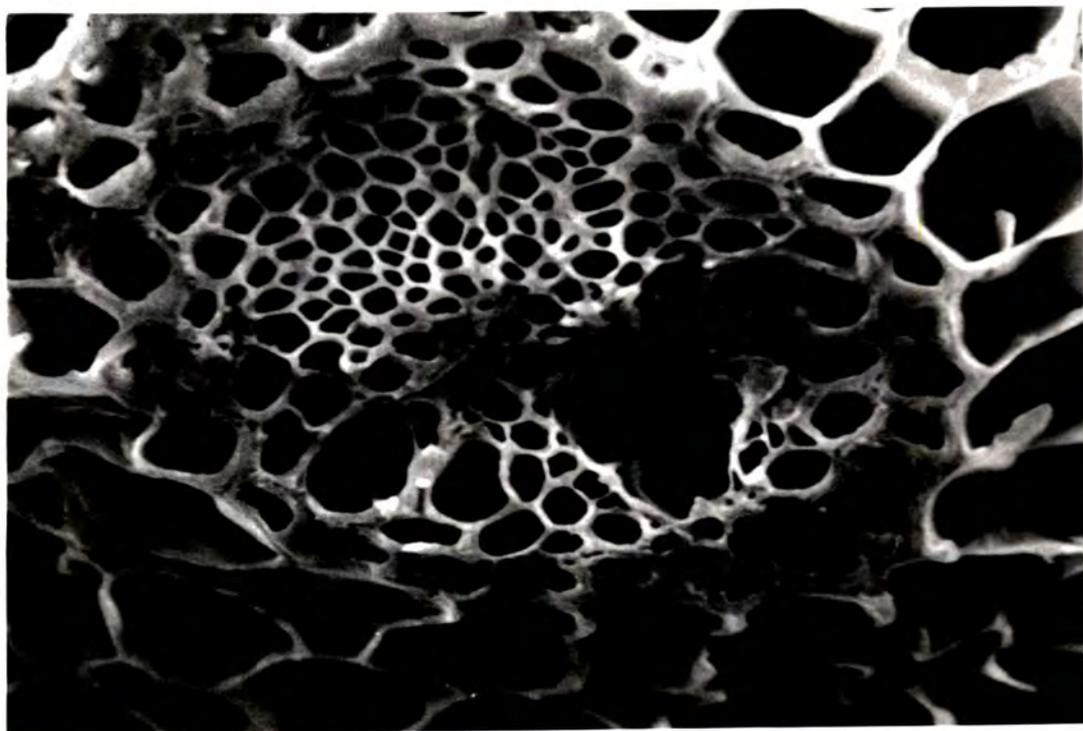
X 1000

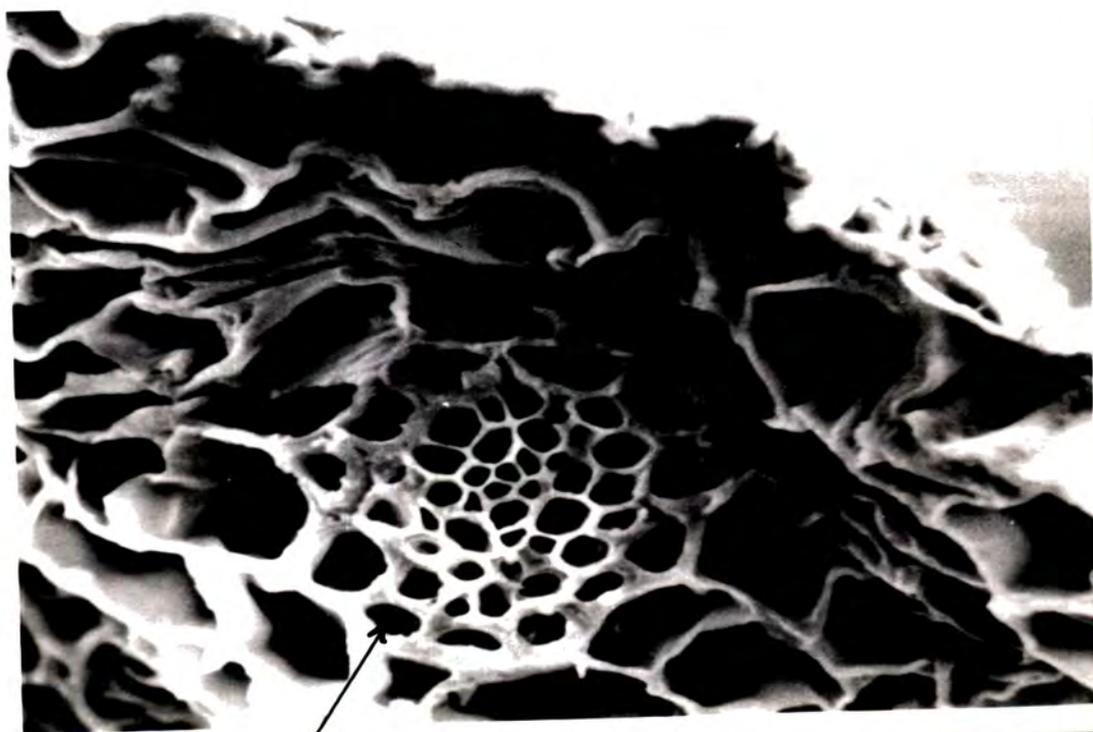## FIG 20 b

Fig. 20 (a)   TS of Ligule region – young leaf

20 (b)   TS of vascular bundle 1

20 (c)   TS of Ligule region of yellowing leaf

20 (d)   TS of Vascular bundle from 20c

20 (e)   TS of Vascular bundle from 20c

20 (f)   TS of Vascular bundle of ligule region of

old leaf
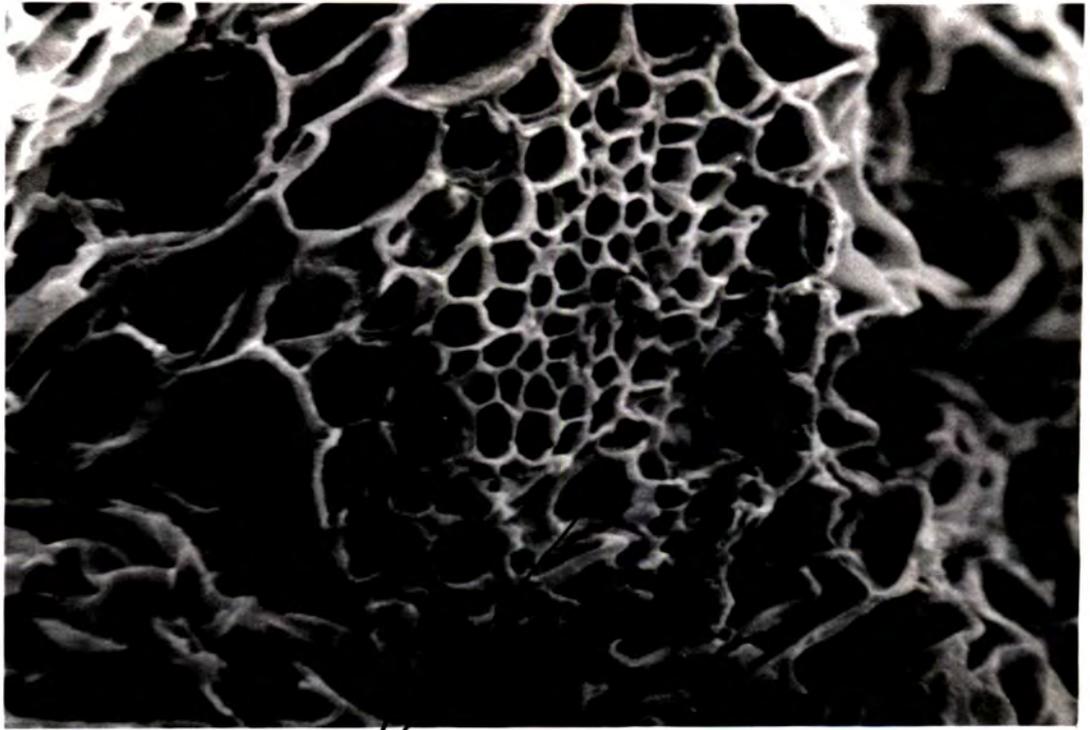
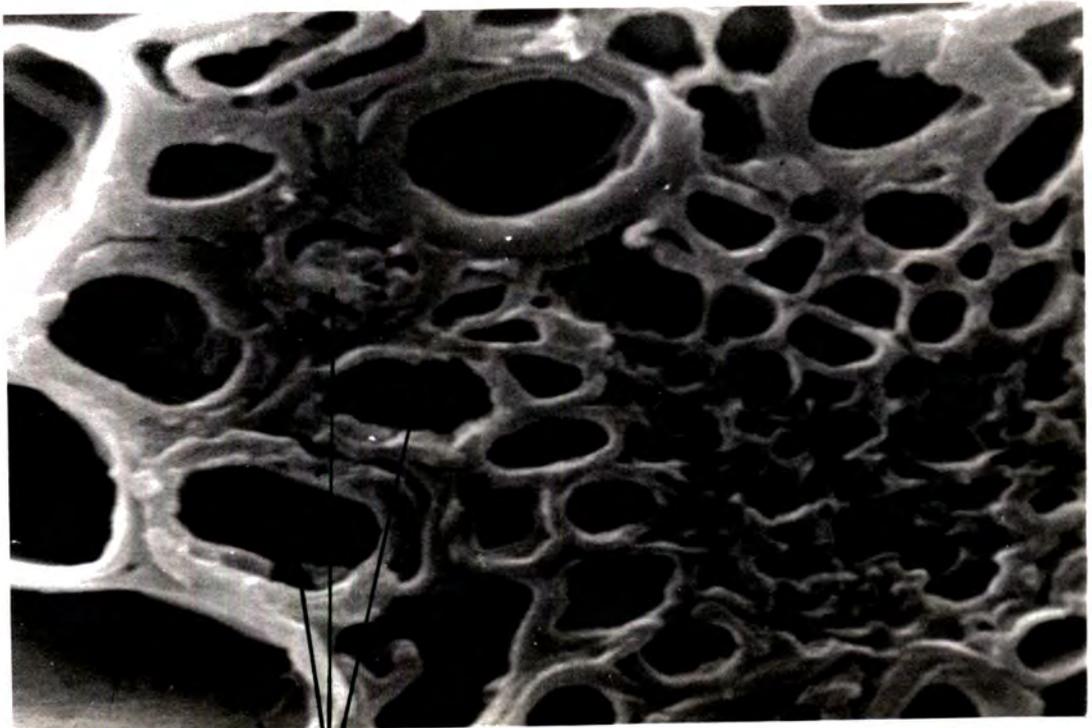x 20

FIG 20 c

tyloses

x 1000

FIG 20d.

tyloses

X 1000

FIG 20e

tyloses

X 2000

FIG 20 f

DISCUSSION

Initial studies of the anatomy of young and senescing leaves yielded little information or insight into the mechanism of senescence in Festuca pratensis. They did however show that gross cellular breakdown did not occur even in the oldest tissues although once a leaf had matured (ligule present) the leaf sheath showed a loss of packing tissue. This indicated that once the leaf blade developed fully it acted as a site of metabolic activity for the rest of the plant.

The study of chlorophyll levels within the leaf from the tip to the base yielded more information regarding the senescence process. If chlorophyll loss can be taken as a measure of the degree of senescence then the results show that the distal parts of the leaf senesce first. This may be due to the cells having a finite age after being produced by the basal meristem or it may involve an isolation mechanism or a retardation of nutrients reaching these cells resulting in their subsequent death.

It has been well established in dicots (Hardwick et al., 1968) that an isolation zone occurs producing senescence and this is followed by the production of an abscission zone in the petiole. The possibility existed that a similar system could exist in monocots without the process of

abscission and leaf fall.

The presence of a structural "abscission zone" has not been shown in monocots and in Festuca there was no evidence of such a zone in the leaf sheath or blade. It seemed most probable that if a functional abscission zone existed then it would occur at the transition from the low photosynthetically active sheath and the active blade i.e. in the ligule region.

This possibility was investigated and the results of the transport of dyes and radiochemicals indicated the presence of a barrier in this region as there was an accumulation and retardation of these components at this point as the leaf aged. It was however shown that the older leaves did transport materials out of the blade and so this blockage must have been selective in its nature. This selectivity presumably could be of economic importance to the plant for as the leaf ages so the useful compounds within the leaf are broken down and transported into the body of the plant for future use and the transport of raw materials into the leaf for the formation of new tissues are retarded as the leaf ages.

Physical analysis of the ligule region revealed little conclusive information as to the nature of the blockage except that the E.M. work did show the presence of

structures which resemble tyloses

in the xylem vessels of senescing leaves. These appeared

to protrude into the lumen of the vessels and so could act

as physiological control points.

The evidence for the presence of a structural blockage

is not really conclusive at this point in time and so this

requires much more investigation which is beyond the scope

of this present thesis. However it must be recognised that

in many biological phenomena function cannot always be

directly related to structure and hence the significance

of these structures requires further research.

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APPENDIX I

	Wt. of container (gm)	Wt. of container and sample (gm)	Wt. of sample (gm)	Radioactivity of sample in cpm	Radioactivity corrected for weight in cpm
1OA	1.050	1.0515	0.0015	217.4	217.4
1OB	1.050	1.0515	0.0015	2497.0	24970.0
2OA	1.050	1.0512	0.0012	156.8	$150.8 \times \frac{15}{12} = 196.0$
2OB	1.050	1.0510	0.0010	95.0	$95 \times \frac{15}{10} = 142.5$
1MA	1.050	1.0541	0.0041	1324.0	$1324 \times \frac{15}{41} = 484.3$
1MB	1.050	1.0529	0.0029	8666.0	$8666 \times \frac{15}{29} = 4482.4$
2MA	1.050	1.0536	0.0036	78.6	$78.6 \times \frac{15}{20} = 32.75$
2MB	1.050	1.0546	0.0046	129.4	$129.4 \times \frac{15}{46} = 42.19$
1YA	1.050	1.0549	0.0049	571.4	$571.4 \times \frac{15}{49} = 174.9$
1YB	1.050	1.0544	0.0064	680.0	$680 \times \frac{15}{44} = 231.8$
2YA	1.050	1.0535	0.0035	80.0	$80 \times \frac{15}{35} = 34.28$
2YB	1.050	1.0552	0.0032	92.8	$92.8 \times \frac{15}{32} = 63.5$

1+2 = Points of injection   O = Old leaf (Yellowing)
A = Section above liquid   M = Medium age Leaf
B = Section below liquid   Y = Young Leaf

APPENDIX II

Photographs showing fluorescent dye uptake in old and young leaves

     (a) = young leaf }
                        } above ligule
     (b) = old leaf   }

     (c) = old leaf showing ligule region

From the photographs the dye has only migrated in easily detectable quantities above the ligule in the younger leaves again suggesting that the ligule is acting as a barrier to transport in older leaves.
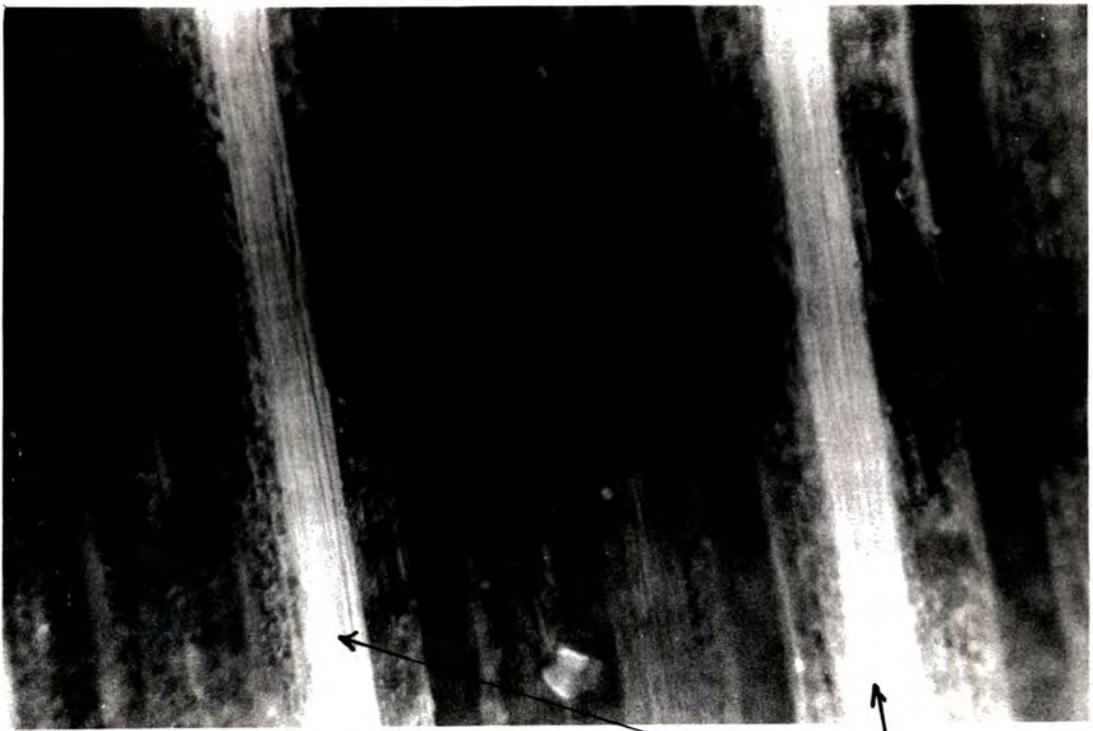
(a)

fluorescent dye
uptake

(b)

(c.)

above ligule

ligule region

below ligule