ASPECTS OF THE BIOLOGY OF RUBUS CHAMAEMORUS L. (BAKEAPPLE) IN NEWFOUNDLAND AND LABRADOR

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ASPECTS OF THE BIOLOGY OF *RUBUS CHAMAEMORUS* L. (BAKEAPPLE) IN NEWFOUNDLAND AND LABRADOR

by

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ABSTRACT

Rubus chamaemorus L. (bakeapple), a common species of nutrient-poor peatlands throughout Newfoundland and Labrador, is harvested for its commercially valuable berries. Management practices in Scandinavia are used to increase natural yields in the field; this study in part attempted to determine if similar practices could be applied to the native population in Newfoundland and Labrador. In sites throughout the province the synecology and autecology of *R. chamaemorus* L. were studied to find the environmental and habitat preferences of the species. Flowering, fruiting and vegetative development were traced through several seasons and found to be particularly sensitive to climate. Although germination can be enhanced by the use of gibberellic acid (GA_3) and kinetin, growth of *R. chamaemorus* L. from seed remained an ineffective proposition. However, results from greenhouse and field studies showed that yields of existing populations could be greatly improved by ploughing the bog surface, reducing the numbers of competitive species, and providing increased shelter from wind.

Using the greenhouse population, plant organs were prepared and examined by optical and electron microscopy. General descriptions of root and rhizome anatomy compared well with Resvoll (1929), Bailey (1941) and Taylor (1971). Previous results (Bal, 1975) indicated that certain cells in the root contained dense material. The electron density was

subsequently identified as due to the presence of polyphenolics. Ferric chloride was used to localize phenolic deposits within the cells and a variety of staining procedures enabled various classes of phenolics to be identified and their distributions in root and rhizome sections determined.

Rubus chamaemorus L. also possesses a varied root microflora, consisting of many bacterial and fungal species. Isolation and culture of root bacteria yielded good results and the rhizosphere medium displayed considerable diversity. It appears that phenolic compounds not only protect the root and rhizome from excessive damage by microorganisms but also control and delineate the association between the root microflora and the root tissue themselves. These two factors acting together could play a major role in the ability of *R. chamaemorus* L. to exploit nutrient-poor environments.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

1. General Background

Rubus chamaemorus L., commonly known as cloudberry or bakeapple in North America, has a boreal circumpolar distribution throughout arctic and subarctic North America, Greenland (Bailey, 1941) and across northern Europe and Asia (Resvoll, 1929; Taylor, 1971). In North America, the southernmost extremity of its distribution lies at 44°N (Resvoll, 1929); in Asia the species has been recorded as far south as Mongolia (Simpson, 1912). Throughout its geographical range, *R. chamaemorus* L. is usually found in peaty *Sphagnum* bogs, especially on raised or blanket bogs with good drainage, although it has also been reported from spruce swamps (Mäkinen and Oikarinen, 1974) and from drier locations covered with gravel or mineral soil (Resvoll, 1929). Such localities are characterized by their low nutrient status and low pH, usually between 3.5 and 4.5 (Pollet, 1972).

Peatlands cover approximately 20200km^2 or 17% of the total land surface area of Newfoundland and Labrador (Pollet, 1968). These areas can be of potential value for agriculture and silviculture (Pollet and Rayment, 1973; Richardson et al., 1976), both of which necessitate draining, ploughing or similar alterations to the natural state of the bog. Another economic possibility lies in the management and controlled harvest of the natural plant resources of the peatland habitat, including berries. Because of its high market value as a "luxury crop", the bakeapple is particularly suitable for this sort of approach. Combining these two methods of bog utilization has already proven successful in

Scandinavia, where the harvest and processing of *R. chamaemorus* L. are of considerable economic importance. Prices for berries in Finland are high (ca. 20-30 Fmk/kg = $6.00 - $9.00/kg) and the income from picking is tax-free (Kortesharju et al., 1978). In favourable years yields of approximately 22680 metric tons are possible, of which 90 metric tons are directly utilized by the home processing industry. Of the latter, 45% is used for liqueurs, 30% for canning and 25% for aromatics and semi-finished goods (Mäkinen and Oikarinen, 1974). At present a considerable research effort is being directed towards developing methods to increase natural yields and in field cultivation of the bakeapple plant (Kortesharju et al., 1978). In Newfoundland and Labrador, the economic potential of a similar bakeapple harvesting and processing industry is recognized (Newfoundland and Labrador Development Corporation, 1976) but the development of such an industry is still in its preliminary stages. Prices for the berries have risen steadily in recent years with pickers receiving $2.00/kg in 1973 (NLDC, 1976) and approximately $4.00/kg more recently and the berries retailing for $6.00/kg in mainland markets (Agric. Commod. Coord. Comm., 1975). In order to manage the species effectively, research into its biology in Newfoundland and Labrador is essential and comparisons with Scandinavia must be made. The purpose of this study was to investigate several problems of potential importance to management and control of the yields of *R. chamaemorus* L.

(1) Field observation of the species and its habitat in the province

(2) Growth and cultivation in the field and greenhouse

(3) Seed germination

(4) Pollen morphology and germination

(5) General anatomy of plant organs

(6) Phenolic localization

(7) Root microflora

(1) Field observation of the species and its habitat in the province

Early studies of *Rubus chamaemorus* L. in Scandinavia (Resvoll, 1929) and North America (Bailey, 1941) and later work in the British Isles (Taylor, 1971) have resulted in a considerable body of observations of the species in the wild. Apart from scattered references to the flowering times and range of the species in other works (Polunin, 1940; Porsild, 1964), no comparable study has been made in northern North America. More detailed ecological work has indicated that the morphology, fruiting success and vegetative growth of *R. chamaemorus* L. can vary between male and female plants (Rosanova, 1928) and between plants growing in sheltered and open areas (Lohi and Havas, 1972; Lohi, 1974). Furthermore, the species has been recorded from many habitats other than the "classic" peat bog, for instance from drying swamps (Simpson, 1912) and dry gravel fields (Resvoll, 1929). It was therefore considered important to make similar observations of the species in as wide a range of habitats as possible in Newfoundland and Labrador in order to compare the population of *R. chamaemorus* L. in eastern North America with those in Europe.

(2) Growth and cultivation in the field and greenhouse:

Much of the previous experimental work on growth and productivity of *R. chamaemorus* L. has taken place in Scandinavia and has largely been directed towards the increase and stabilization of natural yields, and towards the propagation of wild rhizome cuttings for horticultural purposes (Mäkinen and Oikarinen, 1974; Kortesharju et al., 1978). Early observations indicate that frosts of around $-2^{o}C$ have detrimental effects on floral development (Resvoll, 1929) as well as on fruit development (Jaakola and Oikarinen, 1972) which can lead to poor yields or to total crop failure. Covering the surface with gravel, ploughing to increase surface temperatures, and afforesting bog edges can be used to stabilize crop levels (Mäkinen and Oikarinen, 1974). In addition, tree cover acts as a windbreak to improve both temperature regime and pollination. Fertilization of *R. chamaemorus* L. stands with a variety of compounds and enhancement of the competitive ability of the species in its natural habitat can improve natural yields. Nitrogen appears to stimulate vegetative growth while calcium and trace elements have little effect (Østgard, 1964). Phosphorus significantly increases berry weight and total yield (Østgard, 1964), as well as vegetative growth and flowering (Taylor, 1971). Uptake of phosphorus is positively correlated with temperature and the phosphorous level of the substrate (Saebø, 1968 and 1970). However, results vary considerably (Mäkinen and Oikarinen, 1974). Methods presently utilized to decrease competition on *R. chamaemorus* L include use of cover material (Østgard, 1964), direct eradication of dwarf shrubs

(Taylor, 1971), burning of the surface vegetation (Taylor, 1971; Taylor and Marks, 1971; Marks and Taylor, 1972) and ploughing of the bog surface (Østgard, 1964).

R. chamaemorus L. has been successfully propagated from rhizome cuttings in ploughed fields (Mäkinen and Oikarinen, 1974; Østgard, 1964) and in greenhouses (Kortesharju, 1978). Well over 50% of these rhizomes began forming roots shortly after being transplanted, and continued to grow successfully. Other horticultural studies have dealt with the taxonomy and breeding of the species as related to possible hybridization with other edible *Rubi* to produce economically valuable fruits (Pike, 1952; Larsson, 1969; Newton, 1977). The diseases of the *Rubi* have been examined and related to their success as fruit crops (Converse, 1977). Volatile compounds present in the berries of *Rubi* have been compared and contribute towards their differing taste and palatability (Pyysalo, 1976).

Such research, although of practical value in improving economic returns from the bakeapple industry, has often neglected to relate the biology of the species in nature to its success as a managed or cultivated crop. For this study, therefore, the habitat and development of wild populations were studied in conjunction with greenhouse experiments in order to delimit some of the requirements for successful rhizome transplantation. The resulting greenhouse population could then be used for studies of anatomy and physiology.

(3) Seed germination

Propagation of *R. chamaemorus* L. from seeds is difficult. Before germination will occur, seeds must be stratified at 4°C for seven months (Fong and Bal, 1976) and the hard endocarp must be broken or removed (Kerr, 1954). Acid treatment cannot substitute for this time-consuming and tedious process (Rantala, 1976; Warr et al, 1979). Plants raised from seed can take up to seven years to flower (Østgard, 1964). Sexual reproduction is secondary to vegetative reproduction in wild populations and seedlings are rarely found in nature (Resvoll, 1929; Lohi and Havas, 1972; Mäkinen and Oikarinen, 1974). However, germination success can be improved by soaking in gibberellic acid (GA_3) and kinetin (Warr, 1977). Further examination of the effect of hormone treatment and subsequent development of seedlings was considered a useful addition to this study.

(4) Pollen morphology and germination

Insect pollinators of *R. chamaemorus* L. include empedids, syrphids, anthomyids and bumble bees (Dallman, 1932; Østgard, 1964; Taylor, 1971; Kortesharju et al., 1978; Hippa et al., 1978). Windbreaks appear to encourage pollinators and thereby increase fruiting success (Mäkinen and Oikarinen, 1974). The specificity and success of pollen germination is therefore related to crop yields and is directly relevant to the aims of this investigation.

(5) Anatomy of plant organs

The only thorough treatment of the anatomy of *R. chamaemorus* L. is contained in the larger work by Resvoll (1929). In addition, root

microstructure and the extent of the quiescent zone in root tips have been examined by light and electron microscopy (Bal, 1975). To extend these results and compare with them, the anatomy of roots, rhizomes, and leaves were studied.

(6) Phenolic localization

Electron-dense cells have been found in *R. chamaemorus* L. and hypothesized to contain high phenolic levels (Bal, 1975). An important section of this project studied the distribution and nature of polyphenolics in the species using a variety of optical and electron microscopical techniques.

Phenolics can be localized by numerous methods; the earliest tests for lignins used phloroglucenol/HCl and acidified calcium hypochlorite (Seigel, 1953). In addition to non-specific techniques for the phenolics as a group (Brisson et al., 1976; Ramsay and Berlin, 1976; Harris and Hartley, 1976), specific tests (Mace, 1963) have been used to localize various classes of phenolics in root epidermis and cortex (Mace and Howell, 1974; Mace et al., 1974).

Phenolic metabolism is complex, involving two enzyme systems (Mueller and Beckman, 1978), and its importance to plant physiology has been the subject of much discussion. Species with high phenolic levels are generally less susceptible to infection than closely related species with laver levels (Tippett and O'Brien, 1976). Young shoots (Feucht and Nachit, 1977) and root tips (Ginzberg, 1967) accumulate

tannins and related compounds which may facilitate growth and production of callus tissue. Increasing the phenolic content by applying exogenous quinic acid affects the polar transport of auxins, and hence growth, of tomato plants (Marigo and Boudet, 1977).

The relationship between phenolic content and resistance against disease and invasion by microflora is of particular relevance to the study of *R. chamaemorus* L.. Phenolic compounds have definite bactericidal and fungicidal effects (Hegna, 1977; Liszka and Sendra, 1978) and have been implicated in resistance against microorganisms (Parker, 1977), nematodes (Veech, 1977) and insects (Woodhead and Bernays, 1978).

(7) Root microflora

Microfloras are often associated with plant roots (Nicholson, 1967; Royira and Davey, 1974; Sanders et al., 1975; Schenk, 1977) and their role in plant physiology is of special importance to crop plants. Such microorganisms are often located in mucilaginous sheaths (Leiser, 1967; Greaves and Darbyshire, 1972). Mycorrhizas have been described from many groups including pine forests (Ogawa, 1975), grasses (Bartoli et al., 1978) and a range of halophytes, hydrophytes and verophytes (Khan, 1974). Bacterial associations with roots, including symbiotic and assymbiotic nitrogen-fixing bacteria (Evans et al., 1972; Mishustin and Yemstev, 1973), are widely studied. Clover, wheat and rape root exudates enhance the levels of proteolytic bacteria in the rhizosphere (Jacskiewitz, 1977) and most plants are likely to enhance microflora in a similar manner. Studies of bacterial and

fungal associations range from descriptions of their nature (Old and Nicholson, 1973; Patriquin et al., 1979) to more specific examinations of physiological effects produced in the host.

Pinus radiata Don seedlings infected by mycorrhizae are more resistant to the flow of soil water into the roots (Sands and Theodorou, 1978); a similar effect could relate to the sensitivity of *R. chamaemorus* L. for substrate moisture (Resvoll, 1929). The relationship between mycorrhizal infection and phosphorous utilization, considered in the light of the enhancement of vegetative and fruit development observed in fertilized populations of *R. chamaemorus* L. (Østgard, 1964; Saebø, 1968; Taylor, 1971), is of particular interest. Clover (Hall, 1978a), sweet corn and maize (Hall, 1978b) infected with endomycorrhizae competed more successfully for soil phosphorous and showed increased growth and endogenous phosphous levels. Introducing mycorrhizae into the roots of white clover by means of soil pellets produced identical effects (Hall, 1979). The rate of uptake of phosphorous from the soil is increased by the association of mycorrhizae with tree seedlings (Barrow, 1977); in Douglas fir this is due to the role played by fungal acid phosphates in the hydrolysis of complex phosphorous compounds in the soil (Ho and Zak, 1979). The level of external and internal phosphates of the root can control the degree of mycorrhizal infection by a feedback effect (Azcon et al., 1978). Shrubs nodulated with Actonomycetes show enhanced nodulation and nitrogen fixation. When infected with ectomycorrhizae (Williams, 1979). The fungus *Gigaspora margarita* produces a growth inhibitor which could be responsible for the cessation of growth in plants under axenic conditions (Watrud et al., 1978).

In addition to light and electron microscopy of mature roots to determine the nature of bacterial and mycorrhizal associations with *R. chamaemorus* L., radicles and seedling roots were examined to gain some understanding of the development of such associations and their relation to phenolic levels in these developing organs. Preliminary attempts were also made to isolate and identify microorganisms associated with the species in order to assess their possible role in its growth and productivity in natural and controlled habitats.

MATERIALS AND METHODS

1. Field observation and collection

To obtain plants for transplanting in the greenhouse and seeds for germination experiments, as well as various plant parts for electron and optical microscope examination of general anatomy, field collecting was necessary throughout the programme. Collecting was carried out over three growing seasons, from 1977 - 1979, beginning each year when the bog surface was free of frost and the peat itself was no longer frozen, by which time the first buds had usually appeared. Berries were collected throughout the fruiting season whenever possible. Methods of collecting and transplanting rhizomes and of seed germination will be covered in the section dealing with growth experiments. The regular and continuous nature of these collecting trips made general systematic observations of several bogs over the three seasons feasible so that habitat data, as well as information about the synecology of *R. chamaemorus* L. could be compiled and related to similar observations of Scandinavian, British and North American populations.

Although initial collection and observations were confined to a bog on Bauline Line, several other bogs were added to the study in 1977 and 1979.

During the summer of 1977, excursions to Doe Hills and to the Colinet

Experimental Farm were made and observations of the habitats noted. These locations are shown in Fig. 1.

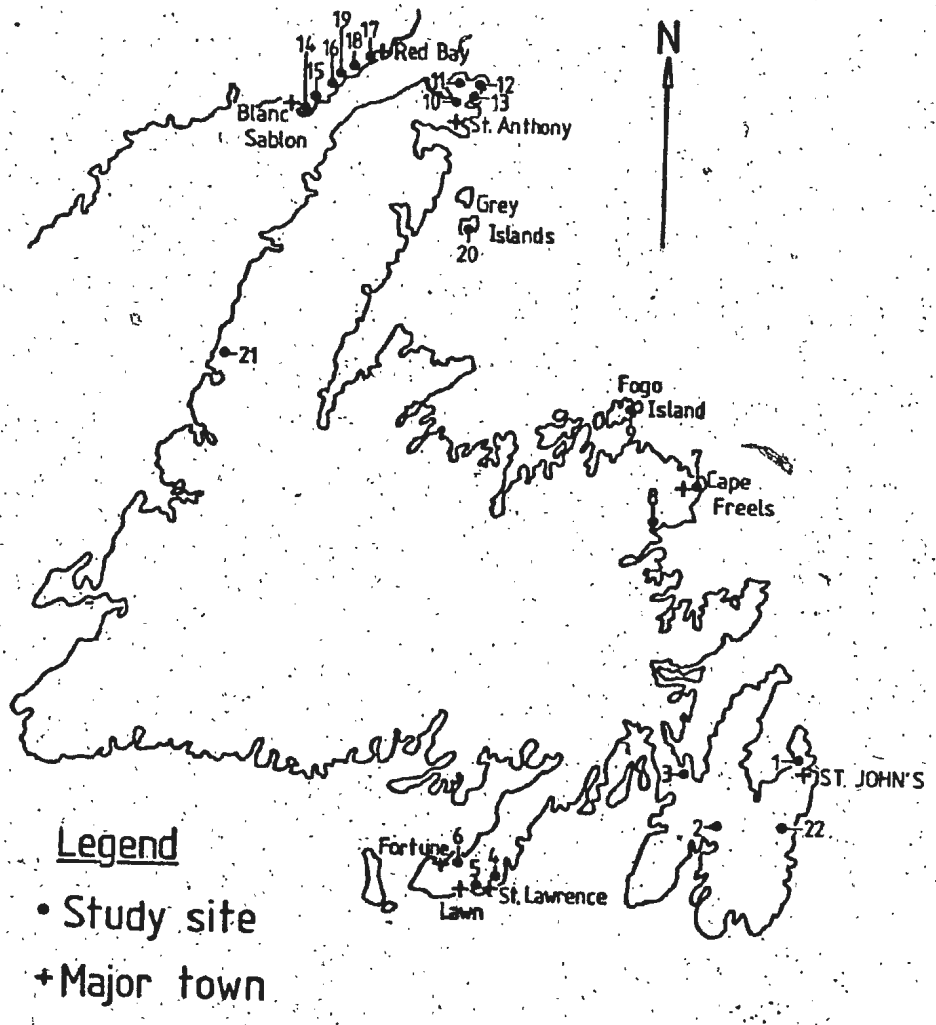
Reports of bakeapple abundance from various sources, mainly from pickers, indicated that although the Avalon Peninsula localities were useful from a practical viewpoint, areas where *R. chamaemorus* L. was more successful and fruit production was higher would also have to be studied. Thus, extensive field work was undertaken throughout Newfoundland and into southern Labrador from August 2nd to 31st, 1979, examining and collecting from seventeen sites (Fig. 1). During 1979 work was also done on several productive bogs in the Witless Bay area: in all these areas, the observations fall into two general categories:-

(1) General habitat and synecology.

(2) The autecology of *R. chamaemorus* L.

(1) Synecology and habitat data

General descriptions of bog morphology, size and topology were made for each locality studied. The peat, or other substrates in several cases, was examined to determine its composition, degree of humification and dampness according to the standards set out by Pollet (1968). Associated vascular and bryophyte species were listed and the degree of cover relative to that of *R. chamaemorus* L. determined. The provincial Department of Agriculture Experimental Farm at Colinet was of special interest in this regard, since it has been part of a study in bog reclamation for pasture and the natural population of



- Figure 1. Location of study sites. Site numbers refer to descriptions given in the results section (pp. 47-79).

R. chamaemorus L. is in competition with the grass species being encouraged there.

(2) Autecology

Flowering times for male and female plants were recorded each year from Bauline and whenever possible from greenhouse plants. During 1979 the bog at Witless Bay was used to study pollination ecology, with flowers being collected for scanning microscopy of pollen germination and insects collected by sweepnetting and 'pootering' the flowers themselves. The ripeness of berries & degree of failed berry development were noted from all the areas studied in addition to general information on 'good' areas for berry picking gained from personal contacts. Berry densities were estimated from the bogs studied in August 1978 in order to measure the success of various habitats in terms of fruit production. For areas studied throughout the programme, comparative estimates of berry production were made each year, as well as general observations of frequency on undeveloped fruits and unpollinated flowers.

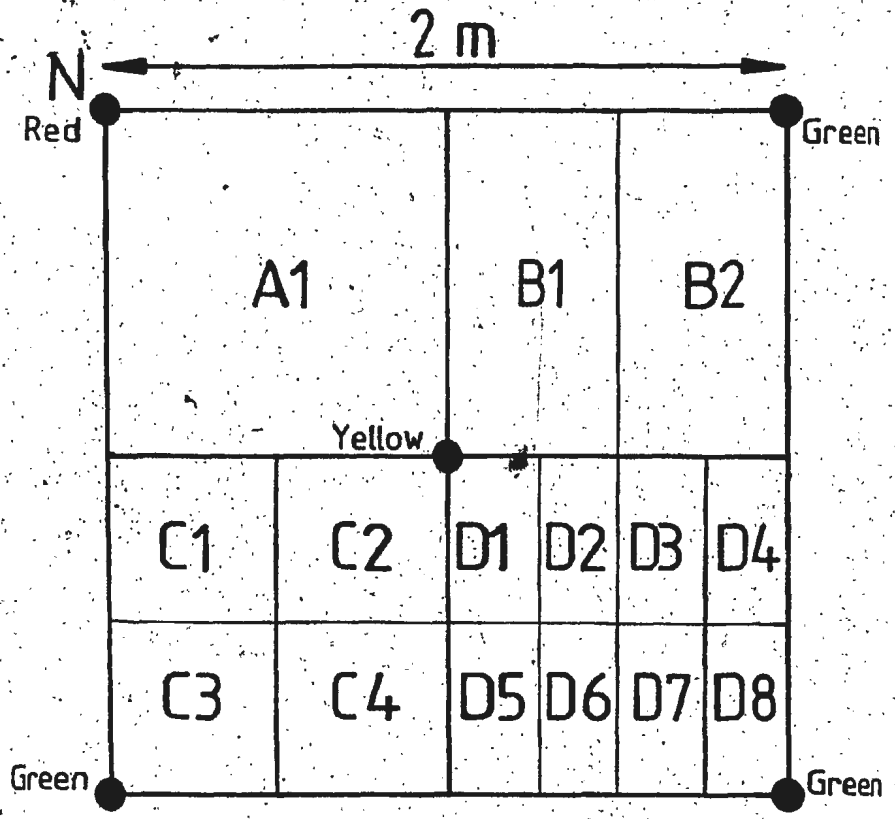
The vegetative growth of the species was also observed in the fields with average numbers of shoots per square metre being recorded for most sites. For all sites the distribution of plants throughout the bog was noted, especially as related to variations in moisture, topography and the degree of shelter. The size and vigour of aerial shoots and the numbers and size of leaves were estimated and compared for various habitats as indications of vegetative success and potential as transplantations.

II. Growth Experiments

Experiments were carried out to determine the effect of bog cutting, rhizome and seedling transplantation, and floral induction on the growth of the species in the field and under greenhouse conditions. The main emphasis in these experiments was the creation and maintenance of a viable greenhouse population of *R. chamaemorus* L. for use in further work on growth under controlled conditions and as sources of material for microscopical examination. In addition, it was possible to study rhizome growth patterns, floral induction and effects of reduced competition in greenhouse-maintained 'wild' plants.

(1) Field experiment

In Scandinavia, ploughing of bogs is frequently employed to break up existing rhizomes, thereby encouraging increased shoot development (Østgard, 1964; Mäkinen and Oikarinen, 1973). An experiment was therefore carried out at Bauline Line to determine the effect of bog cutting on shoot density and to approximate an optimal degree of cutting. On May 16, 1978 three quadrats were set out to the plan shown in Fig. 2, located 10 m apart in the eastern edge of the bog. The corner posts were labelled with the indicated colours of tape and the lines between them were laid out with string and left as markers. These and the internal lines between them were then measured, laid out and cut with a square-ended spade to a depth of approximately 25cm. Fig.3 shows the completed quadrat. The number of shoots per sector were determined for each quadrat before flowering (July 6th 1978), after flowering (July 20th 1978) and after



$A = 1.000\ m^2$

$B = 0.500\ m^2$

$C = 0.250\ m^2$

$D = 0.125\ m^2$

Figure 2. Field experiment; plan of typical quadrat.

Figure 3. Field experiment; completed quadrat at Bauline Line.

flowering the next year (June 29th 1979). It has been established that the growth of new shoots only takes place after flowering has been completed (Resvoll, 1929), so that an intermediate count between flowering seasons was considered unnecessary. The results were tabulated (expressed as shoots per metre^2) and statistically analyzed to determine whether significant increases in shoot density were produced and if there was an optimal sector size for this increase.

(2) Greenhouse experiments

The greenhouse, operated by the Biology Department, M.U.N., was used for the following experiments, as well as housing the experimental population of *R. chamaemorus* L. Except where otherwise stated, all plants were established in 0.6 cm plywood boxes (1m x 0.5m) filled with peat collected from Bauline Line or from Sundew Peat Industries, St. John's. All the boxes were watered daily and checked regularly to remove seedlings of other greenhouse species.

(a) Minimum Rhizome length

To utilize collected rhizomes most effectively, it was necessary to determine an optimal length of rhizome needed for successful regeneration and growth of shoots. Rhizomes measuring 20, 30, 40, 50 and 75 cm from the apical bud were harvested from Bauline Line on May 17 and 23. All non-apical branches were removed and the rhizomes stored in moist peat for transport to the greenhouse. Fifteen rhizomes per length, divided into five replicates of three rhizomes each (R20/1-5, R30/1-5, R40/1-5, R501-5, R75/1-5), were

planted in moist peat in plywood boxes (for 50 and 75) and plastic trays (20, 30 and 40) at a depth of 3-5cm. Daily watering, as above, made the peat excessively wet, so that these and all subsequent plantings were watered on alternate days and observed throughout that growing season and over winter to determine shoot growth in the subsequent season. By June 16, leaves had appeared and were counted; seven days later they had opened fully and measurements of maximum length and width of each leaf could be taken. Two more sets of measurements were taken at intervals of 18 and 28 days from initial opening of the leaves, by which time they had commenced senescence and drying. In the following summers survivorship and growth of the rhizomes were recorded. Analysis of variance and Dunnett's test for comparison of control means to other group means were used to determine the significance of the observed differences in leaf size.

(b) Apical/non-apical generation

Previous work on regeneration from rhizome fragments (Leakey and Chancellor, 1977 a, b; 1978 a, b) indicated that the position of buds in multi-node fragments could influence the pattern of regeneration and that there was a significant dominance exerted upon the growth of other buds by apical buds. The following experiment was performed in order to extend these observations to regeneration of *R. chamaemorus* L. rhizomes and to determine from this if it was necessary to include apical buds in transplanted rhizome fragments. Thirty-centimetre rhizomes from the previous experiment on optimal length were used as controls, since each

fragment included an intact apical bud and several healthy lateral buds. Rhizomes from Bauline Line from June 5th – 14th were cut into 30cm lengths and all branches removed. Five replicates of three rhizomes each were cut, first at the nodes with apical bud removed (N1-N5), and second between nodes, with no apical bud present (IN1-IN5). These rhizomes were planted in plywood boxes in peat at a depth of 3cm, watered every other day and observed for the remainder of the growing season. Patterns of regeneration, differences in leaf size, root development and overall robustness of the shoots were noted. Leaves produced were small, perhaps due to growth regime; measuring their dimensions was considered irrelevant and statistical analysis was not possible.

(c) Seedling growth patterns

A seedling produced during a previous study on seed germination (Warr, B.Sc. thesis, 1977) was transplanted to the centre of a plywood box, as described above, in the summer of 1977. The development of the rhizome system and new shoots was observed throughout the programme and general observations on the pattern of growth were recorded. In addition, another seedling was transplanted into a small box and observed over the same period.

(d) Effects of reduced competition

During a collecting trip at Colinet Experimental Farm (Summer 1977), a complete turf was cut and transferred to a plywood box. In addition to several healthy plants of *R. chamaemorus* L. the

turf contained greater than 75% cover of 'competitive' species, including *Empetrum nigrum* L., *Vaccinium angustifolium* Ait. and several grasses. After establishment in the greenhouse, all the plants except *R. chamaemorus* L. were removed by hand. Over the next three growing seasons, new shoots of these plants were continuously weeded out, the box watered on alternate days and the growth of *R. chamaemorus* L. determined.

(e) Floral induction

Although transplanted rhizomes displayed successful vegetative growth and most plants flowered in their first season, fruits were not developed and very few plants flowered in subsequent seasons in the greenhouse. Chemical parthenocarpy was attempted using a 0.1% solution of indolacetic acid (IAA) in lanolin paste (Gustafson, 1938), which was applied directly to the stigmatic surface of the female flowers produced in the summer of 1977. This method was unsuitable, causing premature senescence and failure to produce fruit. It was thought that the greenhouse plants failed to flower beyond their first season because they were not exposed to normal winter conditions and floral bud development was thereby not initiated. In order to test this, a box of well-established plants was removed from the greenhouse and put into a cold room at 4°C for 24 hours (June 22–23, 1978), replaced in the greenhouse and treated as usual for the remainder of the programme. The treatment proved to be ineffective, although plants survived the chilling period and displayed continuous

vegetative growth.

(3) Establishment of a greenhouse population

In addition to greenhouse specimens produced as a result of the previous experiments, transplantations of rhizomes from as many localities as possible were carried out throughout the programme to establish a population of *R. chamaemorus* L. accessible for the collection of plant parts and treatment of living plants necessary for much of the subsequent work. Rhizomes in the initial season (1977) were collected from Bauline Line, Doe Hills, Colinet and St. Joseph's. In 1978 collections from these areas were augmented with rhizomes collected from the seventeen sites visited during field work in August, as described in the results. In 1979 collecting was largely confined to Bauline Line and Witless Bay. All rhizomes were harvested and transplanted in the same manner. In the field, healthy shoots with well-developed buds were selected and the rhizomes leading from them carefully loosened from the peat as far back as possible from the shoot, including any branches with their attached aerial shoots. The rhizomes thus obtained varied in length from 20cm to several metres in length, the longest being approximately six metres (Fig.4). For transport to the greenhouse, rhizomes were placed in polythene bags with damp peat to prevent excessive drying. They were then planted at a depth of approximately 3cm in peat-filled plywood boxes, as previously described, with any aerial shoots left exposed, care being taken to ensure that the lateral buds remained below the peat surface. The boxes were left under greenhouse conditions, watered

Figure 4. Rhizome collected in Southern Labrador, August 1978.

on alternate days, weeded and treated once a month with commercial fertilizer.

III. Seed Germination

Several means of promoting seed germination and development of healthy seedlings were investigated. It was noted that seedling losses of 41-94% occurred in preliminary germination trials, so that two methods of producing 'sterile' germination conditions were attempted. The continuation of work by a previous student (Warr, 1978 B.Sc. thesis; Warr, Savory and Bal, 1979) showed that treatment of mechanically scarified seeds with gibberellic acid and kinetin could affect germination frequency. Acid scarification was also attempted. Although few seedlings were sufficiently hardy to be transplanted to the greenhouse, subsequent experiments in phenolic distribution and development of microflora were able to utilize the technique thus developed.

In order to supply seeds for the following experiments and to build up a 'seed bank' for future use, berries collected or purchased from various sources were treated to remove the fleshy mesocarp from the pyrene. Throughout this thesis, the term 'pyrene' refers to the bony endocarp plus the enclosed seed. Initially, berries were mixed with water and forced through a sieve to remove most of the fleshy tissue, then air-dried and sorted by hand to remove the pyrenes. However, this proved inefficient and time-consuming. The following alternative method was subsequently developed and improved the processing of berries considerably. Fresh berries were placed in a Waring blender and thoroughly mashed. Clean tap water was added to nearly fill the blender (about 500 ml) and the mixture blended for

several minutes before being left to settle. Exocarp and mesocarp tissues floated to the surface and were easily decanted, leaving the clean pyrenes at the bottom. These were then spread out in Petri dishes and allowed to air-dry, followed by dry stratification at 4°C for at least six months (Fong and Bal, 1976) prior to usage.

(1) Reduction of fungal mortality

(a) Measurements of mortality due to fungal infection

Dry stratified pyrenes from Doe Hills, Trinity Bay were soaked for six hours in distilled water, endocarps removed using a scalpel and forceps and the seed coat nicked at the end furthest from the embryo. Seeds were then placed in petri dishes on several layers of filter paper moistned with five ml of the solutions given in Table 1. Dishes were then placed in a controlled environment chamber (12hr. light, 12hr. dark at 665 lux, 70% relative humidity, 20°C) and the solutions replenished weekly. Germinations and losses due to fungal mortality were recorded for approximately 35 days, with germination defined as successful once the cotyledons or radicle had protruded and the cotyledons had become green. Percentage germination and loss were calculated for each treatment and compared.

Table 1. Germination media for seeds used in measurement of fungal mortality.

Batch #	Number of seeds	Germination medium
1	50	distilled water
2	25	distilled water
	25	4.6×10^{-5}M kinetin
	25	5.7×10^{-5}M GA_3
3	50	4.6×10^{-5}M kinetin
	50	4.6×10^{-6}M kinetin
	50	4.6×10^{-7}M kinetin
4	50	$5.7 \times 10^{-2.5}$M GA_3
	50	4.6×10^{-5}M kinetin
5	25	4.6×10^{-5} M kinetin
6	50	4.6×10^{-5}M kinetin
	50	4.6×10^{-6}M kinetin
	50	4.6×10^{-7}M kinetin
7	95	4.6×10^{-5}M kinetin
8	50	4.6×10^{-5}M kinetin
9	135	4.6×10^{-5}M kinetin
10	38	4.6×10^{-5}M kinetin

(b) Seed preparation under sterile conditions

It was clearly important to develop some method of preparing and germinating seeds which reduced or eliminated the heavy losses due to fungal contamination displayed in the previous experiment. An initial attempt involved the utilization of an ultra-violet microbiological hood in which the procedure could be carried out and the sterilization of all instruments and solutions used. The hood, working surface and environment chamber were first thoroughly washed and wiped out with disinfectant (TOR). All solutions required, including a supply of distilled water, were filtered in a millipore apparatus and stored at 4°C when not in use. Glass petri dishes, scalpels, forceps, pipettes and beakers were autoclaved and stored in the hood under ultraviolet light to prevent recontamination. Pyrenes were soaked in sterile distilled water for six hours and surface sterilized in five per cent calcium hypochlorite for five minutes, the entire procedure from this point taking place in the hood under normal light. The endocarp was removed and the seed coat nicked with sterilized instruments which were kept ready in a beaker of ethanol and flamed after each pyrene had been treated. The seeds were then placed on moistened filter paper in petri dishes and incubated in the environment chamber as above.

Although fungal mortalities were considerably reduced by this method, there was still contamination necessitating regular changing

of the filter papers and removal of infected seeds. Further sterilization solutions were tested including a fungicidal solution of chloramphenicol/cyclohexamine. The preparation of seeds was as above, with surface sterilization taking place in absolute ethanol for ten seconds rather than in calcium hypochlorite. After removing the endocarps and nicking the seed coats, batches of forty seeds were submerged in ten per cent calcium hypochlorite and ten per cent hydrogen peroxide for times of fifteen, ten and five minutes. The resulting six groups of seeds were then washed in several changes of sterile distilled water. Each batch was then incubated under the following conditions in petri dishes; ten seeds on filter paper moistened with sterile water; ten seeds on filter paper moistened with a solution of chloramphenicol/ cyclohexamine (0.05 gm./6.40 gm. per litre of distilled water, BBL Manual of Products and Lab. Procedures, 1973); ten seeds on Difco nutrient agar with the addition of yeast extract; ten seeds on Sabourand's agar (Difco, 1971). Germination was monitored for 30 - 35 days with seeds taken at interyals for use in the phenolic localization experiment.

(2) Promotion of germination using gibberellic acid (GA_3) and kinetin

Berries collected from Cape Race (August 1974) and Doe Hill, Trinity Bay (August 1977) were cleaned to separate the pyrenes from the fleshy mesocarp and stratified by storage in covered petri dishes at $4^{o}C$ for seven months (Fong and Bal, 1976). Stratified

pyrenes were soaked in distilled water for six hours, following which the endocarp was carefully cut away using a sterile scalpel and forceps under an ultra-violet hood and the seed coat was nicked at the end furthest from the embryo. After surface sterilization in 0.5% sodium hypochlorite for five minutes, the mechanically-scarified seeds were washed in sterilized distilled water and replaced in sterilized glass petri dishes on several layers of filter paper moistened with 5 ml of the following solutions:-

(a) 5.7×10^{-7}M, 5.7×10^{-6}M, 5.7×10^{-5}M gibberellic acid (GA_3)

(b) 4.6×10^{-7}M, 4.6×10^{-6}M, 4.6×10^{-5}M kinetin

(c) sterile distilled water (control).

All solutions were sterilized by disposable millipore filter apparatus and stored at 4^{o}C when not in use. For each treatment, four replicates of fifty seeds each were used. The petri dishes containing the treated seeds were placed in a controlled environment chamber under conditions of 12hrs light/12 hrs dark at 665 lux, relative humidities 70% and temperature 20^{o}C. The filter papers were changed weekly to prevent excessive fungal contamination and 5 ml of the appropriate solution added to each dish. The number of seeds germinating (i.e. with cotyledons or radicle protruding) was recorded over a period of thirty days, converted to percentage germinations for each treatment and statistically

analysed using analysis of variance and Dunnett's test for comparing means.

(3) Acid scarification

Dry stratified pyrenes as described above, from Lord's Cove, Burin Peninsula (August 1977) were placed with concentrated sulphuric acid in a conical flask resting in a beaker of ice water and left on a mechanical shaker at 100 rpm for three hours. The seeds were then repeatedly washed with distilled water and rinsed under running distilled water for an additional five minutes. Excess acid was neutralized by a further fifteen minute rinse in a 5% solution of sodium bicarbonate followed by a five minute rinse in distilled water. Seeds were then air-dried at room temperature in covered petri dishes for 24 hours before planting in moist Sphagnum and incubation in environment chambers as previously described. Four replicates of fifty seeds received this treatment; in addition, control groups of fifty seeds were treated as follows:-

(i) pyrenes were soaked for six hours in distilled water, then sown as above with endocarps left intact;

(ii) pyrenes were soaked in distilled water for six hours and the endocarps removed before sowing.

All seeds were watered regularly and observed for germination over a period of three months.

IV. Pollination study

During the summer of 1977, flowers were collected from Bauline Line for scanning microscopy of pistils, anthers and pollen grains. In the following summer female flowers were similarly observed to determine the success of pollen germination. In both cases, flowers and floral organs from wild plants in the field were immediately fixed in Karnovsky's solution (Karnovsky, 1965) for one hour. Following fixation, the tissues were washed repeatedly over a period of twenty-four hours in phosphate buffer (ph 7.2) then osmicated in osmium tetroxide solution for one hour. Specimens were dehydrated to absolute alcohol and taken through a 3:1, 1:1, 1:3 series of ethanol: Freon 113 (trichloroflurethane) to three changes in pure Freon 113. Following transfer to Freon 13 (chloro-triflouroethane), the specimens were dried in a Bomar SPC-90/ex critical point drying apparatus, fixed on aluminium stubs with silver conducting paint and gold-coated in an Edwards vacuum coating unit, Model E12E with continual rotation using a planetary rotation stage. Prepared specimens were viewed and photographed in a Cambridge "Stereoscan" Mark 2A scanning electron microscope.

As previously described, field studies at Witless Bay in 1979 included the preliminary collection and identification of possible pollinating insects.

V. Root and rhizome anatomy

With the establishment of accessible populations of seedlings in

controlled environment chambers and of mature plants in the greenhouse, it was possible to investigate several more specific problems using optical and electron microscopy.

(1) General Anatomy

Initial work involved general observations of the anatomy of a variety of plant organs in both greenhouse plants and 'wild' plants, using paraffin sections for light microscopical examination. Samples were collected from healthy plants and immediately fixed in either FAA (Ethyl alcohol 50% : Glacial acetic acid : 40% Formaldehyde, 18:1:1) or Carnoy's fluid (Absolute ethyl alcohol : Chloroform : Glacial acetic acid ; 6:3:1), FAA generally proving most effective for paraffin embedding (Jensen, 1962). Specimens were then dehydrated in an alcohol - tertiary butyl alcohol series and embedded in paraffin. Sections of 5 - 10 microns were de-paraffinized and stained as follows:-

(i) safranin-aniline blue for general staining
(ii) feulgen method for staining of chromosomes
(iii) safranin-light green for differential staining of cytoplasm/cellulose and nucleic acids/protein
(iv) methyl green/pyronin for staining of DNA and RNA (Jensen, 1962).

Stained sections were permanently mounted in histoclad and photographed.

(2) Quiescent zone studies

In order to delineate the quiescent zone of *R. chamaemorus* L. autoradiography of greenhouse plants was attempted using H_3 thymidine. The root tips were exposed by carefully removing the peat from near growing shoots and submerged in an H_3 thymidine or H_3 thymidine/Triton X aqueous solution with 50mCi/ml radiation content in 50ml beakers, while leaving the root and rhizome intact and covering them with peat as close as possible to the tips. Plants were exposed in this way for 48 hours before the root tips were removed and fixed in FAA. Equal numbers of specimens treated with H_3 thymidine alone and treated with H_3 thymidine plus Triton X to improve absorption were embedded in paraffin and sectioned. After deparaffinization, slides were dipped in photographic emulsion, dried and stored for seven days to expose. They were developed in Kodak D-19 developer, washed in tap water, fixed for six minutes, washed again and dried. Developed slides were stained using Azure B (Jensen, 1962) and examined under the optical microscope.

This initial method gave slides with too much background exposure to be of use and the exposure was therefore lowered to a 24 hour 'pulse' using H_3 thymidine alone, followed by a one hour wash in 'cold' 1% thymidine solution. It was also decided that thinner sections of the specimens could prove useful in the exact location of the quiescent zone. Specimens were therefore fixed in Karnovsky's solution for two hours, washed repeatedly in phosphate buffer

for one hour, dehydrated and mounted in Epon (Spurr, 1969) for ultra thin sectioning before treatment as above and examination using optical microscopy. Results were slightly improved, although background was still present. However, the quiescent zone was found to be evident in whole roots stained with ruthenium red as part of an experiment in detecting the mucigel layer, to be fully described in a later section.

VI. Phenolic localization

Sections of *R. chamaemorus* L. root tips prepared for electron and optical microscopes displayed the metachromatic reaction with toluidine blue characteristic of phenolic compounds (Ramsay and Berlin, 1976; Tippett and O'Brien, 1976). Previous work (Bal, 1975) showed that similar areas of the root meristem contain electron dense cells which were considered to have high levels of such compounds and it was considered feasible to investigate this phenomenon further. The distribution of phenolics both within the root and within individual dense cells was studied using electron microscopy of material stained with ferric chloride. A variety of staining techniques was applied to freehand sections of both roots and rhizomes to further define the chemical nature of the compounds and their distributions. In order to trace the development of dense cells, radicles and seedling roots were examined using electron microscopy.

(1) Localization of phenolics using electron microscopy

In order to determine the effectiveness of ferric chloride in

general localization of phenolics in *R. chamaemorus* L. root tissues, roots were prepared for electron microscopy using the following treatments:-

(i) fixation in Karnovsky's solution for one hour followed by staining in OsO_4 for one hour (Feder and O'Brien, 1968)

(ii) fixation in a 3% solution of $FeCl_3$ in Karnovsky's solution for one hour (Brisson et al., 1976)

(iii) fixation in Karnovsky's solution for one hour with no staining (control).

Specimens from all treatments were then washed in phosphate buffer, dehydrated to 100% ethanol and embedded in Spurr's medium for ultrathin sectioning. Sections were counterstained with lead citrate and uranyl acetate, mounted on grids and photographed in the microscope (Feder and O'Brien, 1968). Both OsO_4 and $FeCl_3$ yielded good overall staining of dense cells, with $FeCl_3$ improving the resolution of iron-phenolic complexes within the cells.

(2) Characterization of phenolic compounds using optical microscopy

Several staining methods were used to isolate and localize various classes of phenolic compounds within roots and rhizome tissue. In all cases, sections were obtained by sampling healthy material from greenhouse plants, immersing them immediately in

the appropriate solutions, cutting freehand sections from them with a sharp razor blade and mounting the sections under cover slips on glass slides in a drop of staining solution for observation after the appropriate procedures had been carried out. Stained sections were observed and photographed using a Zeiss photomicroscope.

Two sets of procedures were used to differentiate between flavonoid polyphenolics (i.e. catechins, gallocatechins) and terpenoid polyphenols (gossypol). Treatment of sections with a 1% solution of 2,4-dimethoxybenzaldehyde in 1:1 95% ethanol and 18% hydrochloric acid (DMB reagent, as per Mace and Howell, 1974) stained catechins and gallocatechins light red. The nitroso reaction, in which equal volumes of 10% sodium nitrate, 20% urea and 10% acetic acid are added to fresh sections before treatment with two volumes of 2N sodium hypochlorite (Reeve, 1950), stained catechol tannins cherry red. Treatment with saturated solutions of 2,4-dinitrophenylhydrazine in 2N HCl (DNP reagent) and $SbCl_3$ in 60% $HClO_4$ ($SbCl_3$ reagent) stained gossypol and related terpenoids orange-red and red, respectively (Mace et al., 1974).

Further tests were done on freehand sections to localize phenolics with several more specific chemical properties (Mace, 1963). Gibb's reagent gave a blue-colour on reaction with phenolics with free positions para- to a hydroxyl group and those with carboxyl, sulpho, formyl, nitroso, or nitro groups in the same ring as

hydroxyls. In this procedure, 0.5 ml of a 5% solution of 2,6-dichloroquinone-4-chloroimide in 95% ethanol was added to 10 ml of pH 9.4 borate buffer (50 ml of 0.025M borax in 6.2ml of 0.1M NaOH). Fresh sections were immediately placed in this mixture and left for 20-25 minutes, then transferred to 3% NH_4OH for 10 minutes before mounting. Localization of aniline addition products of quinones derived from naturally occurring phenolics was carried out using aniline KIO_3 reagent, consisting of 0.5 ml aniline, 20 ml of 0.5M KIO_3 and 5 ml distilled water, in which sections were treated for 5 minutes, giving a dark blue reaction product. A solution of 2% aqueous aniline blue gave a similar but more faintly coloured reaction with aniline addition products of o- or p- quinones.

(3) Development of dense cells

Root tips from seedlings grown under sterile conditions in the environment chamber as described in Section III, were prepared for electron microscopy using the standard OsO_4 treatment and Spurr embedding procedure. In addition to electron dense cells and rhizoplane microorganisms, these sections displayed metachromatic staining of the epidermis with toluidine blue indicative of phenolic content. It was therefore decided to examine radicles and seedling roots at other developmental stages and to determine possible changes in the extent and pattern of phenolic distribution.

All specimens were treated using the ferric chloride method

(Brisson et al., 1976), embedded in Spurr's medium and thin-sectioned for viewing and photography in the electron microscope. Radicles from dry seeds were initially treated and yielded good results. In addition, radicles from seeds which had been soaked in sterile distilled water for six hours were sampled and examined. Photographs from these specimens were then able to be compared with those from the above-mentioned seedlings, which had germinated 23 days prior to sampling.

VII. Root Microflora

Greenhouse and seedling root tips, prepared for electron microscopy using $FeCl_3$ (Brisson et al., 1976) were seen to contain microorganisms, both at the root surface and within the root tissue. In order to determine whether these organisms were present in the seeds before germination, or were entirely derived from the environment, seedlings and radicles grown under sterile conditions were also examined using the same procedure. Several further experiments were set up to determine if mycorrhizae were present, using special staining techniques, to determine the presence and extent of a mucigel layer in the roots and finally to isolate and culture microorganisms from the roots of greenhouse plants in order to make preliminary characterizations of the surface rhizoplane and internal microflora.

(1) Mycorrhizal staining

Healthy roots from plants raised in the environment chamber were harvested and treated with a KOH and lactophenol/acid fuschin

clearing and staining procedure (Berch, pers.comm). The fresh roots were gently washed in distilled H_2O and then heated in 10% KOH at approx. 90°C for several hours, until the tissues became transluscent. The specimens were then washed in several changes of water, the last being acidified with several drops of lactic acid and cut into 5mm lengths. Washed root pieces were placed in a drop of 0.05% acid fuschin in lactophenol stain on a glass slide and heated on a hot plate until fumes of lactic acid were visible. Excess stain was removed by transferring specimens to a drop of lactophenol and reheating as above, changing lactophenol as the old solution moved to the cooler end of the slide until the wash was no longer pigmented. Treated specimens were then mounted in lactic acid under a cover slip, gently flattened to spread the tissues and sealed with paraffin for observation of hypal structures. These were then compared with descriptions of mycorrhizae from the literature (Nicholson, 1967; Sanders et al., 1975; Old and Nicholson, 1975).

(2) Detection of Mucigel Layers

Ruthenium red (Leiser, 1968) was used to stain roots from a variety of sources to determine the extent of mucigel layers, if present. Whole root tips were treated from roots in the following locations:-

(a) environment chamber

(b) Witless Bay and Bauline bogs

(c) peat in the greenhouse

In all cases, whole roots were cut at ca. 5mm from the tip and the specimens placed in dilute ruthenium red stain (1:5000 aq. solution) for several minutes, then mounted in water and observed.

(4) Isolation and culture of root microorganisms

Root microorganisms from the surface and tissues of healthy roots were collected from greenhouse plants and were isolated and cultured using the procedure summarized in Fig. 5.

Peat extract agar was prepared by boiling approximately 385g of commercial peat (Sundew Peat Farms) in two litres of distilled water for ten minutes, then filtering the extract through Whatman 1 filter papers. The pH was adjusted to 4,5, 6 and 7 respectively before the addition of 5g of Na_2CO_3 and 20g of Difco trypticase-soy agar to each flask. The agar was then autoclaved and poured into plates for use (Difco, 1971).

Jensen's nitrogen free medium for growth of *Azotobacter* species (Difco, 1971) was also prepared. The following chemicals were dissolved in two litres of distilled water:- sucrose (40.0g), K_2HPO_4 (2.0g), $MgSO_4.7H_2O$ (1.0g), NaCl (1.0g), $FeSO_4$ (0.2g) and $Na_2MoO_4.2H_2O$ (0.01g). The resulting solution was divided

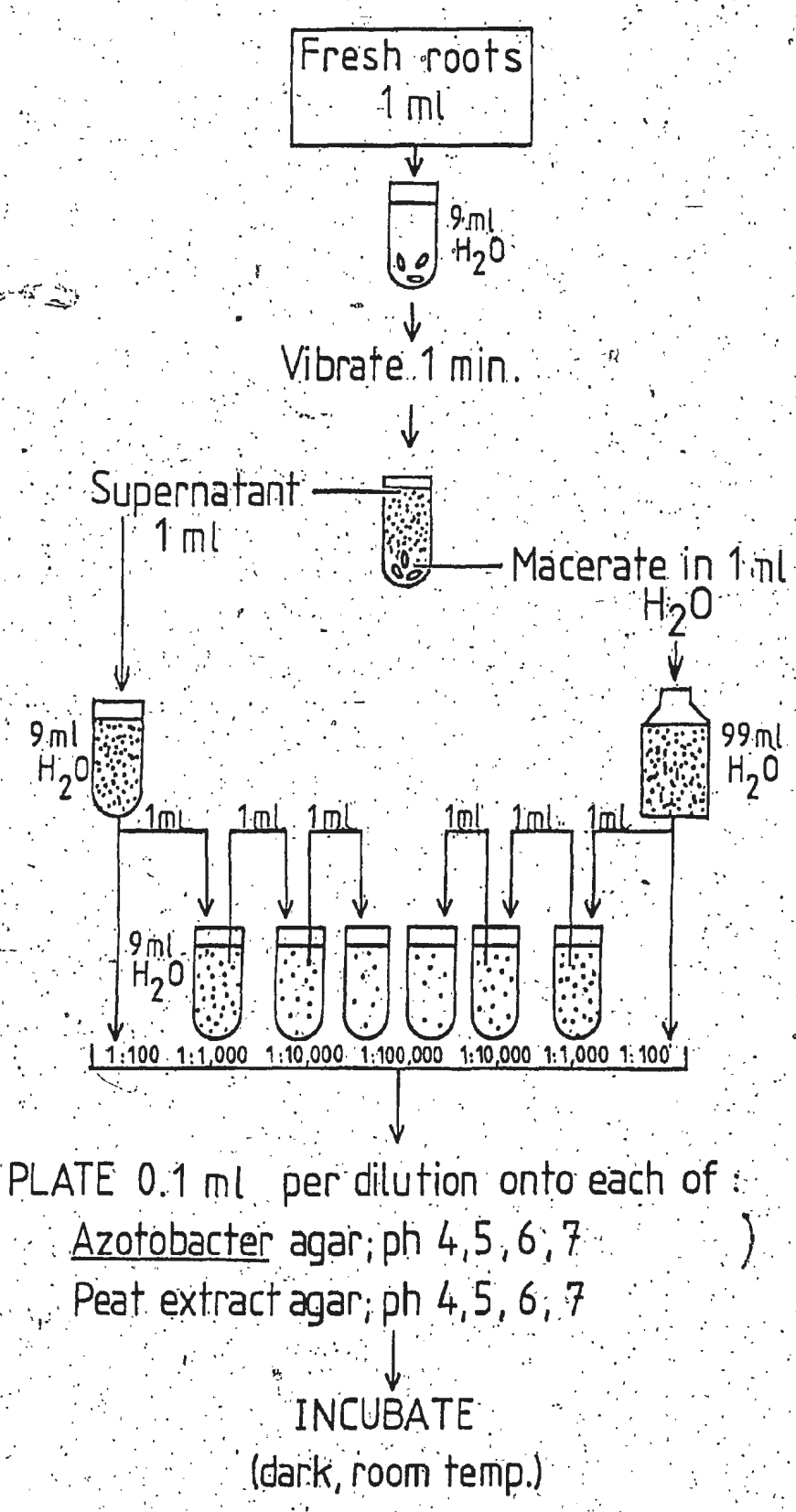


Figure 5. Isolation and culture procedure for root microorganisms.

into 4 x 500 ml lots in Erlenmeyer flasks adjusted to pH 4,5,6 and 7 respectively before the addition of $CaCO_3$ (1gm) and agar (Noble, 7.5gm) to each flask. The medium was then autoclaved and poured. All plates were labelled with the type of medium and pH and stored at 4°C until used. All materials and equipment used in the isolation were first autoclaved and stored under UV light in a microbiological hood. Fresh, healthy roots were collected from greenhouse plants and immediately placed in vials of distilled water.

The remainder of the isolation procedure took place in the hood, with care being taken to avoid cross-contamination of plates. In the initial trial several roots were placed directly on the agar surface of one plate for each medium at each pH, a total of eight plates then being incubated in the dark at room temperature for six days. These plates, however, became rapidly overgrown with overlapping colonies unsuitable for isolation and the procedure was not used in the second trial. The rest of the root material was transferred aseptically to a pyrex macerating tube containing 9 ml of sterile distilled water and vibrated for one minute in a Vortex mixer to loosen the rhizoplane material and associated microflora. One ml of the resulting supernatant was decanted off and added to a test-tube containing 9 ml of sterile distilled water to produce a 1:100 dilution. This procedure was repeated to give dilutions of 1:1000, 1:10000 and 1:100000 following

standard procedures (Salle, 1973). For each dilution, 0.1 ml of solution was pipetted onto plates of both media at the given pH (eight plates in all) and spread using a glass spreader which was immersed in ethanol and flamed between platings. The root tissues remaining once the supernatant had been decanted were then macerated in 1 ml of sterile distilled water and the resulting suspension transferred to a dilution bottle containing 99 ml of sterile distilled water and glass dispersion beads, to give an initial dilution of 1:100. The above dilution and plating procedure was repeated to give eight plates for each of the four dilutions of macerated tissue.

All plates were incubated in the dark at room temperature for six days, until well-isolated and developed colonies were visible. During this time, culture tubes of Jensen's and peat extract agar of pH 4,5, 6 and 7 were prepared as above, autoclaved and made into slants for culture of the isolated microorganisms and a descriptive table and key for describing the colonies was set up. Once colonial growth was sufficient, typical colonies were described, asceptically picked from the plates (under the UV hood) and streaked onto slants of the appropriate medium and pH which were then labelled with a code giving the medium type (A or P), pH and colony number. In addition, slants derived from macerated tissue dilutions were labelled M. For instance, culture A62 would consist of material from the second colony sampled from a Jensen's *Azotobacter* plate of pH6, innoculated with supernatant material, while culture P4M1

would be from the first colony sampled from a peat extract plate of pH4, innoculated with macerated material. The slants were incubated in the dark at room temperature until good growth was established and then transferred to storage at 4°C in the refrigerator to slow the growth. Once these slants had been obtained from the first trial, the process was repeated to give two replicates of the isolation.

Characterization of the isolated cultures could now take place and preliminary classifications be attempted. The initial step involved preparing air-dried, heat-fixed slides of all isolates for Gram staining (Salle, 1967; Cowan, 1974). Stained slides were read, the Gram reaction and cell shape noted and the slides mounted in histoclad for future reference. Slants displaying little or no growth were checked using the stained slides and those with no apparent bacteria present were discarded. Some Gram-stained material was found to contain rods with numerous mycelia, indicative of *Actinomycetes* species. These cultures were set aside for additional tests useful in the identification of this group. As described in Williams and Cross (1971), cover slip cultures of suspected *Actinomycetes* were innoculated, allowed to grow for seven days and observed for characteristic structures. Most of these cultures appeared to grow poorly on slants and an attempt was made to improve their growth by reinnoculating them into the fluid medium (Norris and Jensen, 1958) with moderate success. In addition, catalase and oxidase tests as described below, were

performed on these cultures.

All cultures, except suspected *Actinomycetes*, were then carried through a test protocol modelled after Cowan (1974) to give some initial data for characterization.

The following tests were performed:-

(a) Acid - fast stain (Salle, 1967; Cowan, 1974).

(b) Spore stain (Salle, 1967; Cowan, 1974).

(c) Capsule stain, using india ink as a fast method giving good results (Hodder, pers.comm.).

(d) Catalase test (Salle, 1967; Cowan, 1974).

(e) Oxidase test (Salle, 1967; Cowan, 1974).

(f) Motility and flagellation (Mayfield and Innes, 1977).

Results from these tests were tabulated so that groups of cultures displaying identical patterns of characteristics could be determined. By reference to a standard microbiological test (Bergey, 1974), these groups were identified as far as possible.

RESULTS

I. Field Observations

(1) Field site descriptions

Vascular plant identifications are based on Fernald (1950) and Peterson and McKenny (1968); bryophyte identifications are based on Crum (1973).

Site 1 - Bauline Line (47°42'N,52°52'W)

This bog was located approximately 5km south-east of Bauline, Conception Bay, running along the eastern side of the roadway. It was an oligotrophic, ombrotrophic surface bog sloping towards the hill bordering its eastern side, with vegetation characterized by dwarf shrubs and *Sphagnum*. The average depth of peat was 2.3m, extending to a maximum of 4.4m (Pollett, 1968) with the surface peat in the range of H1.5-2 on the humification scale of Von Post (1926). The vegetation of the main bog surface consisted of hummocks of *Sphagnum* spp., notably *S. fuscum*, interspersed with mats of *Cladonia alpestris* (L) Rabenh and low shrubs such as *Empetrum nigrum* L., *Kalmia angustifolia* L., *Ledum groenlandicum* Retz., *Vaccinium angustifolium* Ait., and *Andromeda glaucophylla* Link. Small individuals of *Abies balsamea* (L.) Mill., and *Picea mariana* (Mill.) Brit., were found growing towards the edge of the bog as well as dominating the low forest surrounding it. This surrounding area was typical of the coniferous forests of the Avalon Peninsula, with species such as *Cornus canadensis* L., *Viola* sp., *Fragaria vesca* L., and *Rubus idaeus* L. under the low canopy of conifers

and shrubs. The transitional zone between the bog and the surrounding woodland was also of interest, consisting of dense stands of *Myrica gale* L., *Scirpus* sp., and *Eriophorum* sp. in addition to the low shrubs and mosses of the bog itself. Overall the bog appeared to be well-drained and possessed of a varied vegetation despite its poor nutrient status.

Site 2 - Colinet Experimental Farm (47°12'N,53°52'W)

The Experimental Farm at Colinet was part of a programme in bog reclamation for agricultural and grazing purposes being carried out by the provincial Department of Agriculture. The natural bog had been drained and grass cover encouraged, resulting in the reduction of bog species, especially *R. chamaemorus* L. The surface was hummocked but well-drained and therefore the growth of mosses was discouraged, with sedges and rushes dominating the hummock tops and grasses being found between them. Where the ground was damp or drainage was poor, *Vaccinium* spp., *Empetrum nigrum* L., and *R. chamaemorus* L., were found.

Site 3 - Doe Hills (47°38'N,53°49'W)

This locality, visited for collection of berries and rhizomes, was a fairly well-drained peatland on the lower slopes of several hills. The vegetation was similar to that of the Bauline site, with increased dominance by ericaceous shrubs and lichens.

Site 4 - Little St. Lawrence (46°52'N,55°20'W)

This extensive blanket bog was located in hilly terrain approximately 11km from Little St. Lawrence on the Burin Peninsula (Fig.6). Vegetation consisted mostly of grasses and *Carex* spp., with many ericaceous shrubs but few *R. chamaemorus* L. The peat was well-drained and appeared well-humified (H3-4).

Site 5 - Lawn (46°53'N,55°28'W)

This locality, approximately 31km west of St. Lawrence, consisted of a thin blanket peat over semi-exposed rock (Fig.7) with occasional patches of thicker peat. The surface was therefore uneven and vegetation scattered. The peat itself consisted of two layers distinguishable by their degree of humification. The upper zone of H1 peat extended to 6-10cm at which point the peat became more humified (H2-3), continuing in that state for 15-20cm to the rock surface. *Juniperus communis* L. scrub predominated, with considerable cover of *Cladonia* spp. in the drier areas (Fig.8). *R. chamaemorus* L., *Carex* spp., *Chamaedaphne calyculata* (L) Moench., *Vaccinium angustifolium* Ait., *Cornus canadensis* L., *Aster novae-belgii* L., *Habenaria blepharidiglottis* (Willd) Hook., *Solidago* sp., and *Scirpus* spp., were also present.

Site 6 - Fortune (46°55'N,55°66'W)

This extensive blanket bog was located 7.0 kilometres east of the town of Fortune. The bog surface was slightly rolling with some hummocks and several small pools towards the centre of the bog. Various species of

Figure 6. Little St. Lawrence; determining peat humification.

Figure 7. Lawn; bog and surrounding landscape.

Figure 8. Lawn; *R. chamaemorus* L. and associates. *Cladonia* and *Juniperus* are visible in the centre, *Scirpus caespitosus* L. to the upper right.

Sphagnum, notably *S. fuscum*, with the occasional inclusion of *Rhacomitrium* sp. and *Cladonia* spp., formed the basic surface vegetation. A layer of low shrubs dominated by *Juniperus communis* L. and *Empetrum nigrum* L. covered the surface of the hummocks, with the addition of *R. chamaemorus* L., *Myrica gale* L., and *Ledum groenlandicum* Retz. in drier areas. Sedges, grasses and some scattered individuals of *Sarracenia purpurea* L., were found between hummocks. The peat was deep, extending at least 40cm below the surface and well-developed with a steady gradation from 2-3cm of H1 *Sphagnum* peat through 20cm of H2 material to H5-8 at 30cm. Drainage was good and the peat was relatively dry.

Site 7 - Cape Freels ($49^{o}14'N, 53^{o}21'W$)

This site, located at the western end of the causeway between Cape Freels and South Bill, was unusual in that *R. chamaemorus* L. was found in the sandy upper edge of the beach. South of the road, sandy beach graded into a raised bog extending back from the shoreline, with a marshy area at the interface of these zones (Fig.9, foreground; Fig.10). On the opposite side, the bog was replaced by dry meadow vegetation consisting of grasses mixed with typical weed species such as *Taraxacum* sp., *Hieracium* sp., *Matricaria* sp. and others but no *R. chamaemorus* L. (Fig.11). The beach vegetation was typical, with *Potentilla anserina* L., *Mertensia maritima* (L.) S.F.Gray, *Rumex crispus* L. and various grasses present in scattered clumps (Fig.12). The intermediate area supported a mixture of these vegetation types on a sand/soil substrate,

Figure 9. Cape Freels; bog-beach intermediate zone. Large hummocks are characteristic of this zone.

Figure 10. Cape Freels; hummocks in bog-beach intermediate zone. The ground between these hummocks is marshy with many small pools due to run-off from the bog in the background.

Figure 11. Cape Freels; meadow-beach intermediate zone. The transition between meadow and beach is less distinct, but some low hummocks are visible.

Figure 12. Cape Freels; beach vegetation. *Potentilla anserina* L. and associates typically grow in isolated clumps.

with *R. chamaemorus* L., *Carex* spp., and *Sphagnum* spp. in the poorly drained hollows where water could accumulate (Fig.13). On the bog edge south of the road *R. chamaemorus* L. was found primarily in the marshy zone, apparently a run-off area for the main bog. It was characterized by hummocks up to 2m in diameter and 1m high, consisting of grasses, *Ledum groenlandicum* Retz., *Kalmia angustifolia* L., and *Vaccinium angustifolium* L. in addition to *R.chamaemorus* L.(Fig.14). Between these hummocks *Sphagnum fuscum* and *Iris versicolor* L. dominated (Fig.15). The overall topography was flat and drainage was poor.

Site 8 - Indian River (49°06'N,53°38'W)

This blanket bog was particularly notable because of the deposition of mica in the top layer of *Sphagnum*, most likely originating in the granit gneisses common in the area (Price and Douglas, 1972). The peat itself was shallow, from 10-15cm, and overlaid large rocks which made the surface very uneven and hummocked (Fig.16). Most of the peat was barely decayed and would certainly be no more than H1. The vegetation consisted of scattered grasses and shrubs such as *R. chamaemorus* L., *Empetrum nigrum* L., *Ledum groenlandicum* Retz., *Dalmia angustifolium* L., and *Vaccinium* spp. Fig.17 shows the bog surface.

Site 9 - Sandy Cove, Fogo Island (49°39'N53°59'W)

This was one of the few raised bogs located on the coast of Fogo Island, most of the island being dominated by low forests and grassy marshes not suitable for *R. chamaemorus* L. The bog was uneven but hummocked,

Figure 13. Cape Freels; *R. chamaemorus* L. on soil/sand substrate.

Figure 14. Cape Freels; *R. chamaemorus* L. and associates on hummock in bog-beach intermediate zone.

Figure 15. Cape Freels; *Iris versicolor* L. on side of hummock in bog-beach intermediate zone.

Figure 16. Indian River; area view. The bog surface is broken by large underlying boulders, as in the lower left.

Figure 17. Indian River; bog surface. The upper layer of *Sphagnum* is heavily encrusted with mica.

with good drainage (Fig. 18). The vegetation consisted of *Cladonia* and other lichens, *Polytrichum* sp., and *Empetrum nigrum* L. throughout with occasional patches of *Sphagnum* spp. and scattered shrubs such as *Ledum groenlandicum* Retz., *Kalmia angustifolium* Ait., *Vaccinium angustifolium* Ait., *V. oxycoccos* L., and *V. uliginosum* L. (Fig.19). *Eriophorum angustifolium* Honck, *Drosera rotundifolia* L., *R. chamaemorus* L., *Carex* spp. and grasses tended to be restricted to the tops of the hummocks (Fig.20).

Site 10 - Pistolet Bay (51°32'N,55°42'W)

This raised bog, located near Pistolet Bay Provincial Park, contained several central pools bordered by *Carex* spp. Beyond these pools, the surface was somewhat hummocked and well-drained, with a rich flora (Fig.21). *Polytrichum* spp. and some *Sphagnum* spp. were found in scattered patches but the dominant species of the ground flora were *Cladonia* spp. and *Empetrum nigrum* L. *Kalmia angustifolia* L., *Ledum groendlandicum* Retz., *Vaccinium angustifolium* Ait., *V. uliginosum* L., and *V. oxycoccos* L. were scattered throughout the bog (Fig.22). Small individuals of *Larix laricina* (Du Roi) K.Koch, *Juniperus communis* L., *Myrica gale* L., and *Betula* spp. occurred around the edges of the bog (Fig.23). The peat was fairly dry with a humification of H2-3 at the surface and for approximately 25cm below.

Site 11 - Ship Cove (51°33'N,55° 38'W)

This was a raised bog of approximately 10,000m^2 lying about 20m from

Figure 18. Sandy Cove, Fogo Island; area view of bog. The bog slopes downwards towards the coast on the left.

Figure 19. Sandy Cove, Fogo Island; surface vegetation dominated by *Cladonia* and *Empetrum nigrum* L.

Figure 20. Sandy Cove, Fogo Island; bog edge. Hummocks of *Carex* and grasses, as to the left, characterize this area.

Figure 21. Pistolet Bay; dense growth of low shrubs on bog surface.

Figure 22. Pistolet Bay; *R. chamaemorus* L. with associated bryophytes and *Ericaceae*.

Figure 23. Pistolet Bay; low shrubs and conifer community at bog edge.

Figure 24. Ship Cove; area view showing dominance of *Cladonia* (white).

the shoreline on the outskirts of the community of Ship Cove (Fig.24). Topography and drainage were uneven, as evidenced by the presence of irregular wet and dry areas within the bog (Fig.25). Although the peat was not especially deep, reaching a maximum of 50cm,, it was in the early stages of decomposition with ratings of H4-5 at 12-20cm below the surface. Dry areas were similar in vegetation to the previous site, with numerous *R. chamaemorus*L. (Fig.26). The wet areas were dominated by *Carex* spp., *Myrica gale* L., and grasses, with few *R. chamaemorus* L. Dwarf shrubs and conifers bordered these areas.

Site 12 - L'anse-au-Meadows (51°22'N,55°30'W)

This large raised bog circled the L'anse-au-Meadows archaeological site to the southwest and east. Beyond the bog, vegetation consisted of grassy meadowland with *Cornus canadensis* L., *Hieracium* spp., and *Taraxacum* spp. predominating (Fig.27). Vegetation and topography were similar to the previous two sites, except that drainage was slightly reduced and the peat was correspondingly damper (Fig.28).

Site 13 - Griquet (51°27'N,55°28'W)

This raised bog extended for approximately 100m on both sides of the roadway. The surface was hummocky and wet, so that the basic vegetation seen in Sites 10, 11 and 12 was modified to include a greater cover of *Sphagnum* spp. and ericaceous shrubs. *R. chamaemorus* L. was numerous wherever the shrub cover was reduced (Fig.29).

Figure 25. Ship Cove; variation between dry (foreground) and wet (background) areas. *Carex* and *Myrica gale* L. dominate the latter.

Figure 26. Ship Cove; *R. chamaemorus* L., *Empetrum nigrum* L., and associates.

Figure 27. L'anse-au-Meadows; bog margin and adjacent meadow.

Figure 28. L'anse-au-Meadows; surface vegetation. The increased growth of *Empetrum nigrum* L. and *R. chamaemorus* L. indicates a damper substrate.

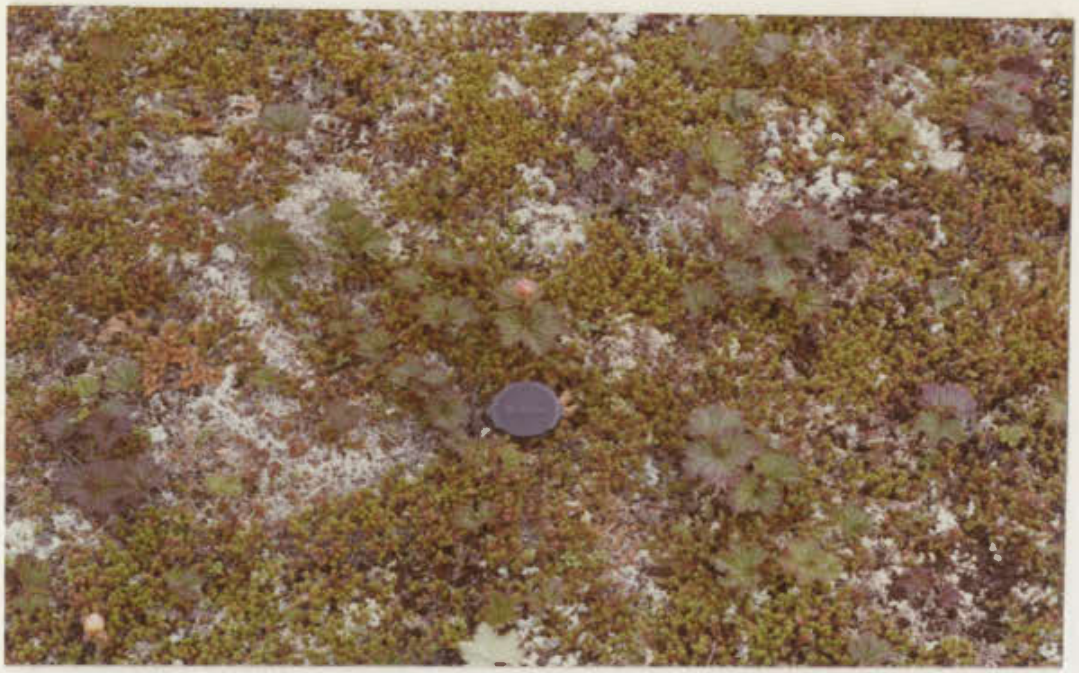


Figure 29. Griquet; *R. chamaemorus* L. in fruit in open area of bog.

Figure 30. L'anse-au-clair; area view of bog and central pond.

Site 14 - L'anse-au-clair (51°25'N,57°02'W)

This site consisted of a hummocky raised bog surrounding a 75m x 25m pond in a valley area 0.5km from the community (Fig.30). The bog extended for approximately 20m beyond the sides of the pond and 50m beyond the ends, where it graded into a low conifer forest dominated by *Abies balsamea*(L.) Mill. A variety of lichens and mosses including *Sphagnum* spp., *Rhacomitrium* spp., *Polytrichum* spp., and *Cladonia* spp. were present. Most of the ground cover, particularly on the hummocks, consisted of *Empetrum nigrum* L. and *Cladonia* spp., with scattered dwarf *Larix laricina* (DuRoi) K.Koch, *Ledum groenlandicum* Retz., *Vaccinium oxycoccos* L., and *V. uliginosum* L. (Fig. 31). *Carex* spp. dominated the vegetation of the pond edges. The peat was very damp, becoming drier in the areas further from the pond (Fig. 32).

Site 15 - Forteau (51°26'N,56°68'W)

This raised bog of approximately 20,000m^2 was located on a terrace above Forteau Brook (Fig.33). The surface was flat, but dry channels, 0.5-1.0m deep and 1.0-1.5m wide transected the entire bog so that it consisted of irregular hummocks with flattened summits up to several metres in diameter (Fig.34). *Polytrichum* spp. and *Carex* spp. grew in the bases of the channels; *Ledum groenlandicum* Retz. and *R. chamaemorus* L. on their banks (Fig.35). The vegetation of the surface was dominated by *Empetrum nigrum* L. and *Cladonia* spp., with some additional mosses and shrubs as in the previous site. Dwarfed

Figure 31. L'anse-au-clair; *R. chamaemorus* L. and associated species on hummock.

Figure 32. L'anse-au-clair; increased growth of *R. chamaemorus* L. on drier peat upslope from pond edge.

Figure 33. Forteau; area view of terrace above Forteau Brook, with bog in centre.

Figure 34. Forteau; channel and hummock system.

Figure 35. Forteau; vegetation of channel system. *Polytrichum* and *Carex* dominate the channel base. The bank to the upper right is dominated by *R. chamaemorus* L. and *Ledum groenlandicum* L.

Figure 36. Capstan Island; bog surface with fen pools and extensive growth of *Carex*.

Kalmia angustifolia L. was also present. Possibly due to the channeling, the peat was generally drier and less humified than in the previous site.

Site 16 - Capstan Island (51°34'N,56°43'W)

This site, located on the plateau above Capstan Island, was a large, irregular raised bog with inclusions of fen pools and *Carex*-dominated vegetation (Fig.36). Hummocks of *Sphagnum* spp, *Polytrichum* spp., *Rhacomitrium* spp., and *Cladonia* spp. where the peat was deep and well-developed (H3-4) were located between these wetter areas. *R. chamaemorus* L. and *Vaccinium uliginosum* L. were particularly abundant on these hummocks, in addition to less frequent species such as *Empetrum nigrum* L., *Ledum groenlandicum* Retz., and *Vaccinium oxycoccos* L. The drier bog edge was covered with stands of *Myrica gale* L., *Betula* spp., *R. chamaemorus* L. and conifer shrubs.

Site 17 - Red Bay (51°44'N,56°45'W)

This bog was one of the few located in the Red Bay-Pinware River area, since the neighbouring landscape was dominated by barren hills and deep valleys unsuitable for bog development. It was approximately 40,000m^2 and located directly on the outskirts of the community of Red Bay. The bog surface was much more even than in the previous Labrador sites, with little hummocking (Fig.37). Surface vegetation consisted of almost total cover by *Empetrum nigrum* L. and some patches of *Cladonia* spp. (Fig.38) with scattered clumps of *Vaccinium uliginosum* L. and *Ledum groenlandicum* Retz. Low *Juniperus communis* L. and

Figure 37. Red Bay; area view showing limited hummocking of the bog surface.

Figure 38. Red Bay; surface vegetation, dominated by *Empetrum nigrum* L. and *Cladonia*.

ericaceous shrubs bordered the bog but only a few stunted conifers were present. The peat was dry and poorly developed, from H2-3.

Site 18 – Cat County Brook (51°43'N,56°35'W)

This site was a small bog located in a stream valley between Pinware River and Red Bay, surrounded by mature conifer forest (Fig.39). *Empetrum nigrum* L. and typical bryophyte genera predominated, with vegetation and peat development similar to the previous site.

Site 19 – Pinware River Delta (51°37'N,56°35'W)

This large complex bog was situated at the mouth of the Pinware River Valley. Bog pools were scattered throughout the surface and drainage was generally uneven (Fig.40). The surface vegetation consisted of *Empetrum nigrum* L., *Polytrichum* spp., *Rhacomitrium* spp. and some *Sphagnum* spp., with considerable cover of shrubs such as *Vaccinium angustifolium* Ait., *V. uliginosum* L., *V. vitis-idaea* L., *Kalmia angustifolia* L., *Ledum groenlandicum* Retz., and *Juniperus communis* L. (Fig.41). Clumps of *Carex* spp., *Diapensia lapponica* L. and grasses were largely restricted to drier areas. Peat development was good, the peat being well-stratified and decomposed (H4-5).

Site 20 – French Cove, Grey Islands (50°40'N,55°35'W)

This large blanket bog, approximately 1km long and 200m wide, circled the cove between a belt of *Alnus* scrub at each level and the more

Figure 39. Cat Country Brook; area view. The surrounding vegetation is markedly different from that of the bog.

Figure 40. Pinware River Delta; area view showing bog pools.

Figure 41. Pinware River Delta; *R. chamaemorus* L. in association with *Empetrum nigrum* L., *Cladonia* and ericaceous shrubs.

Figure 42. French Cove, Grey Islands; western edge of cove, with bog in foreground. The reddish color is entirely due to senescing *R. chamaemorus* L. leaves.

barren hills behind the cove (Fig.42). The bog sloped upwards towards the hills and grassland around the abandoned settlement to the south-east but was otherwise of uniform gently hummocked topography. *R. chamaemorus* L. accounted for close to 100% cover over the bog surface, mixed with fairly continuous cover of *Empetrum nigrum* L. and *Cladonia* spp. (Fig.43). Only a very few scattered ericaceous shrubs were otherwise present. The peat was poorly developed (H1-3) although fairly moist and was less than 50cm deep in most instances.

Site 21 - Parson's Pond ($58^{o}00'N, 57^{o}45'W$)

This large blanket bog extended for several kilometres along the shores of Parson's Pond (Fig.44). The surface showed well-developed hummocks and hollows but peat was generally shallow (less than 50cm), dense, and well-humified (H6+). Hummocks were dominated by *Empetrum nigrum* L., *Eriophorum angustifolium* Honk., *Scirpus* spp., *Carex* spp. and lichens in addition to some moss development in patches. Low shrubs such as *Myrica gale* L. *Ledum groenlandicum* Retz., *Kalmia angustifolia* L., and *Vaccinium* spp. were present, as well as scattered stands of dwarfed *Picea mariana* (Mill.) Brit. A summary of the more important characteristics of these sites is given in Table 2.

(2) Autecology and development of *Rubus chamaemorus* L:

(a) Flowering

Data on flower times and development is largely drawn from observations in Bauline Line, Witless Bay and the greenhouse, since most other bogs were visited during the fruiting period. Flowering at Bauline generally

Figure 43. French Cove, Grey Islands; surface vegetation dominated by *R. chamaemorus* L. and *Empetrum nigrum* L.

Figure 44. Parson's Pond; southern edge of bog with well-developed hummocks and stands of *Carex* and grasses.

TABLE 2. Summary of key site characteristics from field observations

Status of *Rubus chamaemous* L.	Sites	Peat humification	Topography	Dominant Vegetation
1. Poor (<10 berries/m^2; 20-40 shoots/m^2	1. Bauline	H1.5-2	hummocked	Ericaceae/Sphagnum/Cladonia
	7. Cape Freels	Sand-N.A.	"	Cyperaceae/Sphagnum/Gramineae
	8. Indian River	H1	uneven, rocky	Ericaceae/Gramineae
	9. Sandy Cove	H1-2	hummocked	Empetrum/Cladonia/Polytrichum
	18. Cat County Brook	H2-3	low hummocks	Empetrum/Cladonia
2. Average (10-25 berries/m^2; 6-50 shoots/m^2)	2. Colinet	H3-4	hummocked	Cyperaceae/Gramineae
	4. Little St. Lawrence	H3-4	"	Cyperaceae/Gramineae/Ericaceae
	5. Lawn	H1-3	uneven, rocky	Juniperus/Cladonia
	6. Fortune	H2-8	hummock/pool	Sphagnum/Juniperus/Empetrum
	10. Pistolet Bay	H2-3	" "	Caldonia/Empetrum
	13. Griquet	H4-5	hummocked	Sphagnum/Ericaceae
	14. L'anse-au clair	H2-3	"	Bryophyta/Cladonia/Empetrum
	15. Forteau	H2-3	channel/hummock	Empetrum/Cladonia
	16. Capstan Island	H3-4	hummock/pool	Bryophyta/Cladonia
	17. Red Bay	H2-3	low hummocks	Empetrum/Cladonia
	19. Pinware River	H4-5	hummock/pool	Empetrum/Bryophyta
	21. Parson's Pond	H6+	hummocked	Empetrum/Cyperaceae/Ericaceae
3. Excellent (>25 berries/m^2; 40-50 shoots/m^2)	3. Doe Hills	H3	Sloping	Sphagnum/Cladonia/Ericaceae
	11. Skip Cove	H4-5	uneven, pools	Cladonia/Empetrum/Sphagnum
	12. L'anse-au-meadows	H4-5	uneven, pools	Cladonia/Empetrum/Sphagnum
	20 French Cove, Grey Islands	H1-3	gently hummocked	Empetrum/Cladonia

occurred from 2-3 weeks after the bog had thawed. In 1977 and 1979, flowering had occurred by the first week in June, contrasting with a relatively late flowering in the second week of July 1978. This coincided with a fairly long winter, in which the bog surface remained frozen until early June. Male flowers at Bauline, as in other bogs studied, opened approximately one week prior to the females each year, with some overlap.

During 1979 more detailed observations of flower development were made on the more productive bog at Witless Bay. Open male flowers were first observed on May 23 and began to abscis on May 31. At this time several premature females were noted but it was not until June 27 that numerous females had opened. Many insects were present during this period, in particular species of Formicidae and Syrphidae. Ants were often found on the flowers, apparently feeding from the base of the petals, where nectaries were subsequently seen in microscopical examinations. By July 18 most flowers had either developed into mature berries or dried up, with the incidence of undeveloped female flowers reaching 70-80%.

Flowering occurred in greenhouse transplants only in their first season, from floral buds which had developed prior to transplantation. No new buds were produced in any greenhouse plants, despite successful vegetative development, even after cold-shock treatment. Records of flowering times were kept during 1977 for transplants from Bauline Line

to compare with natural flowering and are presented in Table 3. Twenty-four flowers were produced from May 24 to June 14, considerably earlier than at Bauline and extending over a longer period. Flowering took place from eight to nineteen days after transplantation with an average of fourteen days. As shown in Fig.45, rhizomes collected later in the season tended to flower more quickly once they had been transplanted. Male and female flowers developed at approximately the same average time, 13.4 and 13.5 days after transplanting, with 10 males and 14 females produced. Of these 70.8% were tetramerous, 25.0% were pentamerous and 4.2% were trimerous, relative frequencies remaining similar for males and females.

(b) Fruit Development

The maturation period for berries tended to overlap with the flowering period of female flowers, with the first berries found from two to four weeks after the last female flowers had opened. Mature fruits were first noted at Bauline Line on August 18, 1977; July 20, 1978 and July 18, 1979 and at Witless Bay on July 18, 1979.

Table 3. Flowering data for rhizomes collected during 1977

Date Collected:	Date of Flowering:	Details:
May 12	May 24	Male, tetramerous
May 12	May 26	Female, pentamerous*
May 17	May 26	Female, tetramerous*
May 12	May 27	Female, pentamerous*
May 17	May 30	2 males, tetramerous
May 12	May 30	Male, tetramerous
May 12	May 30	Female, tetramerous*
May 12	May 30	Deformed male, tetramerous
May 18	May 30	Male, pentamerous
May 17	May 30	Male, tetramerous
May 17	June 1	Deformed male, tetramerous
May 17	June 2	Female, tetramerous*
May 18	June 3	Male, pentamerous
May 17	June 6	Female, tetramerous*
May 18	June 6	Male, tetramerous
May 30	June 10	Female, tetramerous
June 2	June 10	Female, tetramerous
June 2	June 13	Male, trimerous
May 30	June 13	Female, tetramerous
May 30	June 13	Female, tetramerous
May 30	June 13	Female, pentamerous
May 30	June 13	Female, pentamerous
May 31	June 14	Female, tetramerous

* Treated with indoleacetic acid in lanolin

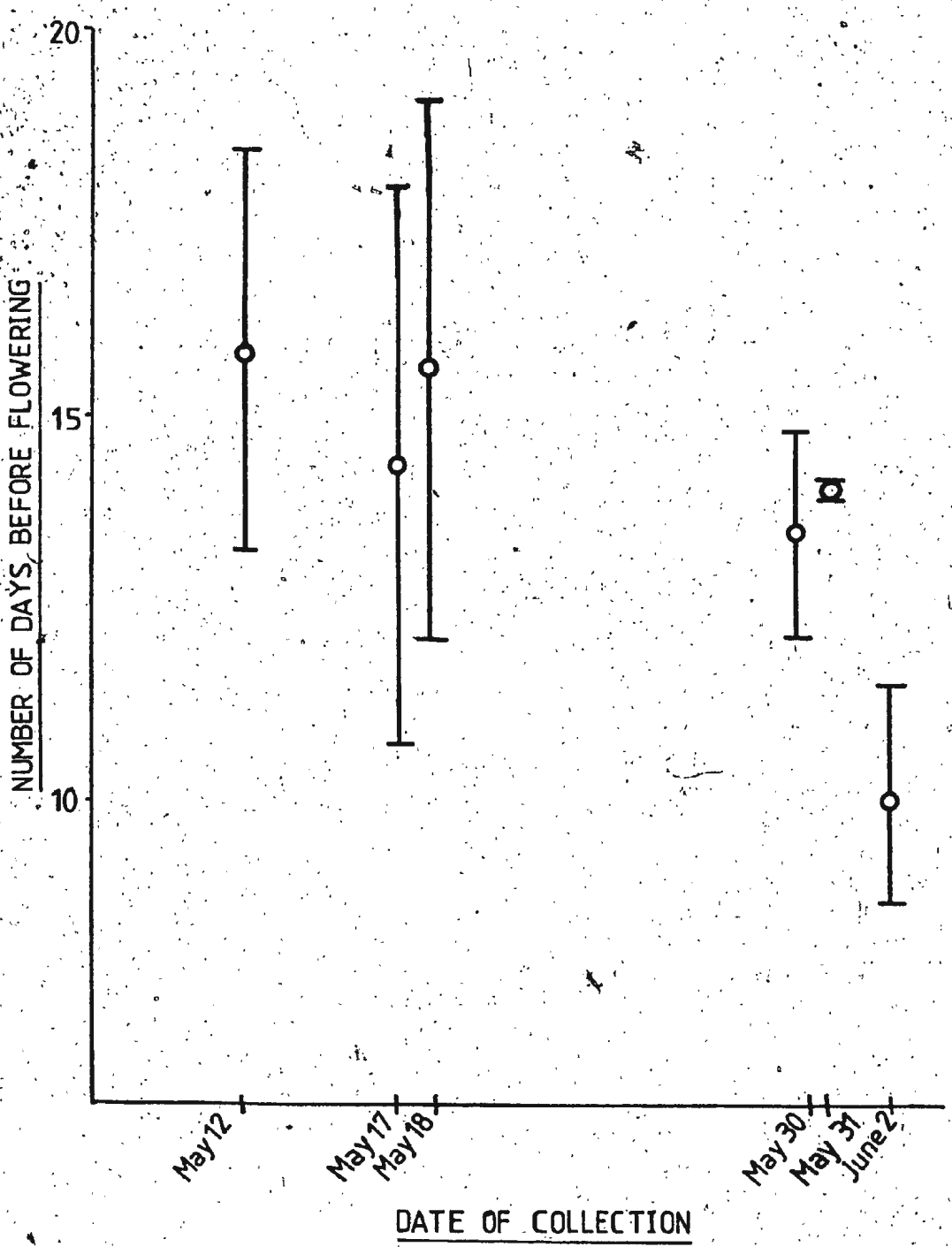


Figure 45. Graph of mean flowering time versus date of collection. For each date of collection, values were averaged and standard deviation computed.

Fruits were collected from Doe Hills on August 11, 1977. Berries were found at all sites examined in August 1978 and in most cases had been ripe for some time previously, as the areas had already been picked over to an appreciable degree. Generally, berries were at the same stage of development in the Great Northern Peninsula and Southern Labrador sites although they were visited two weeks later than the more southerly sites, indicating that their development is somewhat delayed. Berries senesce at the same time as the leaves and usually fall by late September.

Productivity was often difficult to quantify because of the uneven harvesting pressure and was therefore only subjectively assessed. Sites were classified as poor (<10 berries/m^2), average (10-25 berries/m^2), or excellent (>25 berries/m^2). Sites 1, 7, 8, 9 and 18 were poor areas. Excellent sites included sites 3, 11, and 20. Site 20 is especially well-known to residents of the area who report that thousands of litres had been harvested from the bog in good seasons, despite its inaccessibility. All other sites showed average berry densities and tended to be visited by residents on a regular basis. In general, Cape St. Mary's, the Fortune/St. Lawrence area and St. Anthony/Southern Labrador are considered to be productive areas by residents and considerable effort is expended in harvesting and retailing the berries either through firms such as Bidgood's or on an individual basis (Fig.46).

Figure 46. Bakeapple seller, Lord's Cove. Picking and selling bakeapples to passing motorists is a common source of extra income, especially for school-age children.

Success of fruiting appeared to be low in poor bogs, with a considerable percentage of the flowers remaining undeveloped, whereas few undeveloped flowers were noted in the other two productivity classes. The number of failed flowers was higher at Bauline Line and Witless Bay during the hot, dry summer of 1979. During a visit to Goose Bay in the summer of 1977 bakeapples were seen growing near the town but not fruiting. According to residents, these stands normally produced harvestable quantities of fruit but were unproductive that year due to a severe late spring frost.

(c) Vegetative Development

At Bauline Line and Witless Bay, most vegetative growth of *R. chamaemorus* L. took place after flowering had been completed and berries were maturing. Immediately after most berries were mature in August, new aerial shoots with small leaves were produced in great numbers and new rhizomes extended rapidly. By late September all aerial leaves had withered and developed fall colouration, although most leaves did not detach from the stalk. Floral buds for the next season were visible at the shoot bases just below the surface of the peat. Very little vegetative growth appeared to take place during flower development except for the unfolding of leaf buds just prior to flowering.

Greenhouse plants showed a similar pattern of vegetative development, differing from wild populations in having longer durations

for most stages of growth and a longer growing season overall. Rhizomes transferred to the greenhouse throughout the summer of 1977 developed normal leaves, shoots, roots and rhizomes within the same season. Leaves did not senesce fully until December, three months later than the population from which they had been harvested. Shoots and buds began appearing at the same time and developed throughout the winter with the first leaves emerging in early April, 1978. Vegetative growth continued through the season, increasing in August and September until leaf senescence in November and December. New leaves emerged in early April of 1979 and growth continued throughout that year.

As shown in Table 4, the sites examined in August, 1978 varied in shoot density, the highest value occurring at Grey Islands and corresponding to nearly 95% cover. In general, the northern bogs located near the coast had higher shoot densities, while marginal or atypical habitats such as Cape Freels, Lawn and Indian Cove had lower densities. Distribution of plants also varied with differences in bog topography, especially hummocks and bog pools. Cover was uniform on sites with fairly even surfaces; for instance those on the Burin Peninsula, L'anse-au-clair, Red Bay, Cat Country Brook and Grey Islands. Where hummocks occurred, as at Cape Freels, Capstan Island and Pinware River, *R. chamaemorus* L. tended to display increased density and cover on the hummock surfaces. In bogs with alternating bog pools and drier areas, the density of

bakeapple increased with distance from the pool edges and damper ground.

Size and vigour of shoots, especially of the leaves, did not appear to vary considerably with habitat.

Table 4. Table of shoot densities and distribution of *R. chamaemorus* L. in study sites, August 1978.

Site	Shoots per m^2	Distribution
St. Lawrence	12–20	Dispersed
Lawn	6	Scattered
Fortune	12–20	Scattered
Cape Freels	20–30	Mostly on hummocks
Indian River	20	Scattered
Sandy Cove	40	Scattered
Pistolet Bay	40	Numerous in open areas and away from pools
Ship Cove	40	In drier patches
L'anse-au-Meadows	40	In drier patches
Griquet	40–50	Cover varies
L'anse-au-clair	20–30	Fairly even
Forteau	30–50	More at channel edges
Capstan Island	40–50	Continuous on hummocks
Red Bay	20–40	Scattered
Cat Country Brook	20–40	Scattered
Pinware River	20–40	Hummocks and drier areas
Grey Islands	50	Almost continuous
Parson's Pond	20	Scattered

However, in more sheltered areas of a given bog, larger and more robust shoots could be found, as in Fig.47. Here, at L'anse-au-clair, *R. chamaemorus* L. grew well into the scrub woodland at the edges of the bog. Leaves of these individuals measured approximately up to 10cm, twice as large as leaves of plants growing in the open.

II. Growth Experiments

(1) Field experiment

The results of this experiment on shoot growth after rhizome cutting in the field are given in Tables 5, 6, and 7. Table 5 shows the number of shoots per sector for all quadrats immediately after cutting, before flowering had occurred. Tables 6 and 7 show the shoot counts taken after flowering in the same and subsequent season.

Analysis of variance at a significance level of 0.05 was performed on each set of averages per sector type (i.e. A, B, C, D) to determine if there was a significant difference between the sector types in the number of aerial shoots produced. As expected, no significant difference was detected between the types for the initial control counts. However, no significant difference was detected for either of the subsequent counts, although there is a noticeable upward trend in the data. In both cases, the variation due to error as reflected in the error mean square value was high,

Figure 47. *R. chamaemorus* L. in scrub bordering L'anse-au-clair bog, with leaves approximately 10cm wide.

suggesting that differences between the plots may have affected the overall results. To test this, two procedures were used.

Table 5. Field experiment. Number of shoots per sector prior to flowering (July 6, 1978)

Sector		Quadrat 1	Quadrat 2	Quadrat 3	Total	Average
A		36	24	17	77	25.7
B	1	25	18	5	48	16.0
	2	22	15	8	45	15.0
TOTAL:		47	33	13	93	31.0
C	1	13	10	1	24	8.0
	2	4	8	3	15	5.0
	3	3	16	4	23	7.7
	4	2	11	9	22	7.3
TOTAL:		22	45	17	84	28.0
D	1	1	1	1	3	1.0
	2	3	4	3	10	3.3
	3	1	3	1	5	1.7
	4	3	1	1	5	1.7
	5	2	2	7	11	3.7
	6	4	1	5	10	3.3
	7	5	2	3	10	3.3
	8	3	3	2	8	2.7
TOTAL:		22	17	23	62	20.7

Sector A = 1.000m^2 Sector C = 0.250m^2
Sector B = 0.500m^2 Sector D = 0.125m^2

Table 6. Field experiment. Number of shoots per sector after flowering. (July 20, 1978)

Sector		Quadrat 1	Quadrat 2	Quadrat 3	Total	Average	%Increase
A		37	24	26	87	29.0	12.9
B	1	31	22	9	62	20.7	29.4
	2	24	21	11	56	18.7	24.7
TOTAL:		55	43	20	118	39.3	26.9
C	1	13	10	3	26	8.7	8.7
	2	6	13	4	23	7.7	54.0
	3	5	17	5	27	9.0	16.8
	4	4	16	10	30	10.0	36.9
TOTAL:		28	56	22	106	35.3	26.2
D	1	1	3	3	7	2.3	130.0
	2	3	9	4	16	5.3	60.6
	3	4	7	1	12	4.0	135.3
	4	3	9	1	13	4.3	152.9
	5	5	6	7	18	6.0	62.2
	6	5	3	7	15	5.0	51.5
	7	6	4	3	13	4.3	30.3
	8	6	5	4	15	5.0	85.2
TOTAL:		33	46	30	109	36.3	75.8

Sector A = 1.00m^2
Sector B = 0.50m^2
Sector C = 0.25m^2
Sector D = 0.125m^2

Table 7. Field Experiment. Number of shoots per sector after flowering, second season (June 29, 1979)

Sector		Quadrat 1	Quadrat 2	Quadrat 3	Total	Average	% Increase
A		49	32	15	96	32.0	24.8
B	1	33	22	16	71	23.7	48.1
	2	29	25	14	68	22.7	51.3
TOTAL:		62	47	30	139	46.3	119.5
C	1	22	13	3	38	12.7	58.7
	2	11	15	8	34	11.3	126.0
	3	14	20	5	39	13.0	68.8
	4	8	23	12	43	14.3	95.8
TOTAL		55	71	28	154	51.3	83.3
D	1	6	8	2	16	5.3	430.0
	2	6	7	2	15	5.0	51.5
	3	7	7	1	15	5.0	194.1
	4	7	10	0	17	5.7	235.3
	5	13	7	8	28	9.3	151.3
	6	8	3	9	20	6.7	103.0
	7	8	3	10	21	7.0	112.1
	8	2	10	7	19	6.3	133.3
TOTAL:		57	55	39	151	50.3	143.5

Sector A = 1.000m^2
Sector B = 0.500m^2
Sector C = 0.250m^2
Sector D = 0.125m^2

Total and percent increases per sector of quadrats (Table 6) are close to five times as great for Sector D than for Sector A, with a percent increase of 143.5 for D versus 24.8 for A. Therefore, analysis of variance was performed on the increase in number of shoots per sector type as given in the table. In this case, a significant difference was indicated at a significance level of 0.05. Dunnett's test was used to determine which sector types showed significant difference from the control Sector A.

In addition, analysis of variance was used to test for significant differences between the quadrats. At a significance level of 0.05, the quadrats did not show a significant difference in the initial counts. However, analysis of the subsequent counts showed that although there was again no difference between the quadrats in the first season, a significant difference was present between the quadrats in the second season. Quadrat 3 showed significantly lower numbers of shoots in all sectors. This quadrat was poorly drained, which may have affected its response to the experimental procedure.

Significant increases were produced by the cutting procedure, maximum results being obtained with sectors of $0.125m^2$.

(2) Greenhouse experiments

(a) Minimum length

Measurements of maximum leaf length and width (Fig.48) at 7, 18 and 28 days after the initial appearance of leaves are given in

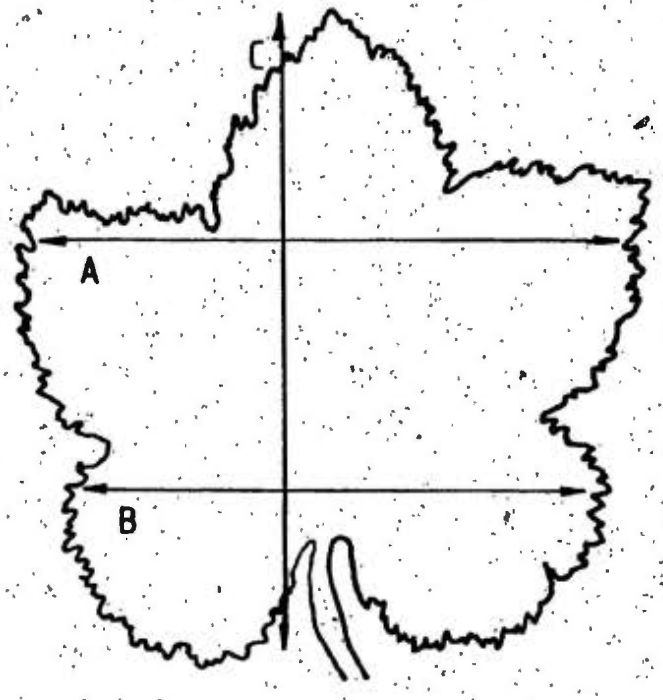


Figure 48. Minimum length experiment. Lines used for leaf length and width measurements.

Table 8. It should be noted that many leaves began to senesce soon after their first appearance and therefore some measurements decreased over time. On the whole, average lengths and widths increased for each group and with increased length of rhizome for each set of measurements.

Analysis of variance and Dunnett's test for measuring the difference between group means and control means were performed for each set of measurements at a significance level of 0.05. Both mean lengths and mean widths showed a significant difference between the various rhizome length groups for all sets of measurements. Dunnett's test, using 20cm rhizomes (R20 group) as control, showed mean lengths and widths of the R75 groups to be significantly higher for all sets of measurements. The R40 group displayed significantly higher mean lengths and widths at 7 days and the R50 group displayed a significantly higher mean width at 18 days. Otherwise, no group was significantly different from the R20 group.

In the following summer, only R50 and R75 groups produced healthy shoots and roots. Both these groups continued to grow successfully and produced new rhizomes, roots and aerial shoots in subsequent years although no flowers were produced. Figure 49 shows the difference in number and size of shoots produced by R30 and R40 rhizomes.

Table 8. Average length and width (mm) of leaves produced by rhizomes of given lengths at 7, 18 and 28 days after transplantation

Rhizome length (cm)	7 days	18 days	28 days	% increase	7 days	18 days	28 days	% increase
20	21.3	19.9	26.1	22.5	22.2	25.6	26.1	17.6
30	24.7	21.8	33.4	35.2	31.4	31.5	33.4	6.4
40	27.9	26.6	40.6	45.5	37.3	37.8	40.4	8.3
50	25.8	24.9	41.2	59.7	32.8	35.1	41.2	25.6
75	33.3	30.9	50.4	51.3	45.5	46.4	50.4	10.8

Figure 49. Minimum length experiment; growth of R30 and R40 rhizomes. Numbers and dimensions of leaves from R30 rhizomes (top) are noticeably less than from R40 rhizomes (bottom).

(b) Apical/non-apical regeneration

Since all leaves produced in the first season by both N1-5 and IN1-5 rhizomes were small, it was decided that measurements of leaf size would be irrelevant and observations were restricted to patterns of leaf development. Shoots were emerging from all rhizomes by 16 days after planting and continued to develop throughout the season until senescence. Maximum growth was reached approximately one month after planting. At this time there was no difference in regeneration between N and IN rhizomes. In rhizomes cut at the nodes, the bud next to the apex developed first, whereas any bud of the internodal cuttings seemed likely to develop. Although no clear apical dominance was established, the development of one shoot appeared to inhibit successful growth of subsequent shoots. Even where several buds adjacent to the dominant produced shoots (Fig.50), these withered completely before the dominant. Development of shoots appeared to be favoured closest to the original growing tip of the rhizome (Fig.50, bottom). Root growth showed similar trends and was most successful at the dominant node. Overall the plants in N and IN groups were not as vigorous and leaves were smaller than those in the control group.

(c) Seedling growth patterns

Growth of seedlings in large and small boxes was similar. In the first growing season, vegetative growth was restricted to the area closest to the original plant, with several new shoots being produced. By the

Figure 50. Apical/non-apical regeneration. Patterns of shoot regeneration in internodal (IN) and nodal (N) rhizomes.

end of the first season, rhizome growth from the original plant had occurred with new buds present at the rhizome tips only. Rhizomes were put out in several directions and grew to the maximum possible length (i.e., to the edges of the box) before developing buds. In the following spring, all buds developed fully and shoots produced new buds. However, in the third season the buds from the original plant failed to develop and the rhizomes linking it to the daughter shoots began to wither. Meanwhile the daughter plants had produced healthy roots and shoots as well as new rhizomes. These did not extend as far as previously and by the end of the season there were several clusters of healthy plants around the edges of the box, all extending rhizomes towards the centre. Figure 51 shows the smaller box; the original shoot was located just below the tape measure in the centre of the box. Of special interest is the presence of a bryophyte, *Bryum argenteum* (det. G.Brassard), which colonized and quickly overgrew the surface during the second season. Growth of the plants after this colonization appeared to be improved, although qualitative measure of this increase was not attempted.

(d) Effects of reduced competition

By selectively weeding out competitive species, the growth of *R. chamaemorus* L. in the turf sample collected at Colinet was considerably enhanced. New shoots and rhizomes were produced several months after transplantation, nearly doubling the original

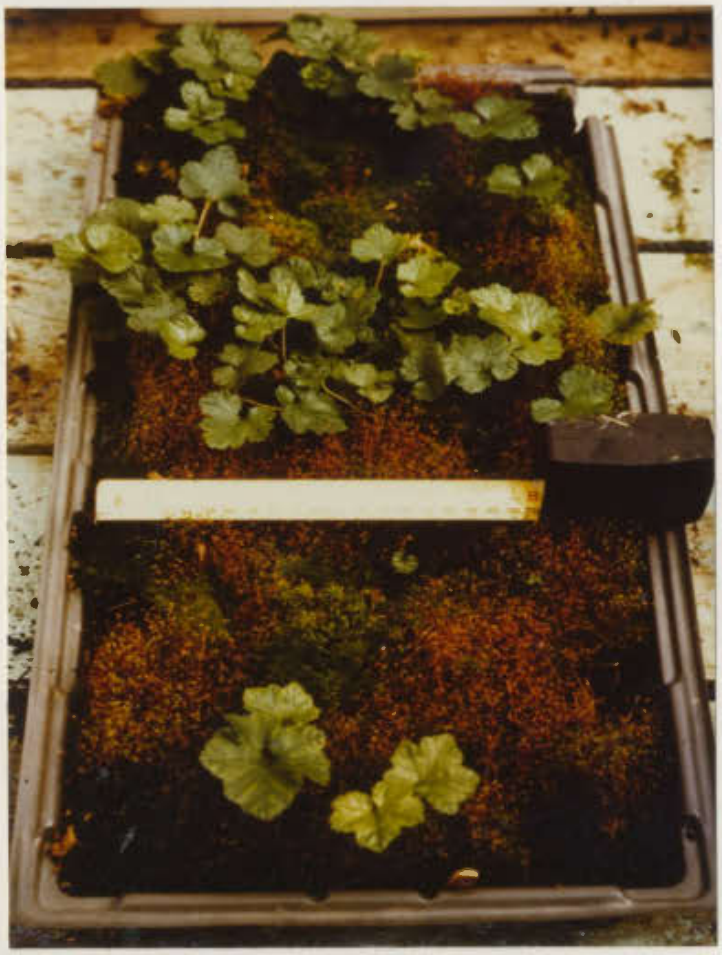


Figure 51. Seedling growth pattern. The original seedling shoot was located below the center of the tape; the visible shoots are daughter plants produced in the second year after transplantation.

cover. In the second growing season, shoots of *Empetrum nigrum* L., *Kalmia angustifolia* L., and *Vaccinium angustifolium* Ait. continued to regenerate but by the third season, regeneration of the original competitive species had nearly ceased and most of the 'weeds' removed were young seedlings of other greenhouse species. At this time cover of *R. chamaemorus* L. was close to 75% with numbers and size of leaves showing parallel increases.

(e) Floral induction

Attempts to induce fruit development by the exogenous application of indoleacetic acid were unsuccessful. Six flowers were treated with IAA in lanolin within one day of flowering. In all cases, petal abscision occurred within a week, followed soon after by withering of the carpels. No fruits were produced.

Chilling experiments were ineffective in inducing carpel development.

(3) Establishment of a greenhouse population

By the final summer of the programme a viable greenhouse population of *R. chamaemorus* L. had been established and was utilized for many experiments. The majority of transplants originated from the Bauline Line site with the addition of rhizomes collected from all study sites. There did not appear to be any variation in the ability of plants from different localities to grow in the greenhouse. Flowering of established plants often occurred in the same season but floral buds were not produced in the greenhouse.

and no fruits developed.

III. Seed Germination

(1) Reduction due to fungal infection

(a) Measurement of mortality due to fungal infection

Table 9 gives germinations and losses for the treatments employed. It must be noted that many of the seeds became infected only after germination so that there is overlap between germinations and losses. As was expected, germination percentages were higher for GA_3 and 10^{-5} (stock) kinetin than for the other treatments, although there was considerable variation in results. However, losses due to fungal mortality were consistently higher than 50% in all treatments, with no apparent difference between them.

(b) Germination of seeds under sterile conditions

The initial test method reduced fungal mortalities by approximately 50% in subsequent germination trails, although it was still necessary to replace the filter papers and remove infected seeds periodically. Fewer seedlings tended to become infected after germination, as in the previous experiment.

Results from preliminary trials with cyclohexamide/chloramphenicol and pre-sterilization methods were encouraging. Only the dish containing seeds treated in calcium hypochlorite for five minutes and incubated in distilled water showed any fungal growth. Germinations were largely successful although proportions were lower

Table 9. Mortality, due to fungal infection for seeds incubated with water, kinetin and GA_3 (gibberellic acid) for 30 days.

Batch No.	Incubation medium	Percentage fungal mortality
1 + 2	H_2O 10^{-5} kinetin, 10^{-5} GA_3	*
3	Kinetin 10^{-5}	58.0
	10^{-6}	94.0
	10^{-7}	50.0
4	Stock GA_3	84.0
	Stock kinetin	92.0
5	Stock kinetin	60.0
6	Kinetin 10^{-5}	82.0
	10^{-6}	94.0
	10^{-7}	72.0
7	Kinetin 10^{-5}	64.2
8	Kinetin 10^{-5}	98.0
9	Kinetin 10^{-5}	79.0
10	Stock kinetin	92.1

* Environment chamber malfunction caused loss of all seeds and no valid measurement of fungal mortality was possible.

than in GA_3 and kinetin groups above. Plates of Sabourand's agar showed no fungal growth, while bacterial colonies were present on the yeast extract agar in all treatments.

(2) Promotion of germination using gibberellic acid (GA_3) and kinetin

Results of this experiment, presented in Table 10, indicated that germination was promoted by both kinetin and gibberellic acid with the latter having more pronounced effects. Analysis of variance demonstrated that there was no significant difference between the treatments, although the calculated F value of 2.35 was very close to the theoretical F value of 2.57. The application of Dunnett's test shows that only treatments with 10^{-6} and 10^{-7} GA_3 produce germinations significantly higher than the controls. However, a trend towards higher germination percentages is certainly indicated for both kinetin and gibberellic acid.

(3) Acid Scarification

No germination occurred, and the experiment was therefore considered unsuccessful.

IV. Pollination study, using scanning electron microscopy

Electron micrographs of the gynoecium of *Rubus chamaemorus* L. show

Table 10. Percentage germination in seeds soaked with water (control), kinetin and GA_3 (gibberellic acid) after 30 days incubation.

Incubation medium	Percentage germination			Total %	Average %
Water (control)	20	30	10	60	20.0
Kinetin					
4.6×10^{-7} M	10	10	30	50	16.6
4.6×10^{-6} M	30	30	20	80	26.6
4.6×10^{-5} M	60	50	30	140	46.6
GA_3					
5.7×10^{-7} M	30	70	50	150	50.0
5.7×10^{-6} M	70	70	60	200	66.6
5.7×10^{-5} M	50	70	80	200	66.6

several interesting features. The stigmatic surface (Fig.52) is considerably furrowed and pitted, with flakes of material present which would appear to be adhesive. The base of the style and surface of the carpels are covered with hairs (Fig.53) which, under high magnification, are seen to be glandular unicellular trichomes (Fig.54).

Examination of the whole anthers showed that a considerable amount of pollen was released by the dehisence of longitudinal slits in the pollen sac (Fig.55). The surface of the pollen grains was covered irregularly with short hooked spinules (echinae) and papillae (bacullae) (Fig.56); possibly enabling the grains to cling to the hairs of pollinating insects. Generally, the grains are not of a complex form, having no elaborate pores or furrows and a simple spherical shape. Germination of the pollen tube took place through a slit in one side of the grain (Fig.57). 'Successful' germination was observed in grains on the stigmatic surface of flowers collected during the early stages of female flowering while some males were still open (Fig.57). However, germinating pollen of a different species (Fig.58) was observed on a bakeapple stigma. This grain was subsequently identified as belonging to the *Vaccinium* sp. indet. type (Oldfield, 1959; Faegri and Iverson, 1974; Moore and Webb, 1978). From this it would appear that the specificity of the stigmatic surface for *R. chamaemorus* L. pollen is low.

V. Anatomy

Contrast and clarity were best in sections stained with Bismarck Brown.

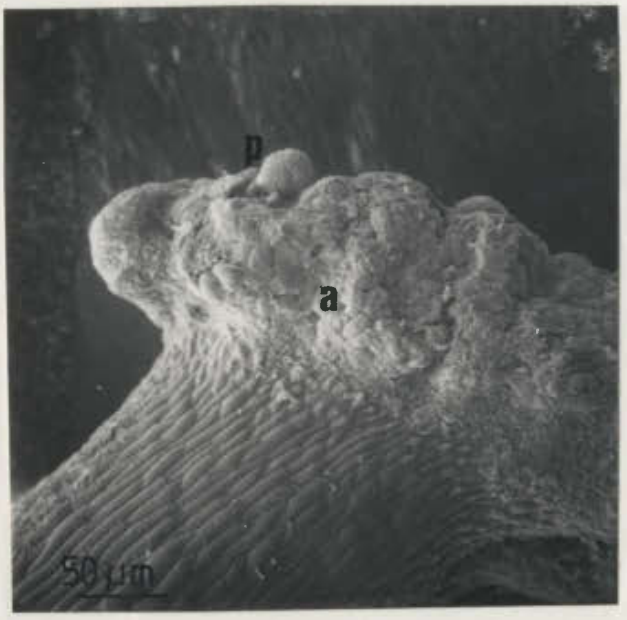


Figure 52. *R. chamaemorus* L., scanning electron micrograph of stigmatic surface. Furrows and flakes of adhesive material (a) are visible around a germinating pollen grain (p).

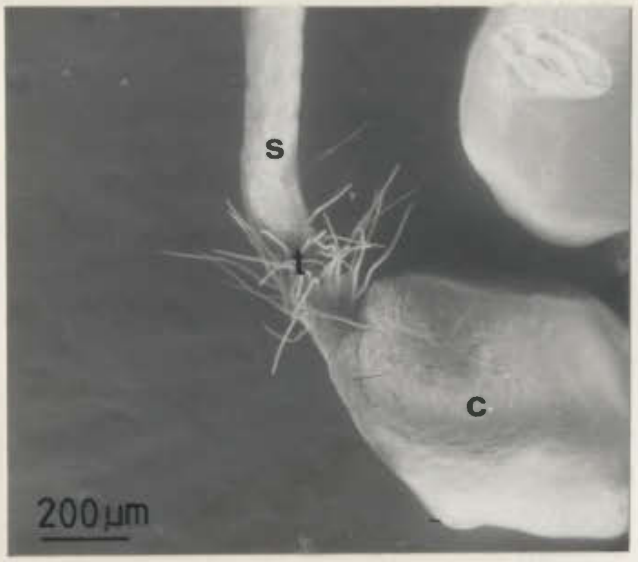


Figure 53. Scanning electron micrograph of *R. chamaemorus* L. gynoecium. Trichomes (t) cover the junction of the style (s) and carpel (c).

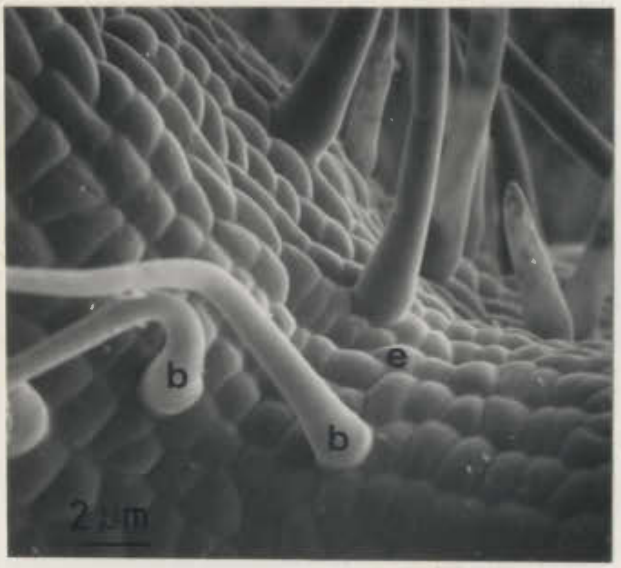


Figure 54. Scanning electron micrograph of trichomes at base of style in *R. chamaemorus* L. The trichomes appear to be unicellular, with swollen bases (b) arising from the epidermis (e).

Figure 55. Scanning electron micrograph of *R. chamaemorus* L. stamen. A pollen grain (p) is visible in the longitudinal opening of the left anther.

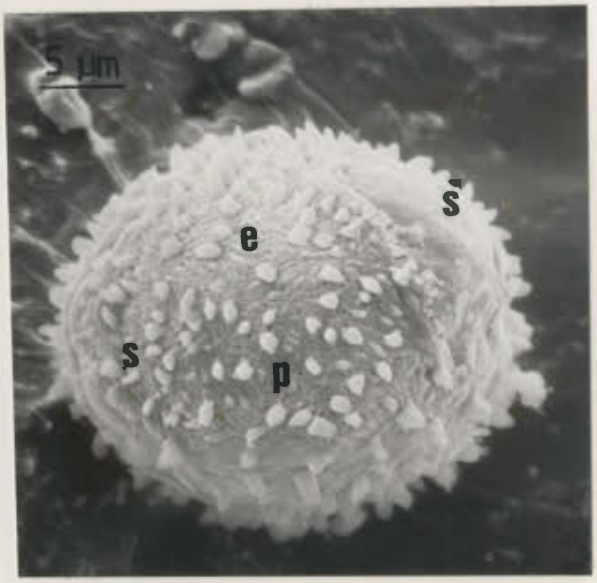


Figure 56. Scanning electron micrograph of *R. chamaemorus* L. pollen grain. In addition to the numerous spinules (s) and papillae (p), the grain possesses an irregularly perforate exine (e).

Figure 57. Scanning electron micrograph of *R. chamaemorus* L. germinating pollen on stigmatic surface. The germination tube (g) has emerged from one of the two visible colpi (c) and entered the stigma to the lower left. Flakes of adhesive material (a) are present on and around the grain.

Figure 58. Scanning electron micrograph of *Vaccinium* sp. pollen germinating on *R. chamaemorus* L. stigma. Tetrads of grains with three long colpi (c) and irregular splits in the exine (e) are characteristic of the genus. The germination tube (g) has clearly penetrated the stigma.

(1) Roots

Root sections display a diarch vascular cylinder (Fig.59) with large central vessels surrounded by smaller thinner-walled vessels, parenchyma and small patches of angular phloem cells (Fig.60). A distinct endodermis and periderm are present around the vascular cylinder, both displaying denser cytoplasm than the adjacent cells. The cortex consists of isodiametric cells with thin walls. Large lacunae are present in the inner cortex. Towards the exodermis, cells decrease in size and the lacunae are absent. The epidermis forms detached patches of cells, the outer two rows of cortical cells forming an exodermis. The outer exodermal cells are small and relatively thin-walled, with slightly thicker outer walls. The inner exodermis cells are enlarged, barrel-shaped and thick-walled; many cells have densely-stained cytoplasm which could be indicative of phenolic accumulation.

(2) Rhizomes

Young rhizomes up to several months old possess a cortex and epidermis outside the developing periderm and secondary xylem. As they develop, these layers begin to degrade and become detached from the rhizome, with the periderm eventually becoming the outer protective lower (Fig.61). The central pith occupies a third of the rhizome diameter and consists of large isodiametric cells which become smaller towards the periphery of the pith. Scattered cells have densely-stained cytoplasm. The disintegration of cells within the pith forms large lacunae in the central

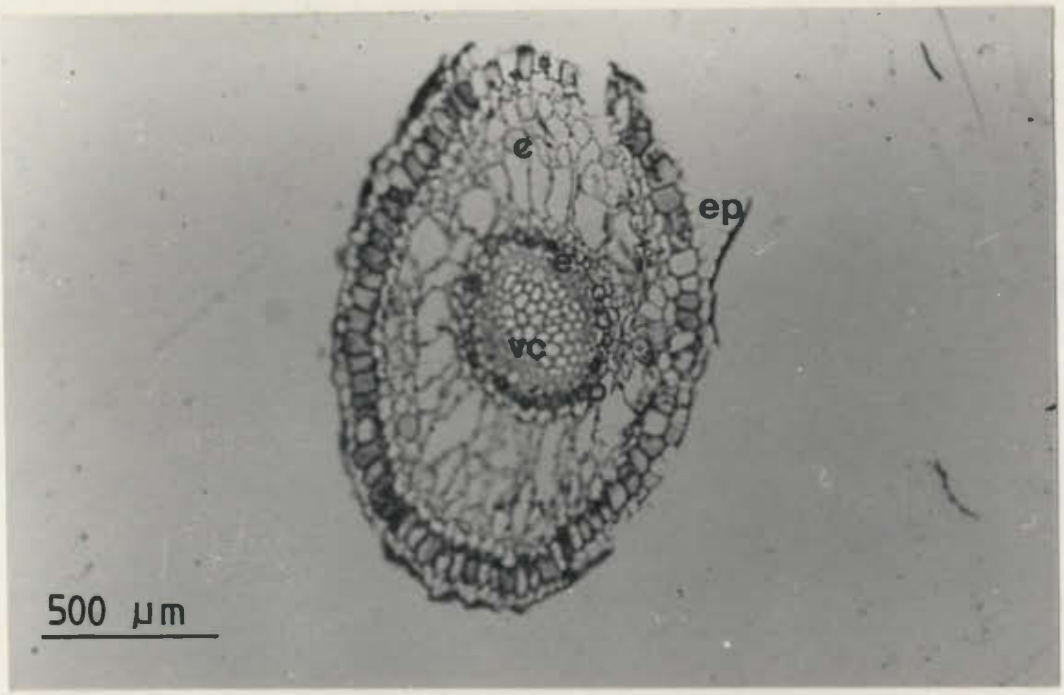


Figure 59. Photomicrograph of 10$^{\mu}$m paraffin section of *R. chamaemorus* L. root stained with Bismarck Brown. Diarch vascular cylinder (vc) surrounded by endodermis (e), periderm (p), cortex (c), and partially detached epidermis (ep).

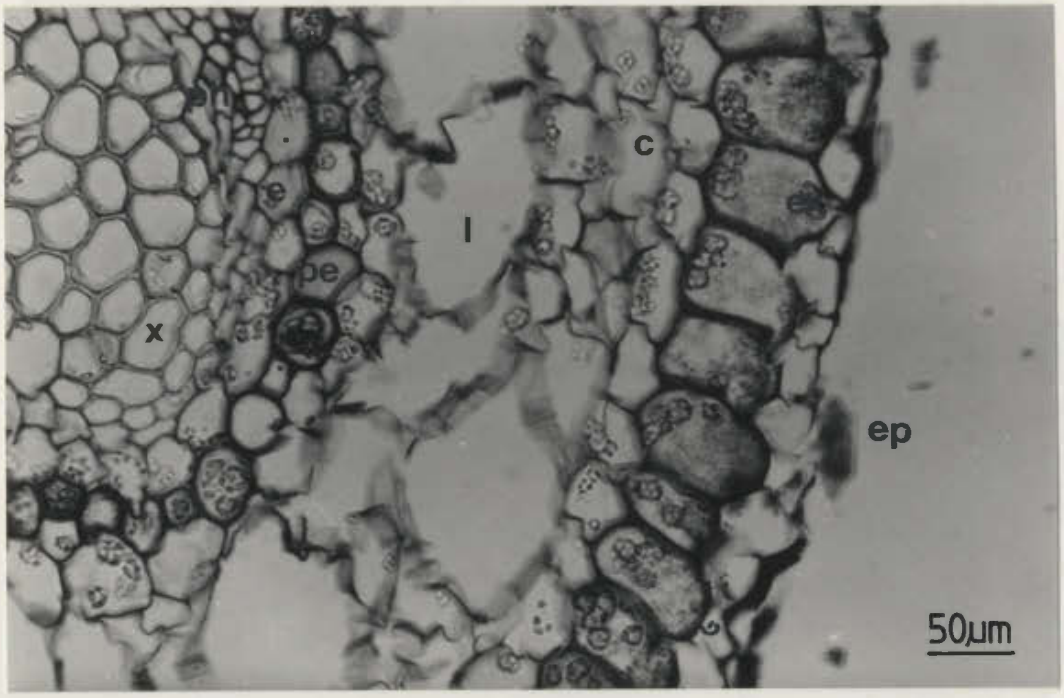


Figure 60. Photomicrograph of 10$^{\mu}$m paraffin section of root stained with Bismarck Brown. Xylem vessels (x), phloem (ph), endodermis (e), periderm (pe), cortex (c), and lacunae (l) are visible. The outer two layers of the cortex form an exodermis (ex) with some remaining epidermis (ep).

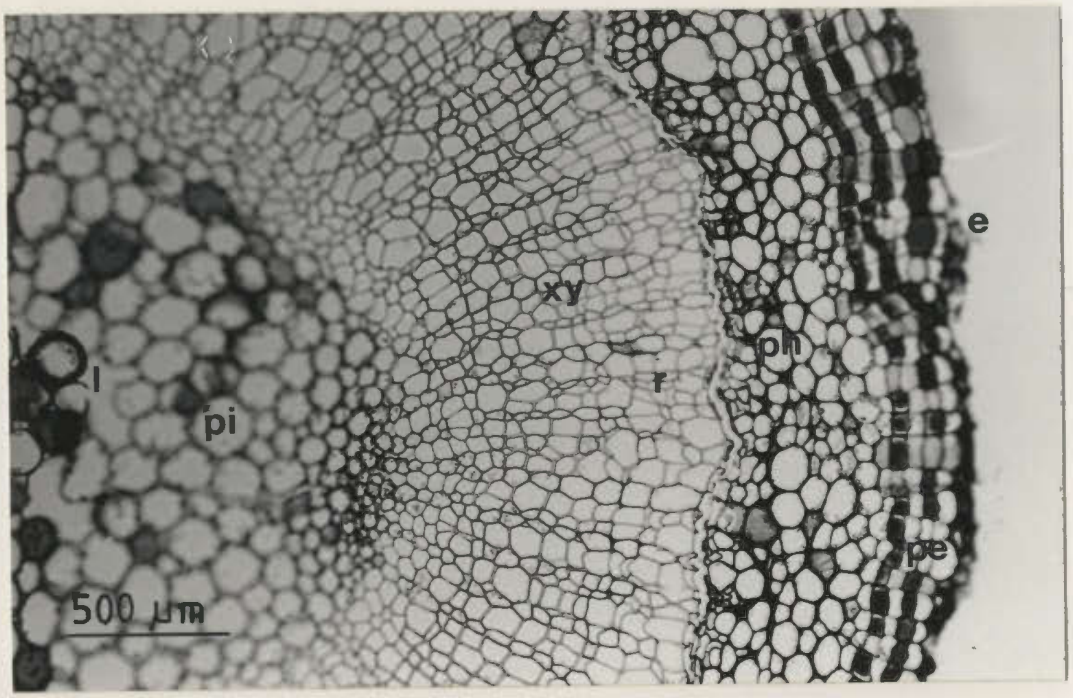


Figure 61. Photomicrograph of 10^{μ}m paraffin section of *R. chamaemorus* L. rhizome stained with Bismarck Brown. The epidermis (e) has almost detached and the periderm (pe) has replaced it. The phelloderm (ph) has partially detached from the secondary xylem (xy) and medullary rays (r). Some cells of the pith (pi) are densely-stained and may contain polyphenolics; large lacunae (l) are present.

area. The secondary xylem shows some degree of annual ring growth in discontinuous zones of the cylinder. Medullary rays consist of single rows of dense, elongated parenchyma cells. Small patches of crushed cells between the xylem and phelloderm could represent secondary phloem (Fig.62). The phelloderm is a band of variable cells in 6-10 layers; overall the cells appear collenchymatous and some have densely-stained cytoplasm. The phellogen consists of 4-5 rows of rectangular cells, with alternative layers staining more densely. The phellem consists of two or three rows of large barrel-shaped cells and an outer row of very thick-walled angular cells containing dense spherical globules (Fig.63). Some remnants of the old cortex can be seen in longitudinal section; the dense deposits in the phellem are clear and the density in the phellogen can be seen to be restricted to the vacuoles (Fig.64). Resvoll (1929) also noted that the medullary rays, phelloderm, and pith "contain plenty of amyle", apparently phenolic.

(3) Leaf

The ribs protrude on the abaxial side with corresponding deep adaxial furrows (Fig.65). Each vein contains one large semicircular vascular bundle with distinct rows of xylem and a continuous endodermis. The surrounding parenchyma is compact and thin-walled. The epidermis consists of small isodiametric cells with occasional unicellular trichomes. Between the veins, the blade is relatively thin, with a few stomata on the abaxial surface and scattered multicellular trichomes. There is some evidence of a division into palisade and spongy mesophyll.

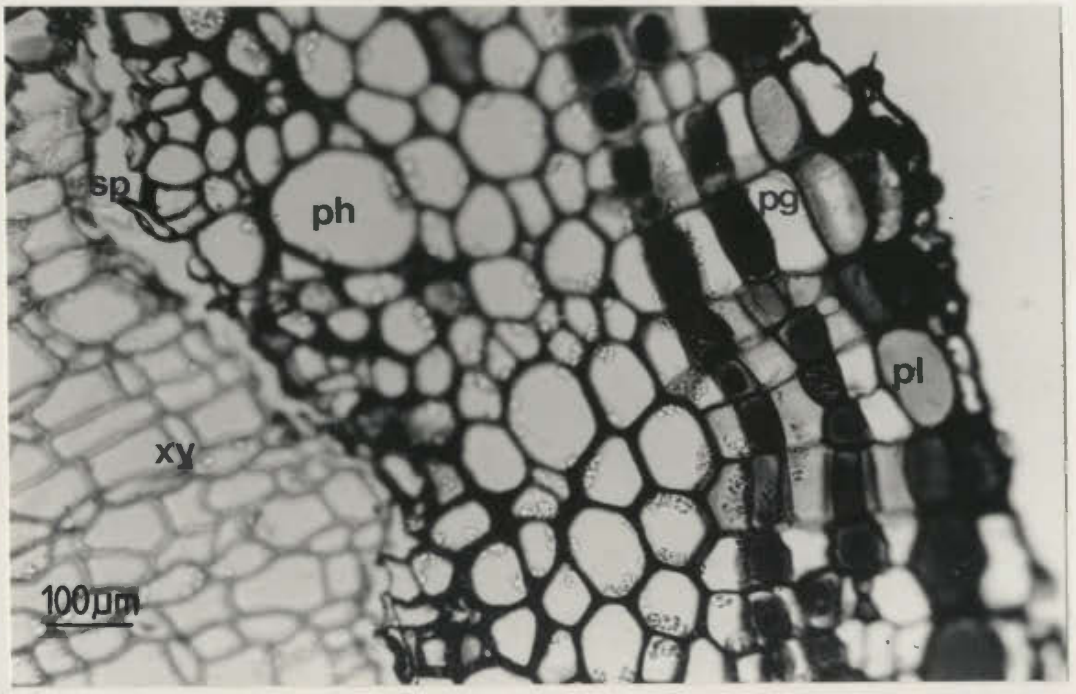


Figure 62. Photomicrograph of outer layers of 10$^{\mu}$m paraffin section of rhizome stained with Bismarck Brown. Crushed cells between the xylem (xy) and phelloderm (ph) could represent secondary phloem (sp). Rows of dense and clear cells alternate in the phellogen (pg); cells of the phellem (pl) also stain densely and may contain polyphenolics.

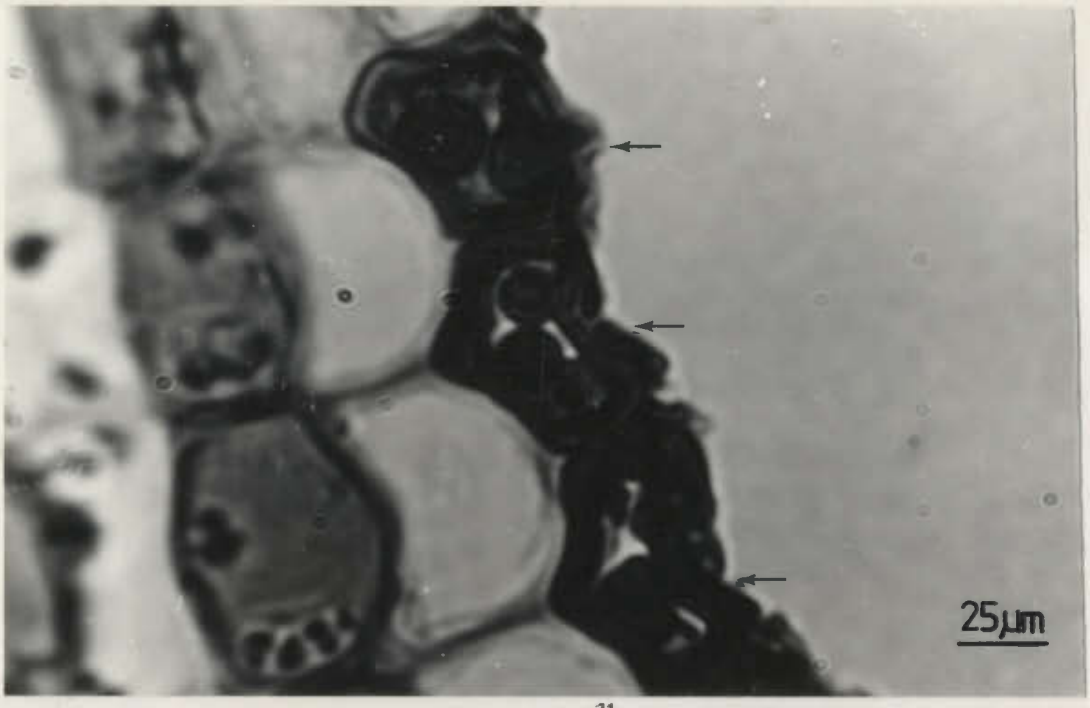


Figure 63. Photomicrograph of 10µm paraffin section of rhizome stained with Bismarck Brown; outer phellem. Dense globular deposits are present within the cells (arrows).

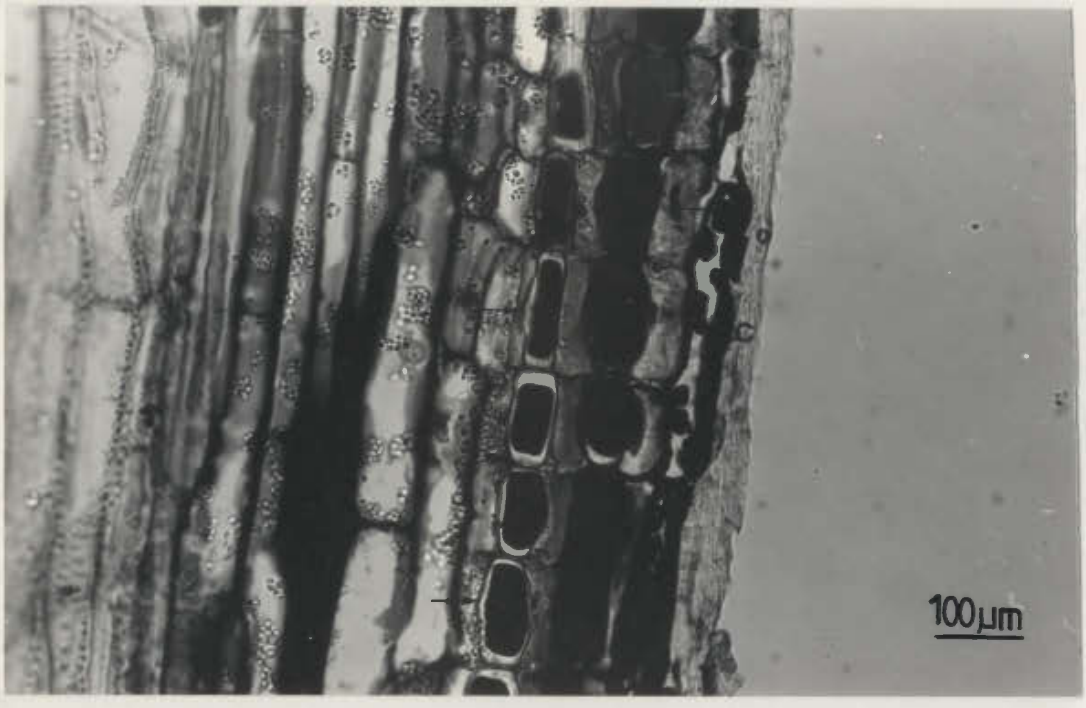


Figure 64. Photomicrograph of 10µm longitudinal paraffin section of rhizome stained with Safranin/light green. Remains of the old cortex (c) are visible, as are dense deposits in the phellem and in vacuoles of phellogen cells (arrows).

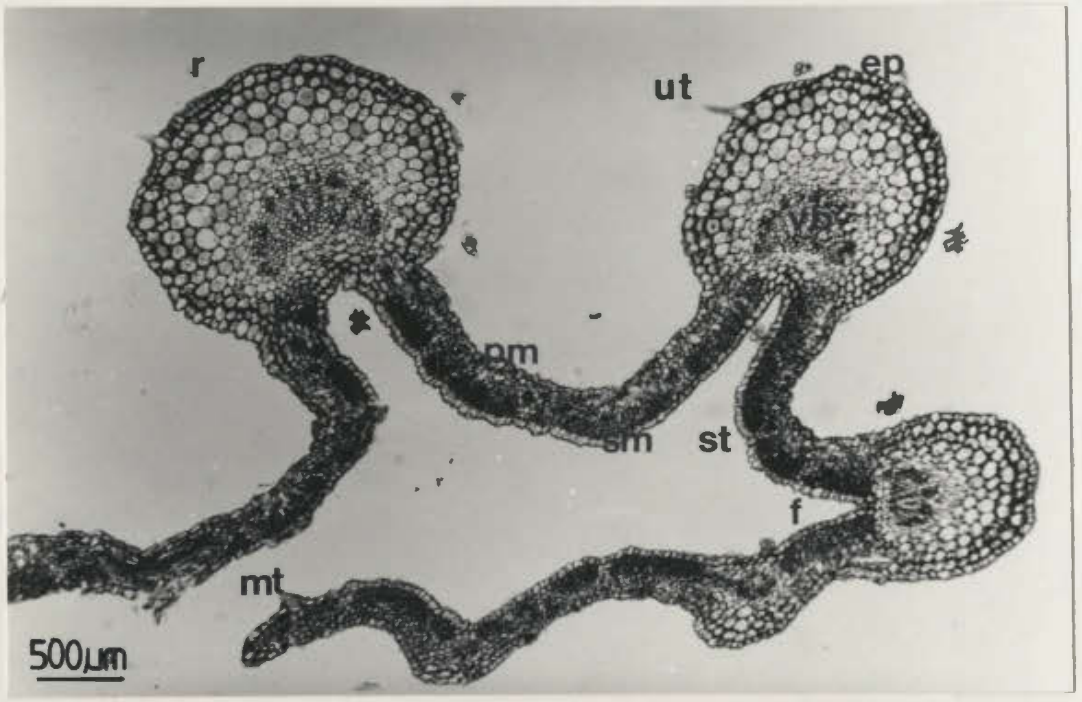


Figure 65. Photomicrograph of 10$^{\mu}$m paraffin section of *R. chamaemorus* L. leaf stained with Bismarck Brown. Ribs (r) protrude on the abaxial side with corresponding adaxial furrows (f). Vascular bundles (vb) contain xylem and a continuous endodermis. Unicellular trichomes (ut) are present in the rib epidermis (ep); multicellular trichomes (mt) and stomata (st) occur in the blade epidermis. Palisade (pm) and spongy (sm) mesophyll are distinguishable.

VI Phenolic Localization

(1) Localization of phenolic compounds using electron microscopy

Fig.66 shows an ultrathin section of a mature *R. chamaemorus* L. root tip stained with toluidine blue. Densely-stained cells can be seen throughout the section, especially in the middle root cap zone. Under white light, this zone displays a pronounced blue-green metachromatic reaction characteristic of phenolic compounds. The reaction appears to be localized in the vacuoles. In addition, rows of cells with more densely-stained vacuoles are visible in the cortex.

Examination of root sections treated with osmium tetroxide and counter stained with uranyl acetate revealed the presence of so-called dense cells within the cortex. In these cells, the cytoplasm itself rather than the vacuolar contents is electron-dense (Fig.67) and the surrounding cells are appreciably less dense throughout. The nucleii of dense cells appear to be only slightly darker than those of 'normal' cells. Evidence of some accumulation of dense material at the cell walls, in the plasmodemata and in the intercellular spaces can be seen in Fig.68.

Treatment with ferric chloride in place of osmium tetroxide increased the resolution of phenolic deposits. Fig.69 shows three dense cells displaying a gradation of cytoplasm density. Phenolic deposits in these cells appear as distinct black globules of ferric chloride scattered throughout the cytoplasm. Some deposition is visible within

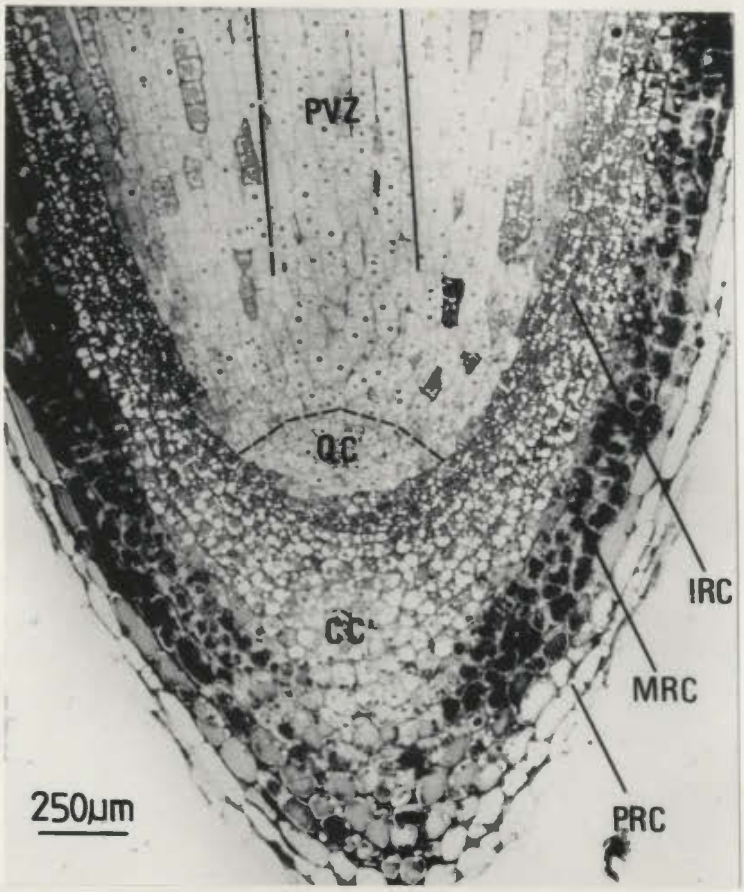


Figure 66. Photomicrograph of 0.5 µm Epon section of *R. chamaemorus* L. root tip stained with toluidine blue. The provascular tissue (PVZ) and quiescent center (QC) contain no dense cells. Cells of the central cap (CC) tend to be less dense than those of the inner (IRC) and middle (MRC) cap. The peripheral cap (PRC) consists of disintegrating cells with no dense deposits.

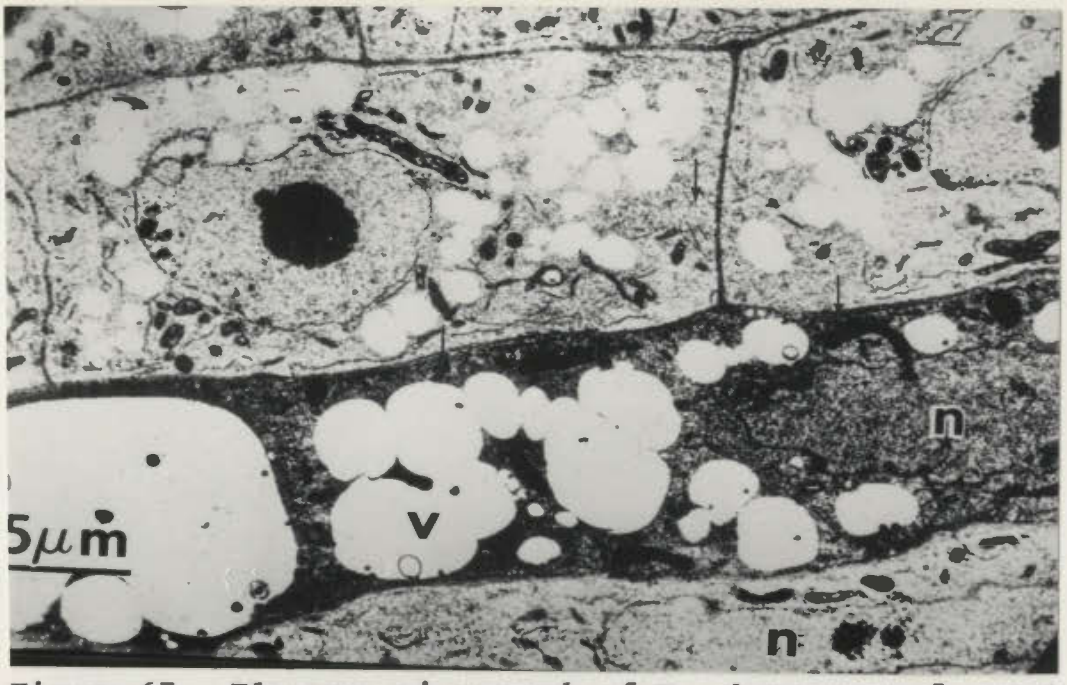


Figure 67. Electron micrograph of *R. chamaemorus* L. root treated with OsO_4. Density is restricted to the nucleus (n) and cytoplasm (arrows) and absent from the vacuole (v).

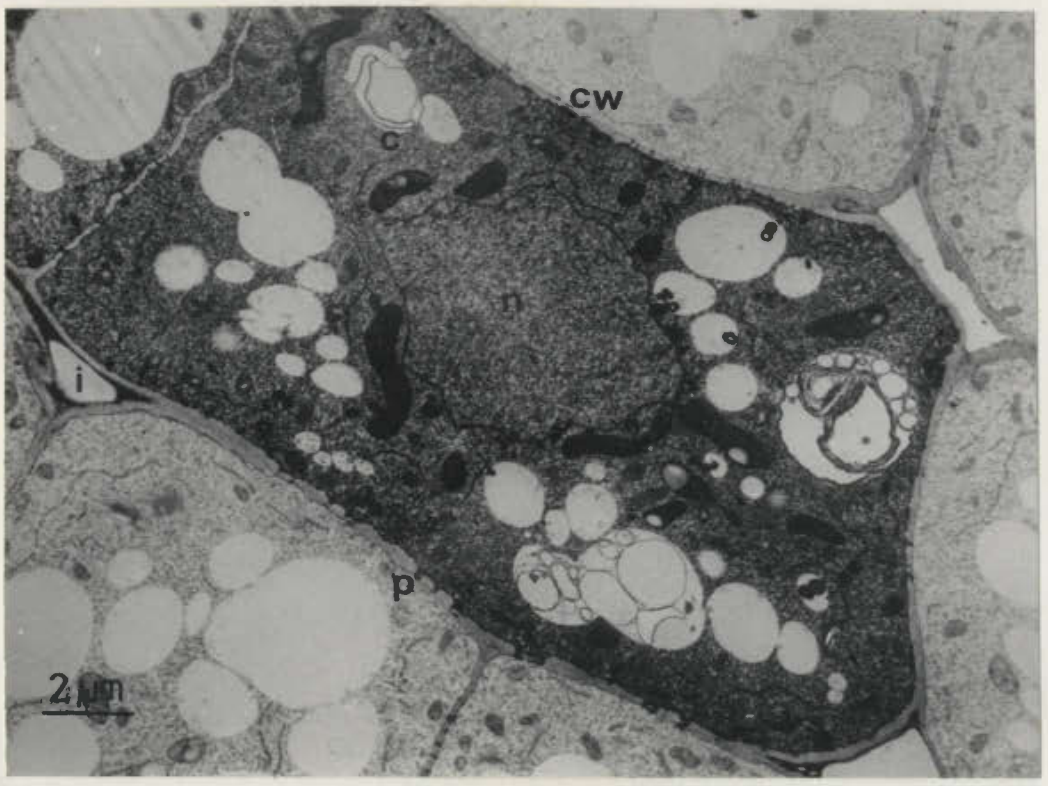


Figure 68. Electron micrograph of dense cell treated with OsO_4. Dense deposits occur at the cell wall (cw), plasmodesmata (p), intracellular spaces (i), nucleus (n), and cytoplasm (c).

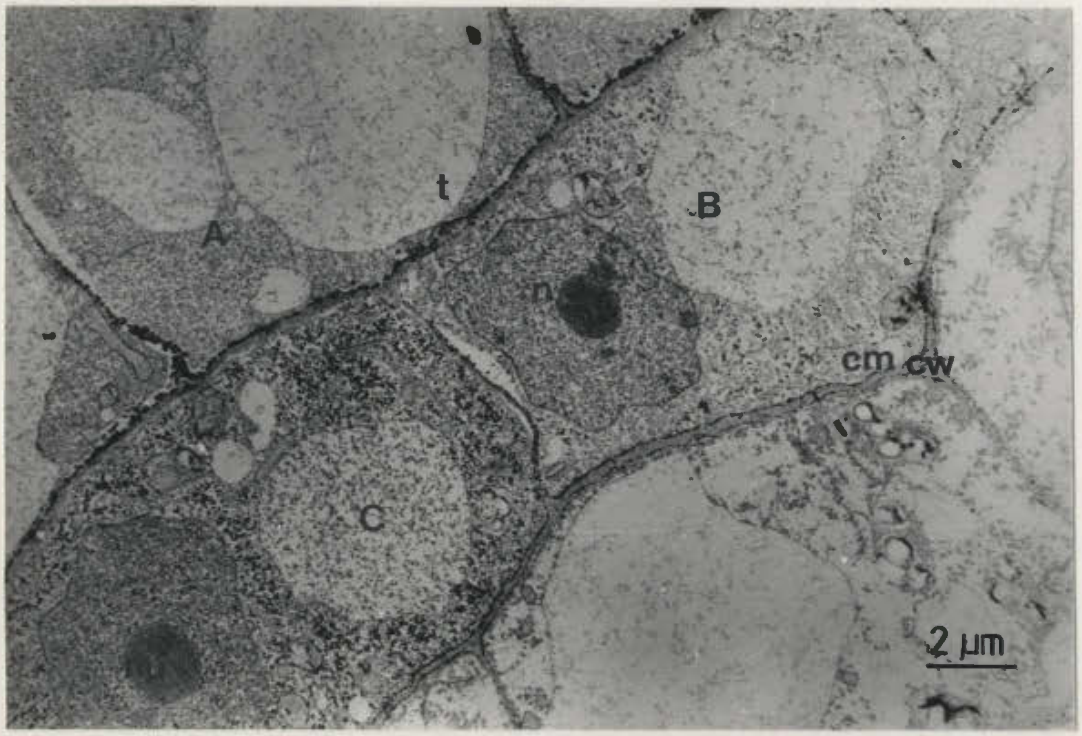


Figure 69. Electron micrograph of dense cells treated with $FeCl_3$. Resolution of phenolic deposits is improved and the increase in density from cell A to cell B and C is clear. Depositions are visible along the tonoplast membrane (t) and between the cell wall (cw) and cell membrane (cm). The nucleus (n) shows some density, but not the nucleolus (nu).

the nucleus but none within the nucleolus. Scattered deposits occur within the vacuoles and concentrate along the tonoplast membrane in the cell on the upper right. Heavy accumulations can be seen between the cell wall and cell membrane, especially of the cells in the upper right, where the concentration of stained particles in the cytoplasm is less. Staining is also evident in the intercellular spaces at the corners of cells and in the plasmodesmata.

Closer examination of the cell wall in preparations stained with osmium tetroxide shows some faint staining at the cell membrane (Fig.70). However, a considerably different result is obtained from ferric chloride staining (Fig.71.). Dense accumulations of ferric material are found between the cell wall and the cell membrane, increasing in thickness at the corners of the cells. These deposits consist of clustered globules of fairly uniform size. The intercellular space is also more dense in these cells. Fig.72 shows less distinct deposits of electron-dense material in a control section without U and Pb counter-staining. Overall resolution of such material is poor, although the relationship of the deposits to the membrane and wall can be seen more clearly.

Phenolics are also localized within the vacuole, as seen in Fig. 73, in addition to heavy accumulation in the intercellular spaces and near the cell walls. Iron and phenolic particles also line the vacuole in close association with the tonoplast membrane, especially visible in the lower right of the cell where the membrane has shrunk away from

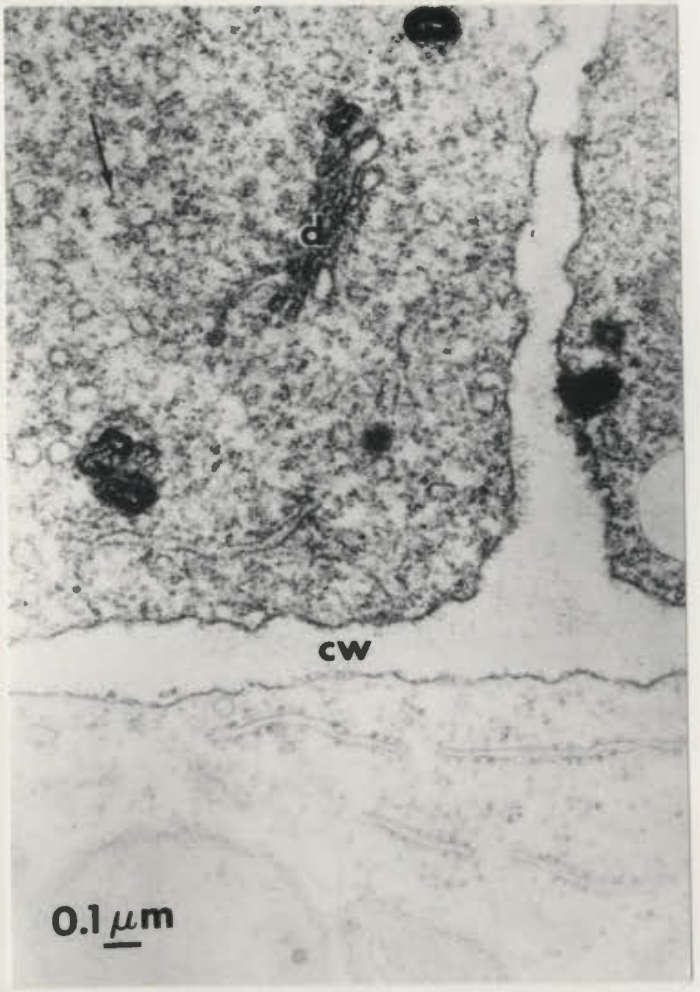


Figure 70. Electron micrograph of root material treated with OsO_4. Some dense material is visible at the cell wall (cw) and in the cytoplasm (arrow). Dictyosomes (d) are also present.

Figure 71. Electron micrograph of root material treated with $FeCl_3$ in place of OsO_4. Accumulations of ferric material are present between the cell wall (cw) and membrane (cm). The intracellular space (i) is also darkened.

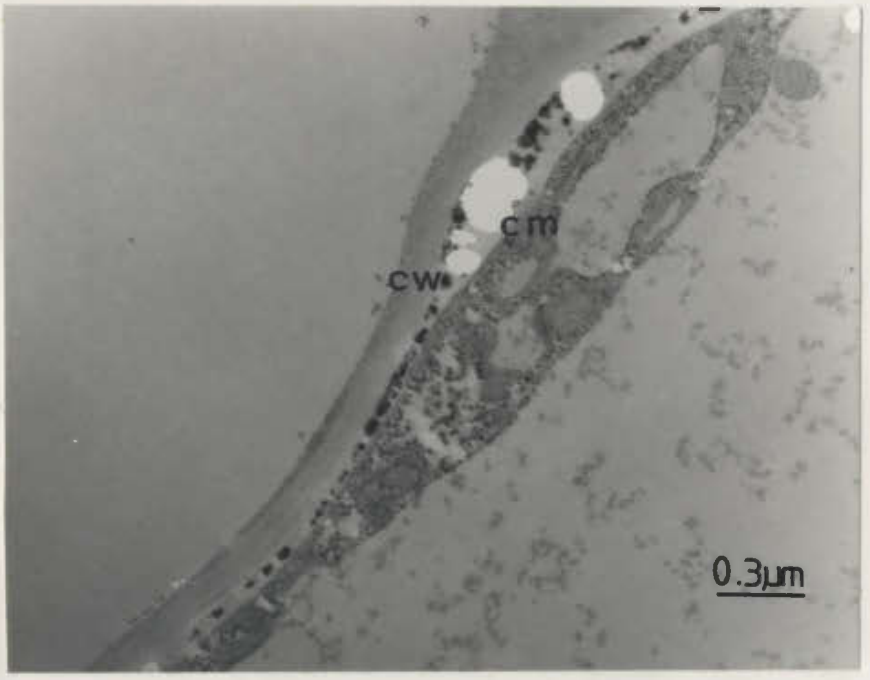


Figure 72. Electron micrograph of root material treated with $FeCl_3$ without uranyl acetate/lead citrate counterstaining. Despite poor resolution, dense ferric deposits are clearly located between the cell wall (cw) and membrane (cm), in close contact with the inner wall.

Figure 73. Root cell treated with $FeCl_3$, showing distribution of ferric material within the cell. Heavy accumulations occur within the vacuole (v) and along the inner edge of the tonoplast membrane, especially where it has shrunken away from the edge of the vacuole (arrows). Deposition also occurs in intracellular spaces (i) and cell walls (cw).

the edge of the vacuole as a result of preparation procedure. Under higher magnification, dense patches within the vacuole are seen to consist of aggregations of ferric material (Fig.74).

(2) Characterization of phenolic compounds using optical microscopy

Tests for characterization of phenolics within root and rhizome sections of *R. chamaemorus* L. are summarised in Tables 11 and 12. It is clear from these results that various phenolic compounds are selectively localized in these organs.

Fig. 75 shows a cross-section of an unstained root. There is some yellowish colour in the outer cortex and epidermis but none elsewhere although the structure of the root is clear and individual cells are distinguishable. An unstained rhizome (Fig.76) displays much more definite darkening in cells of the outer pith, parenchymatous rays and collenchymatous phelloderm which is most probably due to the oxidation of phenolic compounds. Some cells of the periderm are also darkened. The cortex and epidermis are beginning to collapse and detach from the inner rhizome layer, which develops as the rhizome matures.

Dimethoxybenzaldehyde (DMB) treatment of roots and rhizomes stained flavonoid precursors of catachins and gallocatachins light red. The reaction was not particularly strong in the root section (Fig.77), but there is some staining of epidermal and endodermal cell walls, xylem cells and some pith cells. Some cortical cell walls also display a

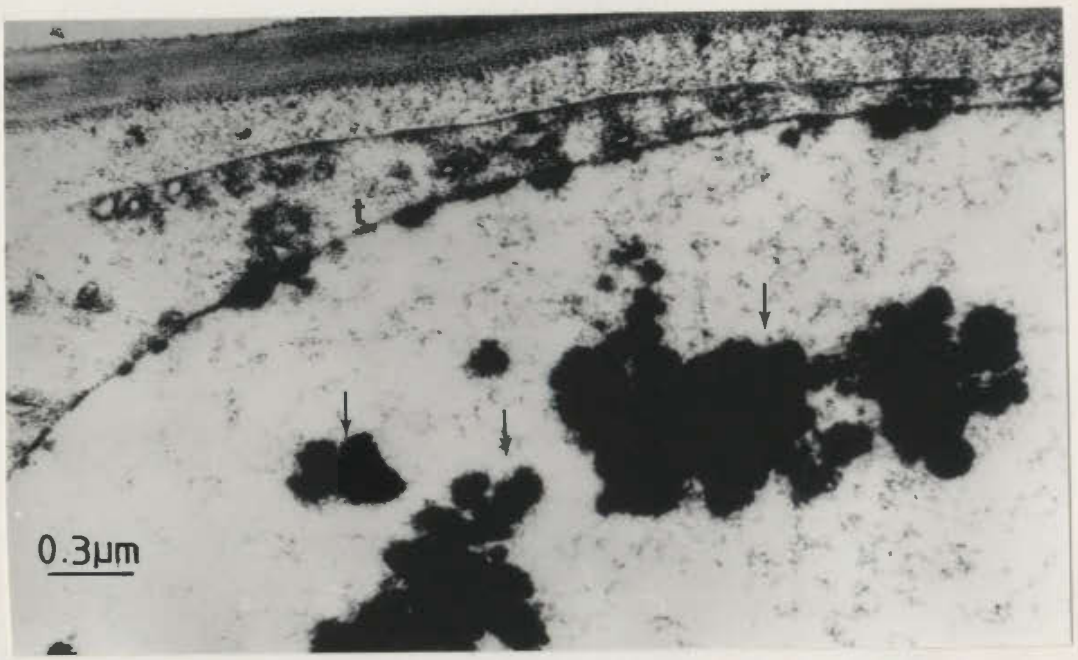


Figure 74. Edge of vacuole and tonoplast membrane in cell treated with $FeCl_3$. Dense patches within the vacuole (arrows) consist of aggregations of ferric-complexed material. Smaller aggregations occur along the inner tonoplast membrane (t).

Table 11. Histochemical tests to distinguish between flavonoid and terpenoid polyphenolics.

Reaction	Polyphenolic	Locations of reaction product
DMB	flavonoid precursors of condensed tannins (catechins, gallocatechins)	cell walls and cell contents of cells in epidermis, cortex, endodermis and xylem
Nitroso	catechol tannins	within cells of epidermis, outer cortex, endodermis; parenchyma cells and ray cells adjacent to xylem and within pith
$SbCl_3$	gossypol and related terpenoids	xylem walls, epidermal cell walls, walls of pith parenchyma adjacent to xylem
DNP	gossypol and related terpenoids	xylem walls, walls of epidermal and outer cortical cells; some staining inside cells of cortex

Table 12. Histochemical tests for specific characteristics of polyphenolics.

Reaction	Phenolic	Locations of reaction product
Gibb's reagent	phenolics with 1. free position para- to a hydroxyl group, 2. no carboxyl, sulfo, formyl, nitroso, or nitro groups on same ring as hydroxyls	limited staining in xylem walls; walls and cell contents of ray parenchyma and scattered cells in endodermis
Aniline-KIO_3	aniline addition products of quinones derived from naturally-occurring phenols	dense stain of cell contents in pith; cells adjacent to xylem, ray parenchyma, endodermis, scattered cortical cells, band of cells in outer cortex
2% aq. Aniline	aniline addition products of some o- or p- quinones	very faint reaction localized same as for Aniline-KIO_3

Figure 75. Photomicrograph of unstained hand-section of *R. chamaemorus* L. root. Oxidized phenolics may account for the yellowing of the outer cortex (oc) and epidermis (ep). The vascular cylinder (vc) and cortex (c) are distinguishable by cell size and thickness of cell walls.

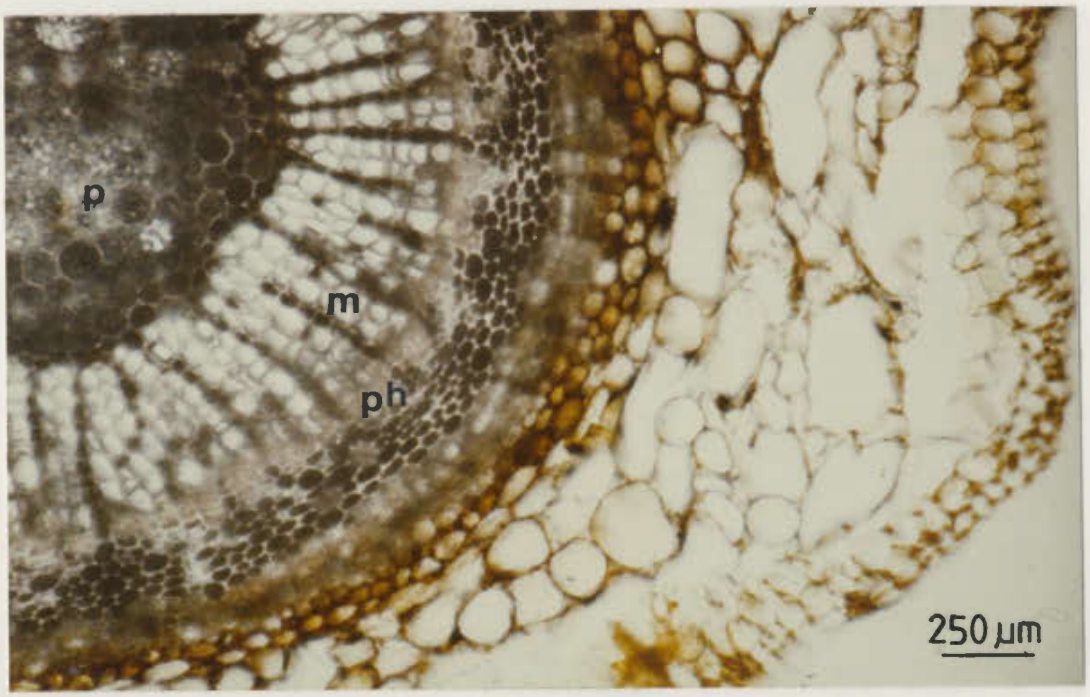


Figure 76. Photomicrograph of unstained hand-section of *R. chamaemorus* L. rhizome. Phenolic oxidation (black) is present in the outer pith (p), medullary rays (m), and phelloderm (ph).

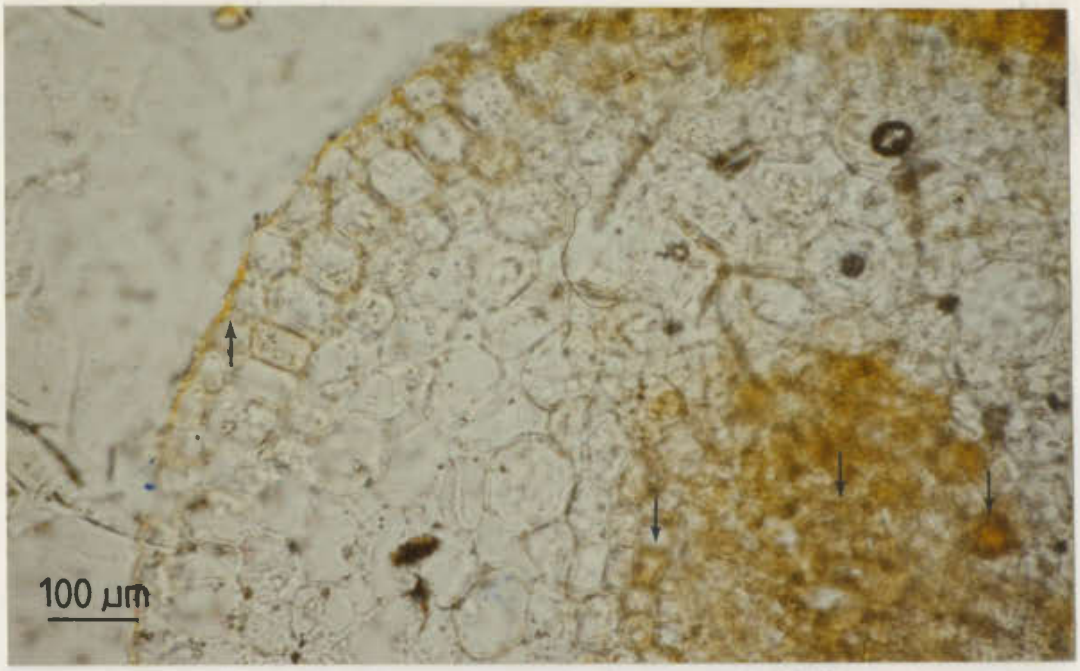


Figure 77. Root hand-section stained with DMB to localize flavonoid presursors of catechins and gallo-catechins. Limited staining occurs in the pith, xylem, endodermis, and epidermis (arrows).

Figure 78. Root hand-section stained with DMB. Some reaction is evident in the cortical cell walls and epidermis (arrows).

slight DMB reaction and there can be darkening of the epidermis (Fig.78). Rhizome sections show a more definite reddish product localized in cells and cell walls of the periderm and in the walls of the secondary xylem (Fig.79). Ray parenchyma cells are also darkened but it is uncertain whether this represents a genuine DMB reaction or phenol oxidation.

The Nitroso reaction also produces a reddish colour on reaction with catechol tannins and is similarly localized. Cell walls and contents of epidermal cells show some reaction, as do cortical cell walls and endodermal cells (Fig.80). In rhizomes, staining can be seen in parenchyma cells of the rays and pith and in walls of the collenchymatous periderm and phelloderm (Fig.81.).

Gossypol and related terpenoids stain orange to red on reaction with both $SbCl_3$ and dinitrophenylhydrazine (DNP) reagents and localize in similar areas. DNP appeared to give clearer, more definite results. Root sections stained with $SbCl_3$ (Fig.82) show positive reaction in the walls of epidermal and outer cortical cells. Xylem cell walls, parenchymatous ray cells and pith parenchyma cells of the rhizome also stain (Figs. 83 and 84).

Blackening of the periderm also occurs but this is likely due to oxidation of polyphenolic compounds rather than to a genuine $SbCl_3$ reaction. DNP reagent stains the outer cortex, epidermis, endodermis and xylem cell walls of root sections (Fig.85). Longitudinal sections show a

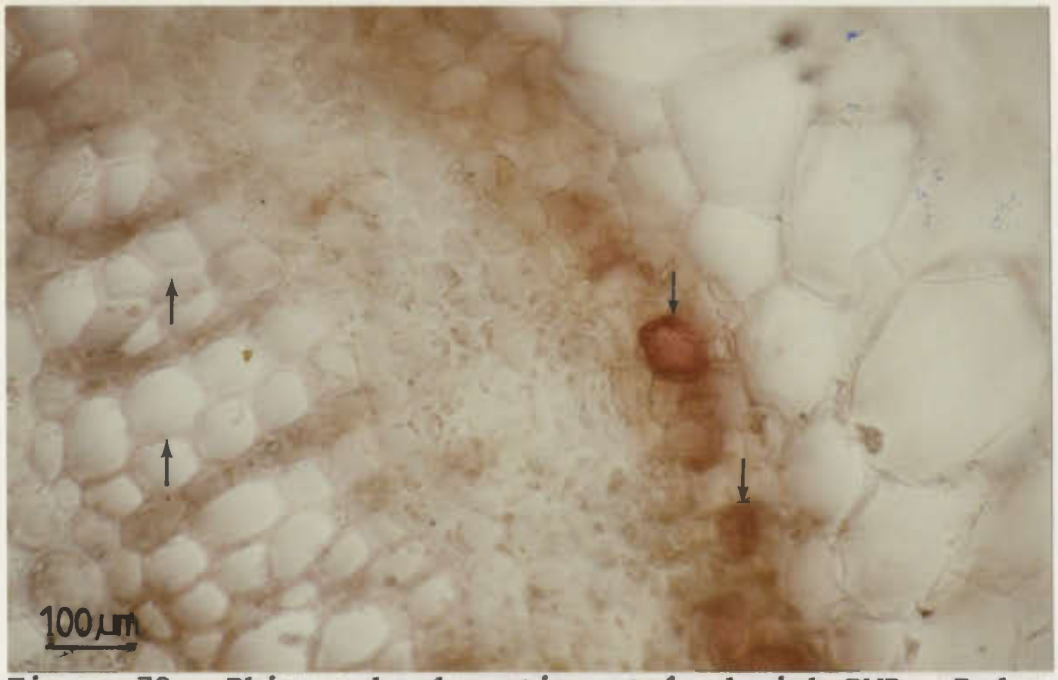


Figure 79. Rhizome hand-section stained with DMB. Red reaction product is visible in cells of the periderm and in secondary xylem walls (arrows).

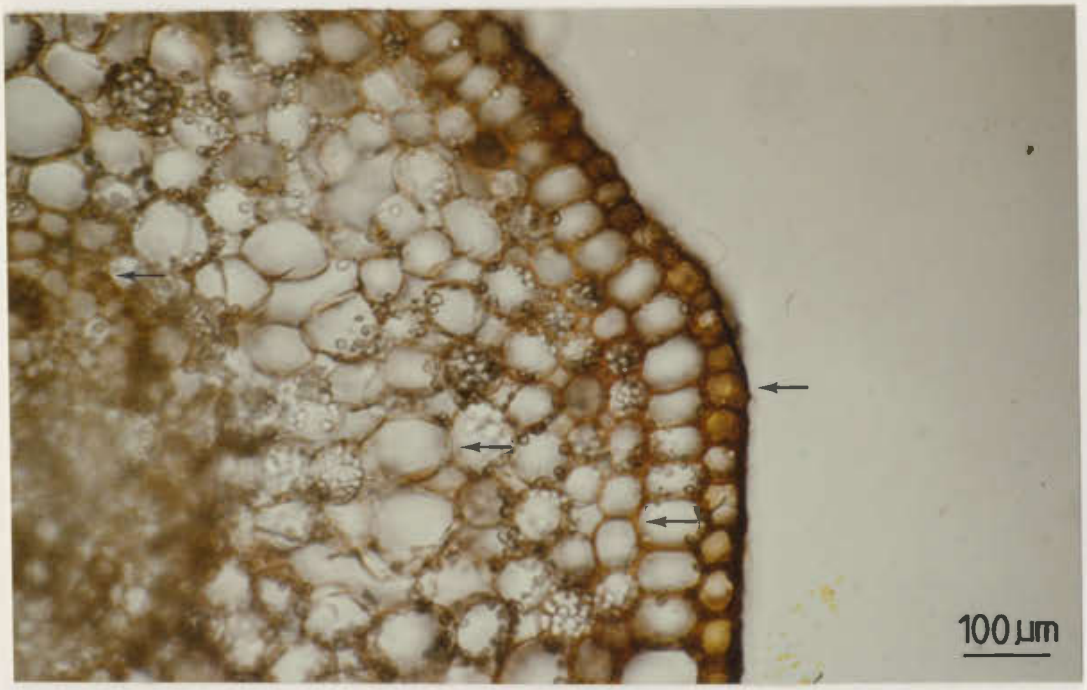


Figure 80. Nitroso reaction in root hand-section. Catechol tannins in the epidermal cells, cortical cell walls, and endodermal cells stain red (arrows).

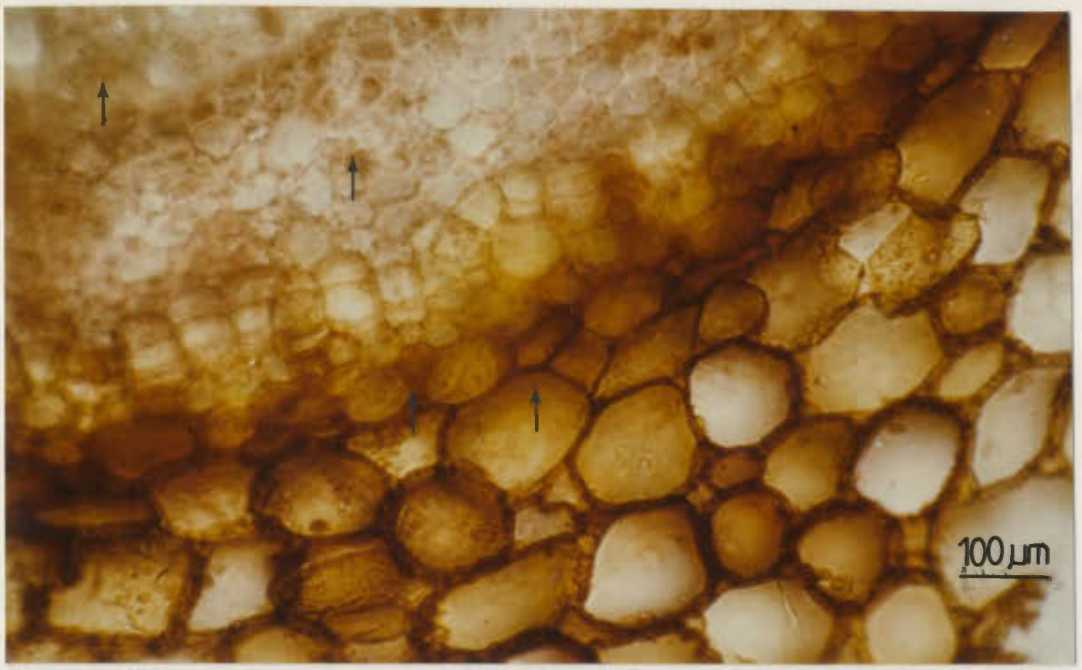


Figure 81. Nitroso reaction in rhizome hand-section. Cells of the pith, rays, periderm, and phelloderm show positive reaction (arrows).

Figure 82. $SbCl_3$ reaction in root hand-section. Orange reaction with gossypol and related terpenoids is largely restricted to the epidermis and outer cortex (arrows).

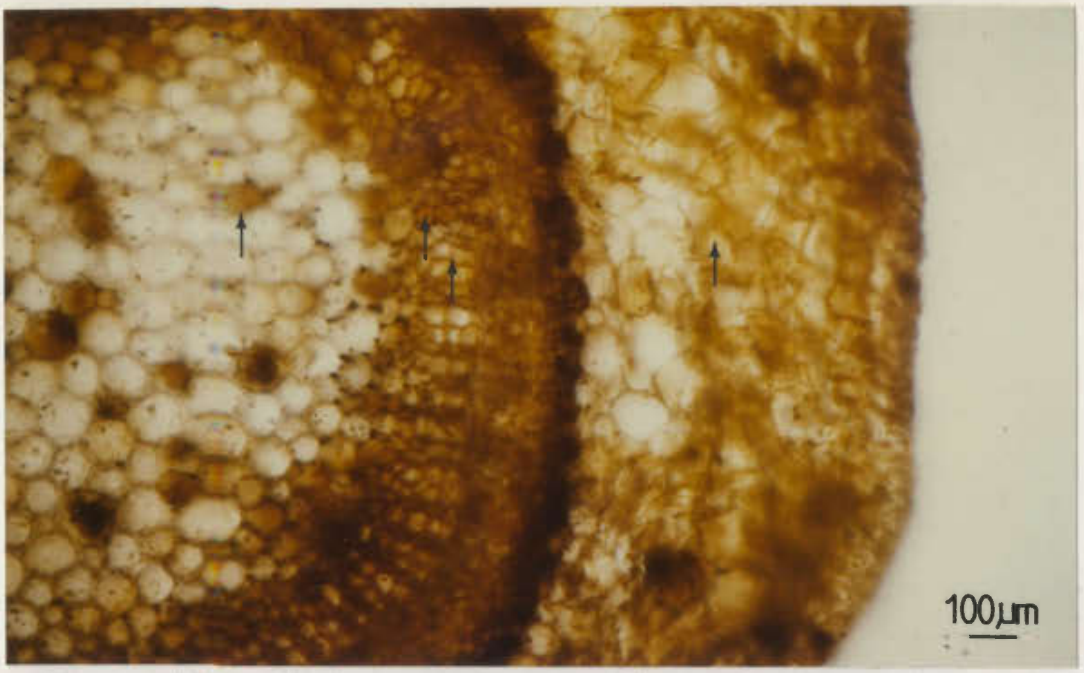


Figure 83. $SbCl_3$ reaction in rhizome hand-section. Staining occurs in the xylem walls, rays, pith, and disintegrating cortex (arrows).

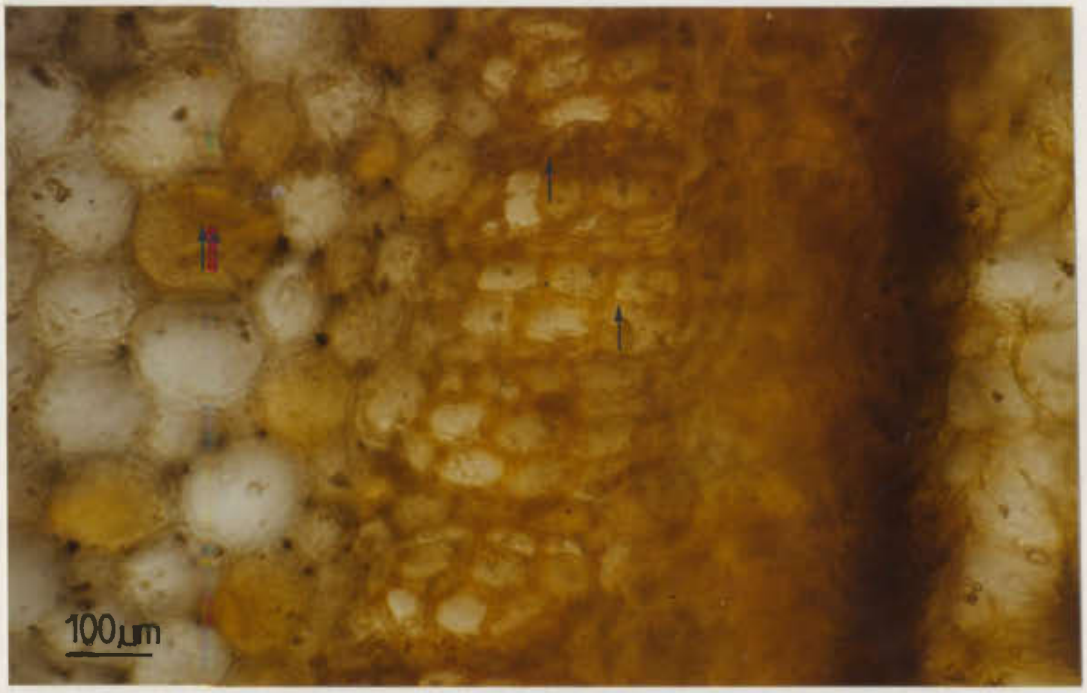


Figure 84. $SbCl_3$ reaction in central rhizome. Xylem walls and contents of pith and ray cells react intensely (arrows). Darkening of the periderm is intense and diffuse; it is therefore more likely due to phenolic oxidation.

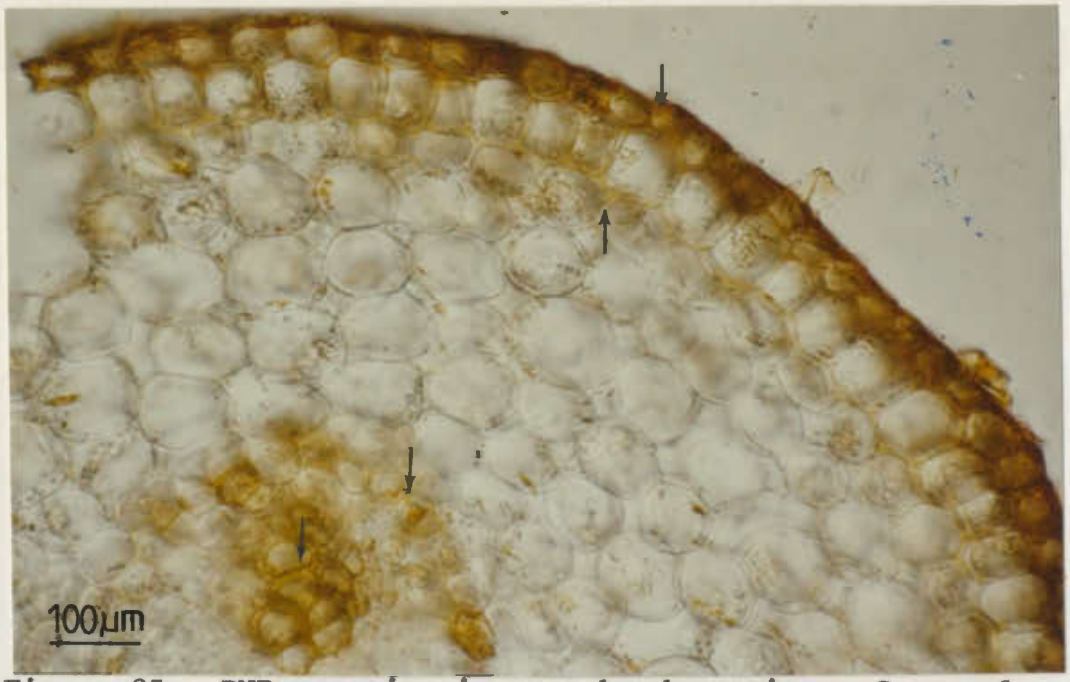


Figure 85. DNP reaction in root hand-section. Gossypol and related terpenoids in the outer cortex, epidermis, endodermis, and xylem walls stain orange-red (arrows).

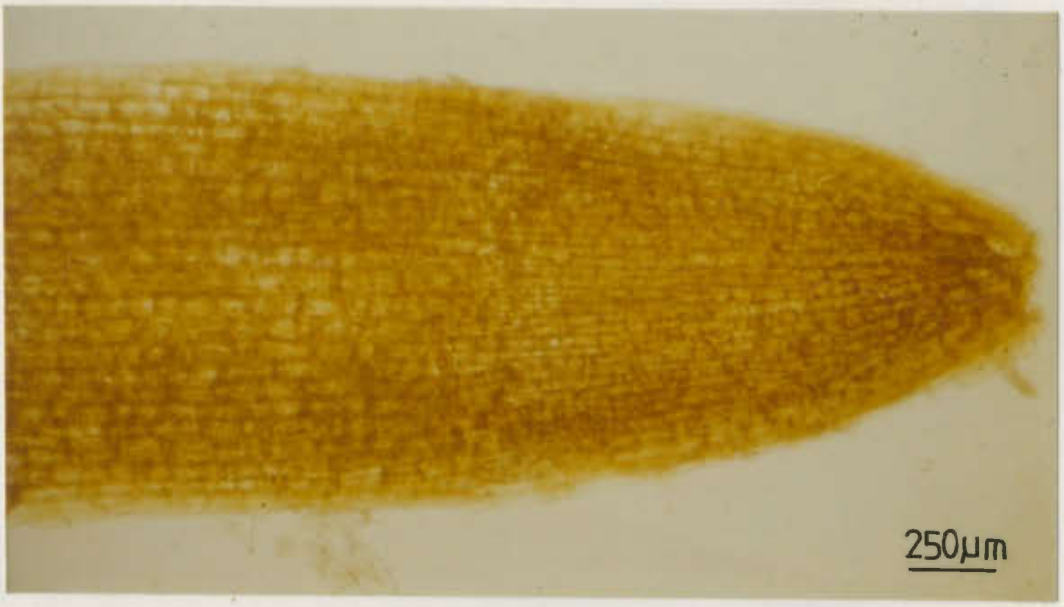


Figure 86. DNP reaction in longitudinal root section. Staining occurs throughout the epidermis, intensifying towards the root cap.

typical DNP reaction colour throughout the epidermis which intensifies towards the root apex and cap zone (Fig.86). Cells of the outer pith, xylem walls, collenchymatous phelloderm walls and periderm cells of the rhizome also show some reaction to DNP staining (Fig.87). This reaction can be faint in some cases (Fig.88) but is still visible in the walls of the outer pith cells and periderm.

Gibb's reagent produces a blue to blue-black colour on reaction with certain classes of phenolics. In root sections, cell contents of epidermal, endodermal and metaxylem cells are stained (Fig.89). The periderm, ray parenchyma and clusters of cells in the collenchymatous phelloderm and outer pith of rhizome sections display Gibb's reaction within the cells; in addition, cell walls of the secondary xylem show a definite bluish reaction (Fig.90). Some epidermal cells also appeared to react with Gibb's reagent, although the colour was less distinct (Fig.91).

Aniline addition products of quinones stained dark blue-black when treated with both 2% aqueous aniline and aniline-KIO_3. Pith cells adjacent to the xylem, endodermal cells, scattered cortical cells and a band of cells immediately below the epidermis of root sections show a strong reaction with aniline-KIO_3 (Fig.92). In rhizome sections (Fig.93) stain is localized in cells of the pith, especially in a band in the outer pith adjacent to the secondary xylem, ray parenchyma cells, clusters of cells in the collenchymatous phelloderm and peridermal cells. Some violet-blue staining of cell walls throughout the rhizome also

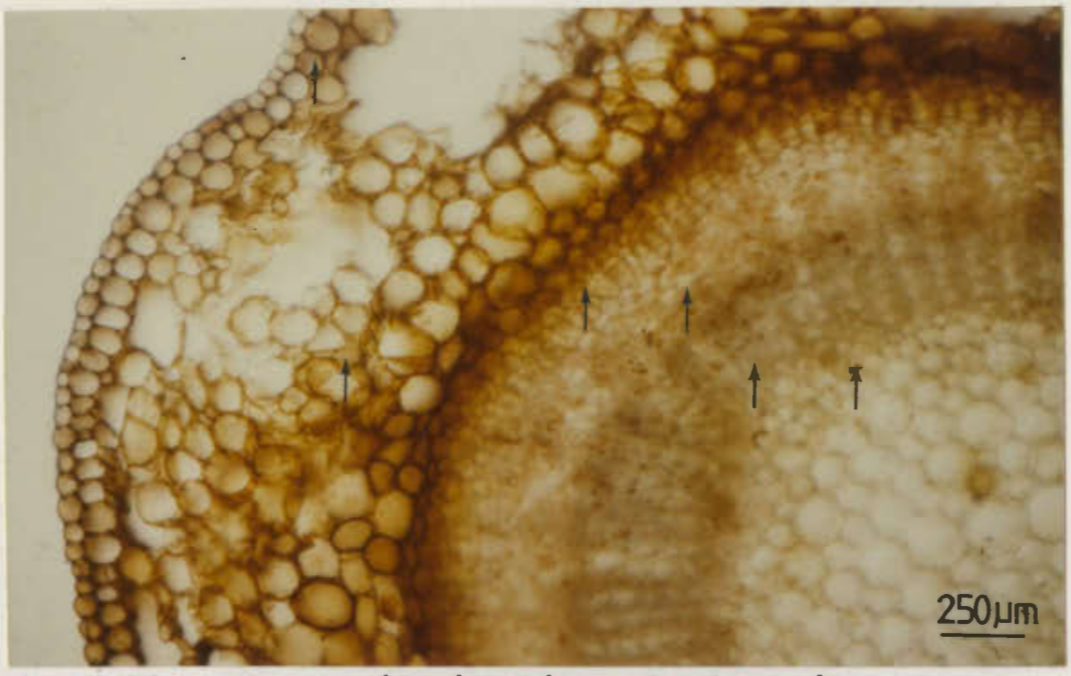


Figure 87. DNP reaction in rhizome hand section. Dense staining occurs in the outer pith, xylem walls, phelloderm walls, and periderm; some reaction also takes place in the old cortex and epidermis (arrows).

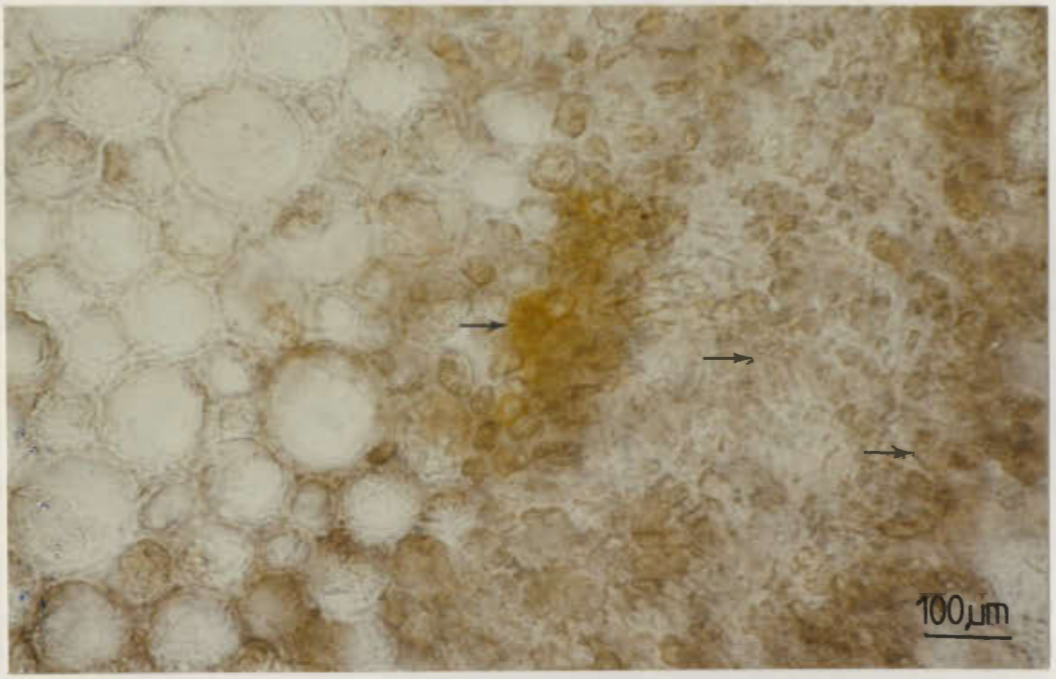


Figure 88. DNP reaction in central rhizome. Faint reaction occurs in the walls of the outer pith and periderm (arrows).

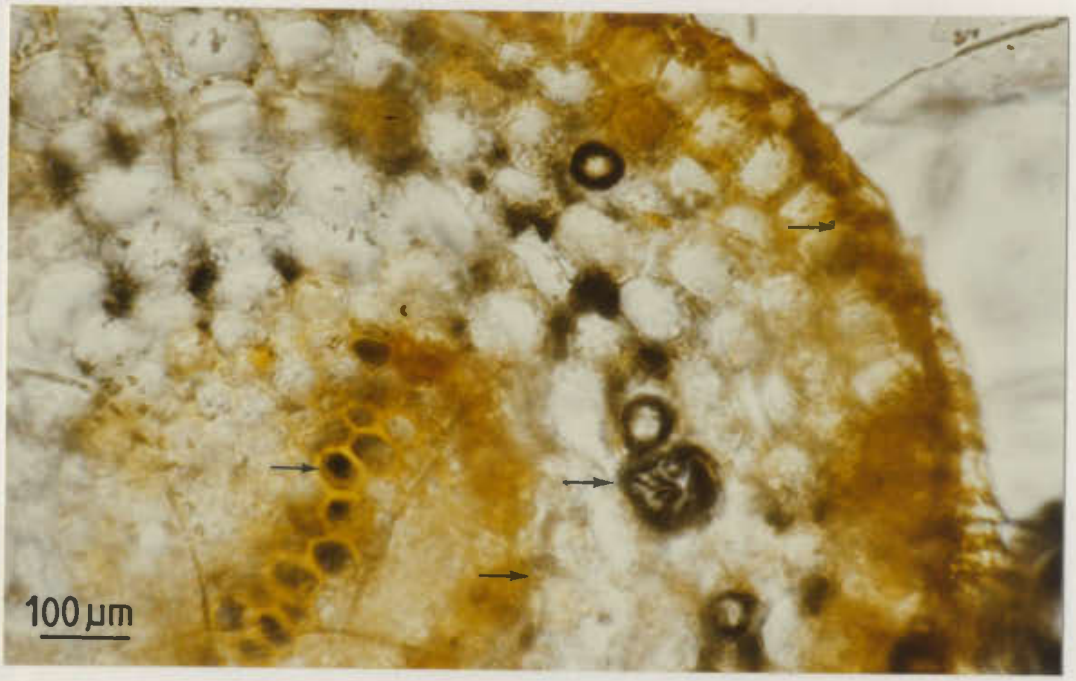


Figure 89. Root hand-section in Gibb's reagent. Reaction with specific classes of phenolics produces a blue-black color in the epidermis, endodermis, cortex, and metaxylem contents (arrows).

Figure 90. Gibb's reagent in rhizome hand-section. Positive reaction occurs in the periderm, xylem, rays, phelloderm and outer pith (arrows).

Figure 91. Gibb's reagent in outer layers of rhizome. Some epidermal cells show faint reaction (arrows).

Figure 92. Aniline-KIO_3 reaction in root hand-section. Aniline addition products of quinones in cells of the pith, endodermis, cortex, and exodermis stain blue-black (arrows).

Figure 93. Aniline-KIO_3 reaction in rhizome hand-section. Pith, rays, phelloderm and periderm react strongly (arrows).

Figure 94. Aniline-KIO_3 reaction in center of young rhizome. Bands of cells in the outer pith and periderm and scattered cortical cells, show positive reaction (arrows).

occurs. The outer pith, periderm and cortical cells of younger rhizomes prior to development of the secondary xylem also react, as seen in Fig.94. A similar pattern is seen in sections of roots (Fig.95) and rhizomes (Fig.96) stained with 2% aqueous aniline but the reaction is much fainter in these cases and is obviously enhanced by the addition of KIO_3.

(3) Development of dense cells

Radicles from dry seeds possess cells with numerous vacuoles and large, well-developed storage granules. Although there is some indication of iron-phenolic complexing in the cytoplasm of these cells, there is no appreciable aggregation near the cell wall (Fig.97). However, sections of radicles which had been soaked for six hours prior to treatment with $FeCl_3$ display accumulation of electron-dense globules at the inner edge of the cell wall and in the intercellular spaces (Fig.98). Vacuoles and storage granules begin to develop a more organized pattern by this time, with the latter enlarging and becoming more dense and the former clustering around them.

Radicles of seedlings sampled 23 days after soaking show increased deposition of ferric material along cell walls and in the nucleus (Fig.99). Some phenolics are also found in the cytoplasm (Fig.100). In most cases the cell wall itself is thinner and less electron-dense and the space between it and the cell membrane is enlarged. Iron-phenolic complexes are associated with the cell membranes rather than the cell wall (Fig.101). Storage granules are still present, each

Figure 95. 2% aqueous aniline reaction in root hand-section. Pith, endodermis, cortex and exodermis (arrows) show a less distinct reaction without the addition of KIO_3.

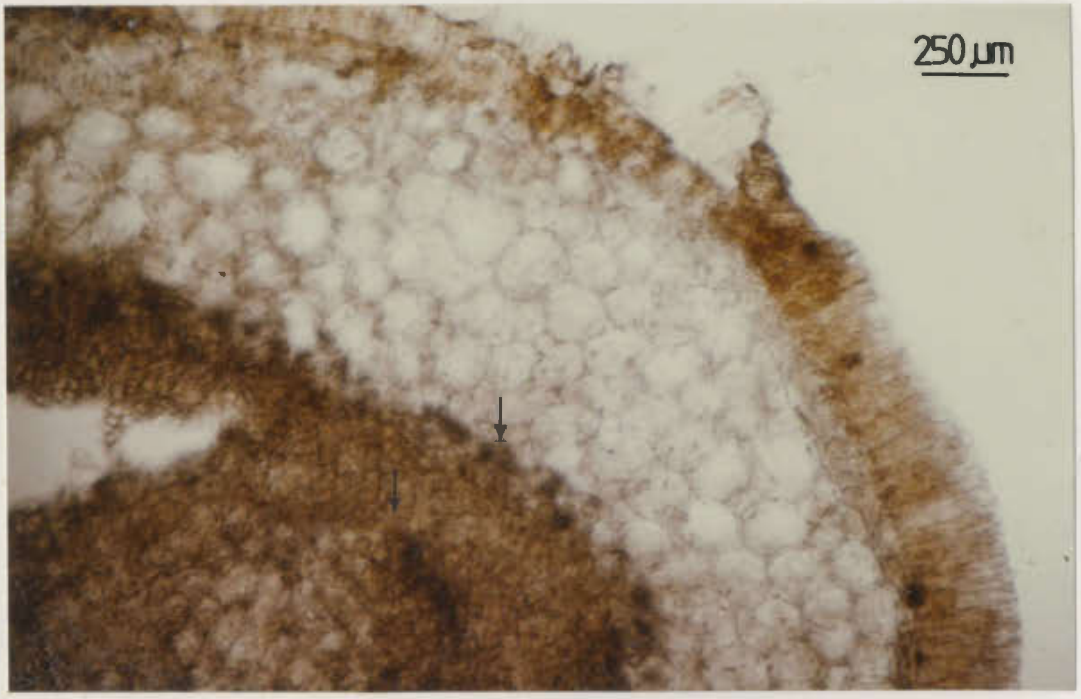


Figure 96. 2% aqueous aniline reaction in rhizome hand-section. Faint staining occurs in the pith and periderm (arrows), but is not as well localized as when KIO_3 is added.

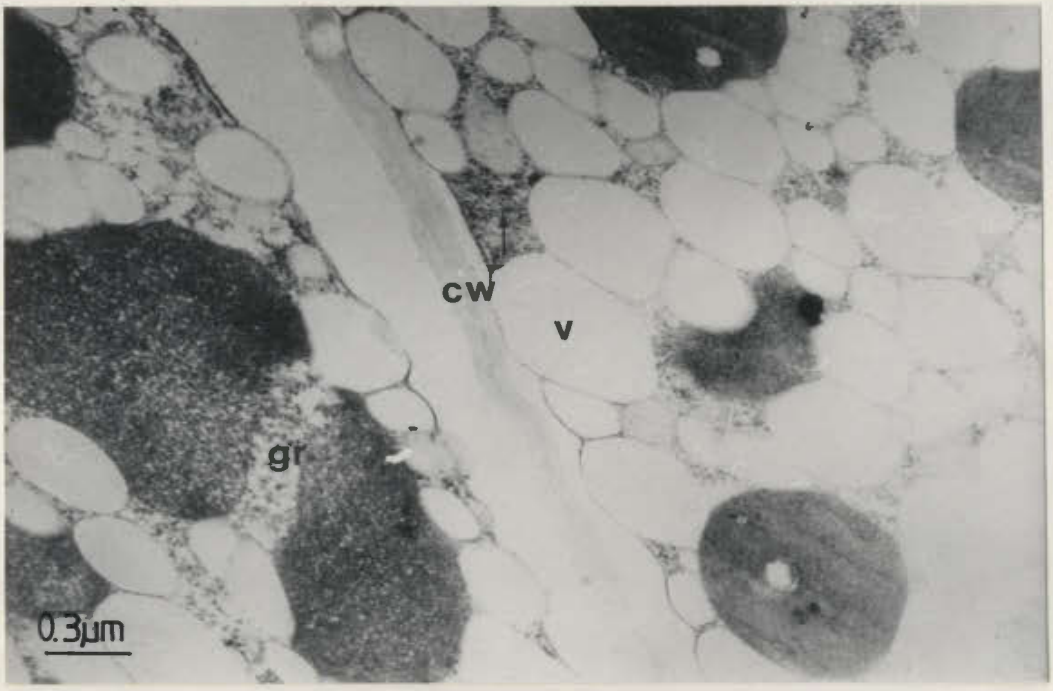


Figure 97. Electron micrograph of cells of radicle from dry seed treated with $FeCl_3$. Well-developed vacuoles (v) and storage granules (gr) are present. Some ferric complexes are visible in the cytoplasm (arrow) but none near the cellwall (cw).

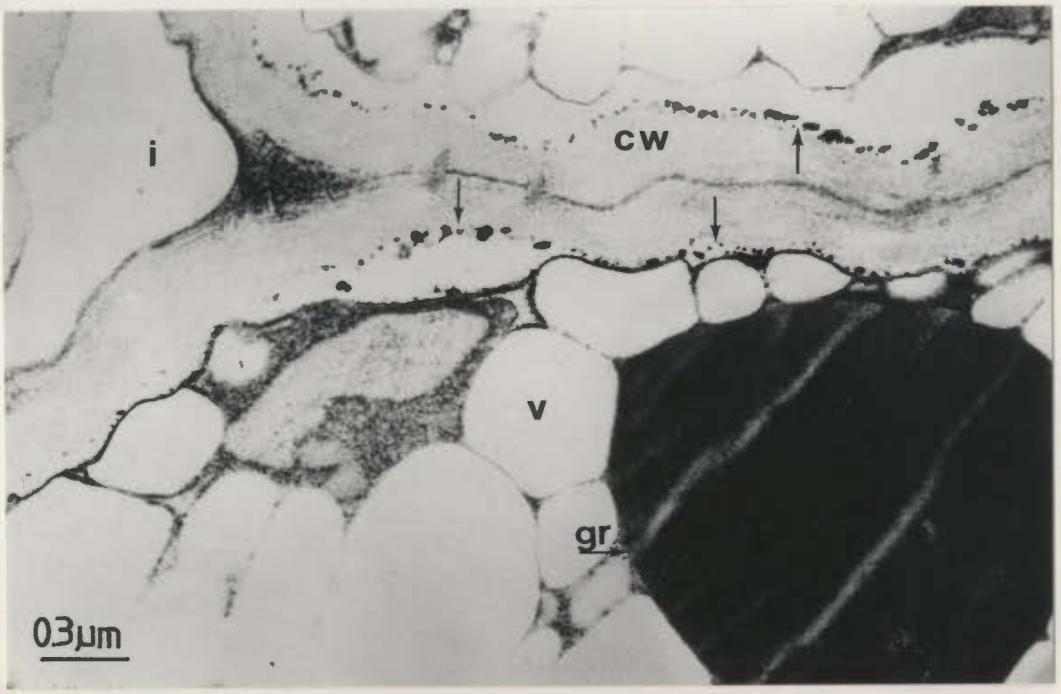


Figure 98. Electron micrograph of cells of radicle from seed soaked for six hours prior to $FeCl_3$ treatment. Ferric complexes (arrows) line the inner edge of the cell wall (cw) and intracellular spaces (i). Storage granules (gr) are more electron-dense and the vacuoles (v) tend to cluster around them.

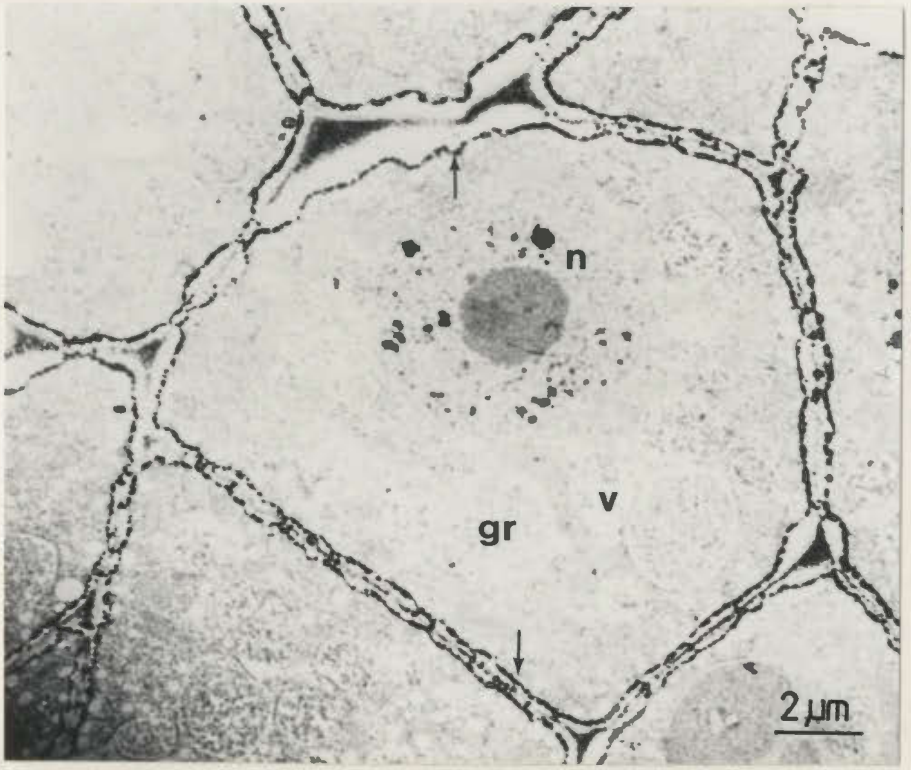


Figure 99. Electron micrograph of cells from radicle sampled 23 days after soaking, treated with $FeCl_3$. Deposition is increased along the cell wall (arrows) and in the nucleus (n). Vacuoles (v) and storage granules (gr) are less distinct.

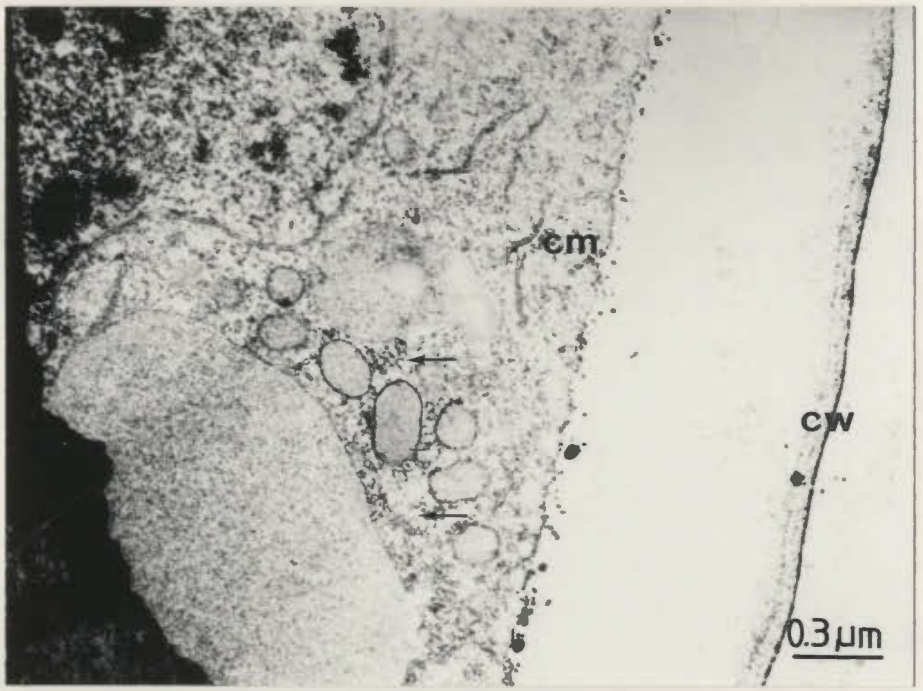


Figure 100. Electron micrograph of cell from 23-day old radicle treated with $FeCl_3$. Ferric complexes are present in the cytoplasm (arrows). The space between the cell wall (cw) and membrane (cm) is increased.

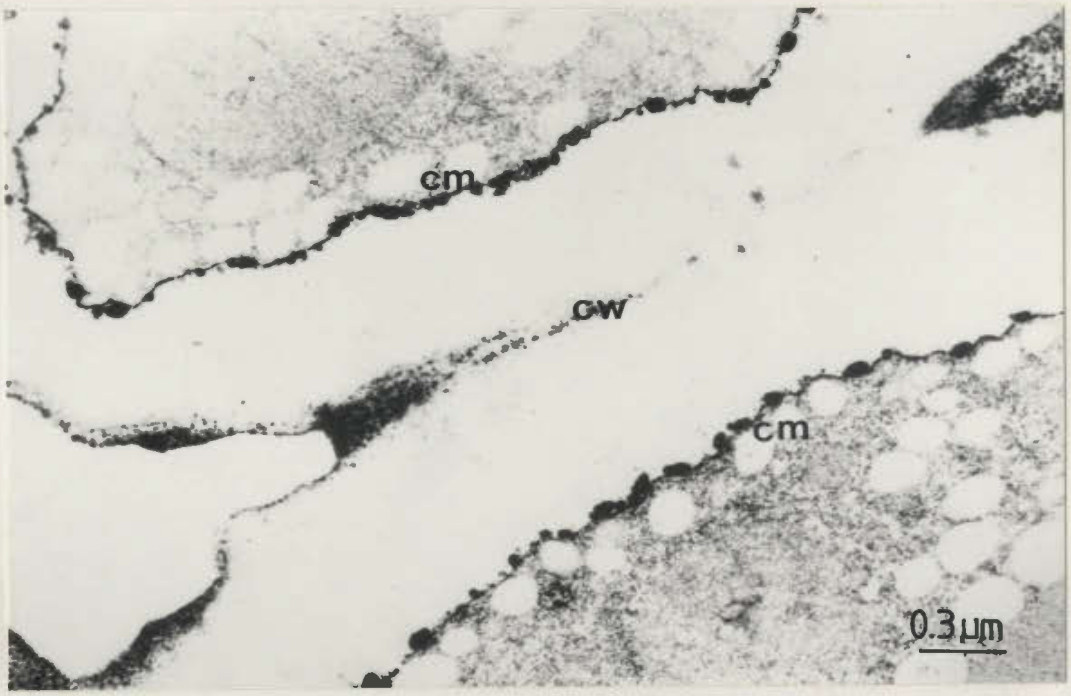


Figure 101. Electron micrograph of interface between two cells from 23-day old radicle treated with $FeCl_3$. Ferric material is clearly associated with the cell membrane (cm) rather than the cell wall (cw).

surrounded by a ring of vacuoles but are less dense than previously (Fig.102). Plasmodesmata also become electron-dense, especially where cell walls are thinner.

VII. Root Microflora

Ultrathin longitudinal sections of bakeapple roots stained with toluidine blue possess an amorphous outer zone possibly corresponding to a mucigel layer, associated with numerous particles (Fig.103). The epidermal cells are noticeably darkened, indicating some phenolic content. An equivalent area viewed under the electron microscope clarifies this association (Fig. 104). There is a distinct zone outside the epidermis consisting of several layers of material of various densities and thicknesses. Towards the outer edge, the material begins to fragment and thin out into the surrounding medium. Below this is an electron-dense area with some indication of a lamellate structure on the right. Between this layer and the outer root cap zone, a layer of variable thickness contains numerous bacterial cells and portions of septate hyphae. Hyphal structures can also be seen just above the epidermal cell wall and one, apparently in cross-section, within the cell.

In a preparation stained with ferric chloride, it is clear that phenolics are present within this outer layer (Fig.105). Although ferric complexes are scattered throughout the area, they cluster around the bacterial cells, which appear to be encapsulated. The lower boundary of the zone appears to have a three-layered structure and to be clearly delineated from adjacent areas.

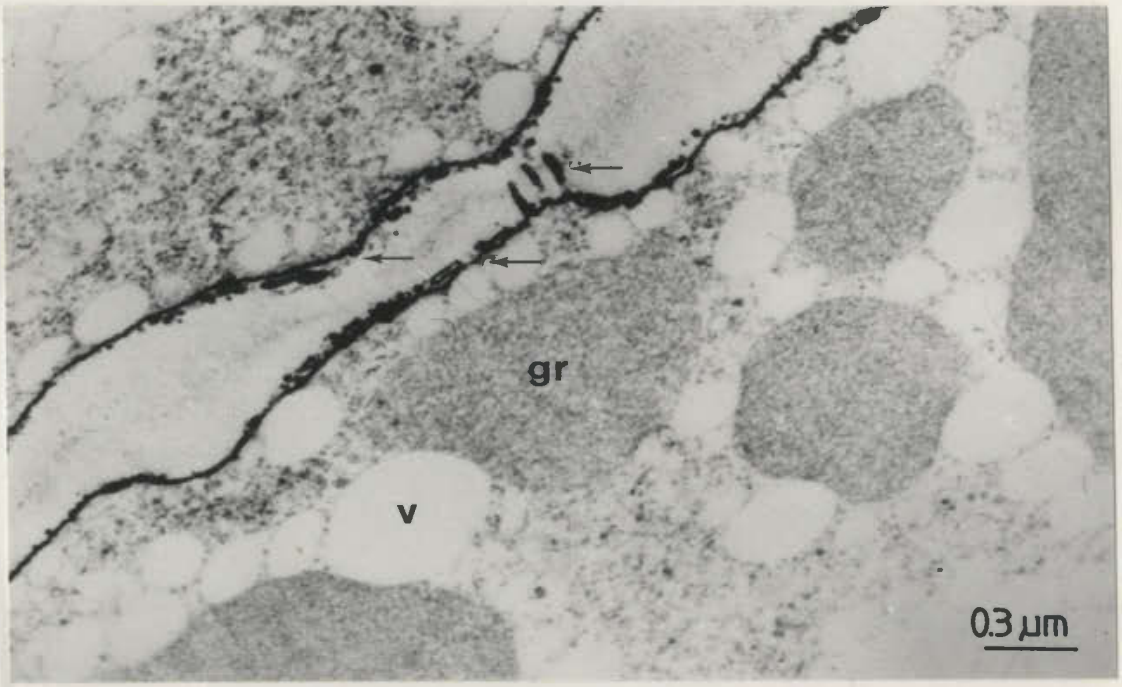


Figure 102. Electron micrograph of cells from 23-day old radicle treated with $FeCl_3$, showing plasmodesmata. Storage granules (gr) and vacuoles (v) are present but less electron-dense. Ferric complexes are localized along the cell membrane and in the plasmodesmata (arrows).

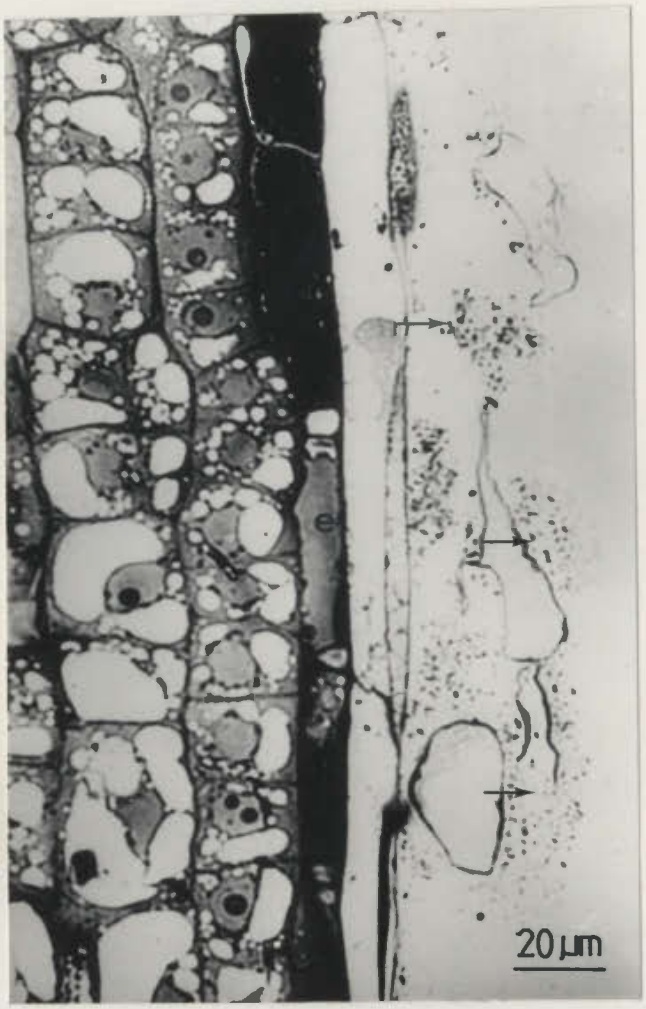


Figure 103. Photomicrograph of ultrathin Epon section of root stained with toluidine blue, showing outer layers. An amorphous layer containing numerous particles (arrows) is visible outside the epidermis (e).

Figure 104. Electron micrograph of root treated with OsO_4, showing outer layers. The outer edge of the amorphous layer (oe) thins out into the surrounding medium. Below this is a dense lamellate region (edr) bordering a variable layer containing bacterial cells (b) and hyphae (h). Hyphae are also present above the epidermal cell wall (cw) and one hyphae in cross-section (arrow) appears within the cell.

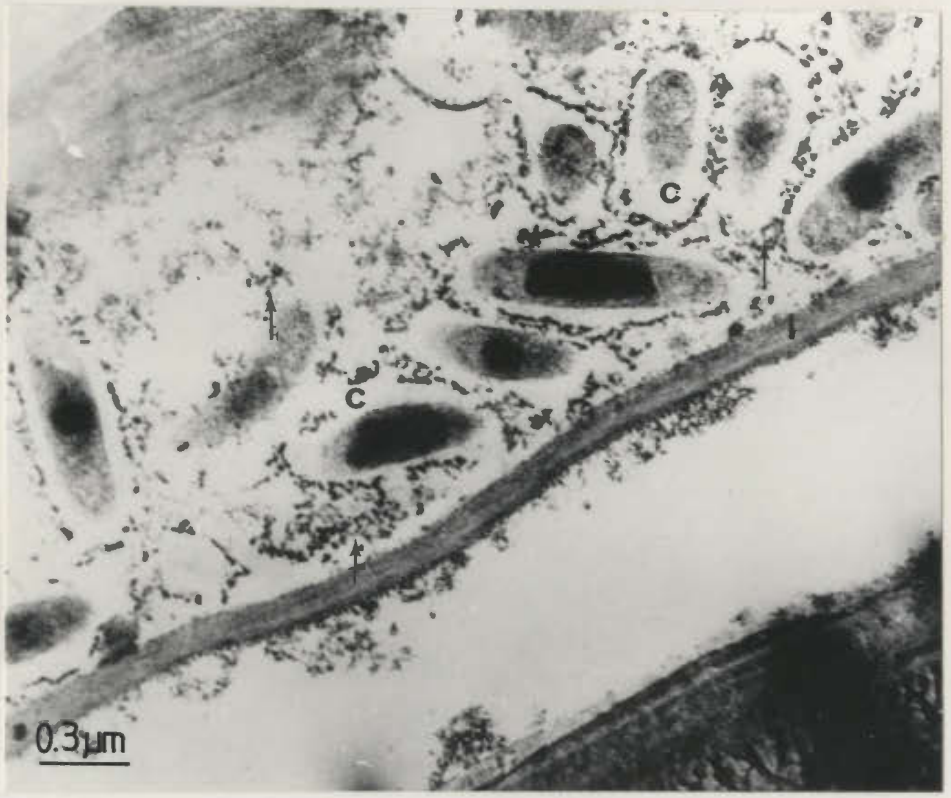


Figure 105. Electron micrograph of root surface in material treated with $FeCl_3$. Ferric complexes (arrows) are present throughout the layers of the amorphous zone, but appear to cluster around the capsules (c) of the bacterial cells. The lower edge of the zone (l) appears to have three layers.

Hyphal structures are also visible between and within root cells in sections stained with toluidine blue (Fig.106) and osmium tetroxide (Fig.107) where they are seen to extend just below the cell wall. A ferric chloride preparation shows hyphae in longitudinal and cross-sections (Fig. 108). The section at the lower centre has a two-layered wall and a distinct septa typical of fungal mycelia. Other hyphae appear to run between the walls of two adjacent cells. In Fig. 109 hyphae in cross-section lie between the cell wall and cell membrane, as well as in the intercellular space. Another hyphae with faintly visible septa lies within the cell on the lower right, apparently surrounded by ferric-phenolic deposition.

Further results do not conclusively identify these organisms, but rather give some indication of the diversity of the root microflora of *R. chamaemorus* L.

(1) Mycorrhizal staining

Fungal structures could be seen in root material stained with lacto-phenol and acid fuschin but were not clear enough to warrant photography of the preparations. Septate hyphaewere visible within several layers of healthy root tissue and slender, threadlike structures were seen within cells and on the surface. However, structures typical of vesicular-arbuscular mycorrhizae(appressoria, arbuscles and vesicles) were not seen in any preparation.

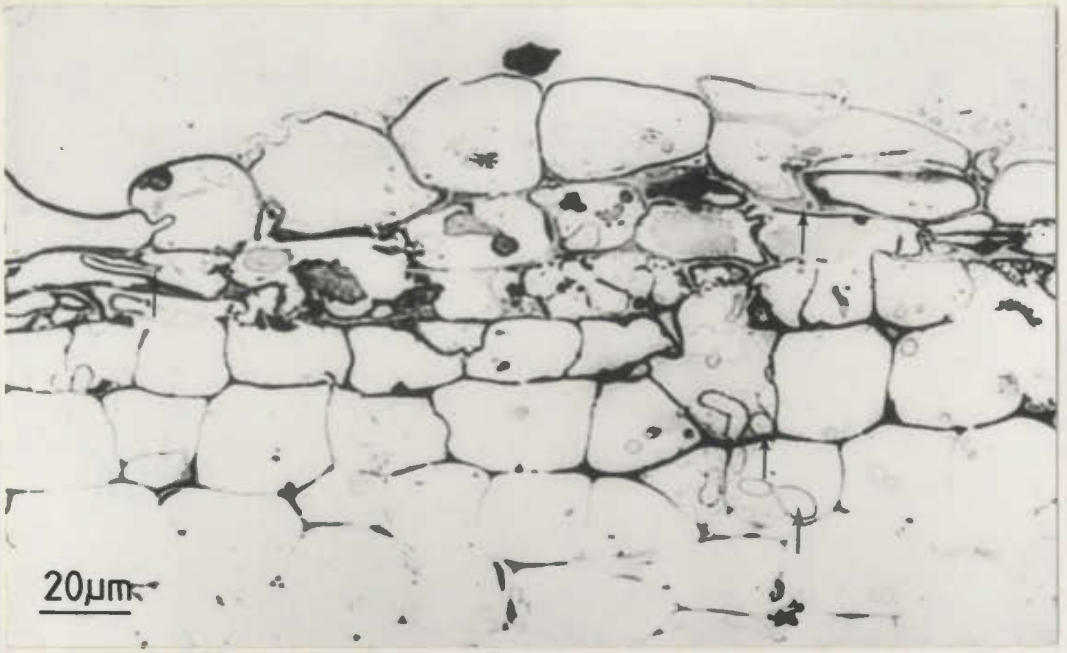


Figure 106. Photomicrograph of ultrathin Epon section of root stained with toluidine blue, showing fungal hyphae in epidermis and cortex. Hyphae (arrows) are visible within and between cells.

Figure 107. Electron micrograph of fungal hyphae in root cells treated with OsO_4. Hyphae in cross and longitudinal sections (arrows) are visible within cell walls.

Figure 108. Electron micrograph of fungal hyphae within root tissue treated with $FeCl_3$. Section A possesses a two-layered wall and distinct septa (arrows). Several other hyphae (B, C, D) appear to run between the cell walls (cw).

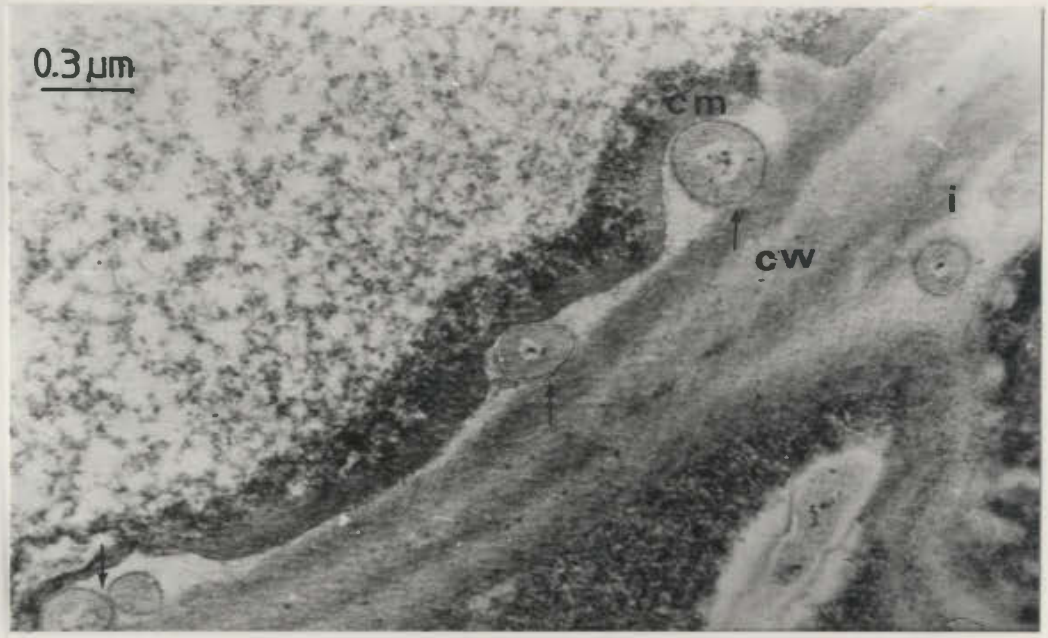


Figure 109. Electron micrograph of hyphae in root cells treated with $FeCl_3$. Several hyphae (arrows) lie between the cell wall (cw) and membrane (cm); one appears in the intracellular space (i). The hypha within the cell to the lower right appears to be surrounded by accumulated ferric complexes.

(2) Detection of mucigel layers

Material prepared in ruthenium red was generally too thick to be photographed effectively. Stained root tips from greenhouse and wild plants growing in peat showed little or no evidence of a mucigel layer. Roots dried before staining, to determine whether there was any dispersal of the material in the stain, showed similar results. Using root tips grown in a liquid medium, a thin layer was visible around the root tip. From these results, it appears that some mucigel is present in *R. chamaemorus* L. roots but at best it forms a very thin layer subject to the medium around the roots.

(3) Isolation and culture of root microorganisms

Examination of Gram-stained preparations enabled the delineation of several large groups: gram-positive rods, gram-positive cocci, gram-negative rods, gram-negative cocci and cultures exhibiting a mycellar structure. The cultures falling into each of these groups are given in Table 13. It is clear that the gram-negative rods account for the largest percentage (55.5%) of the 119 cultures tested. Gram-positive rods and gram-negative cocci account for 19.3% and 14.3% respectively, while only 5.0% of cultures consisted of gram-positive cocci. The remaining small group (5.9%) consists of cultures with positive and/or negative rods and mycelia.

The latter were set aside for cover-slip culture and observation to determine the likelihood of their being *Actinomycetes* species. All such cultures showed negative results, for acid-fast, spore stain,

Table 13. Major groups of cultures from the bacterial isolation procedure

Gram positive rods

A4M2	P44	
A4M3	RP41	
A4M4	P55	
A51	P61	
A5M3	RP4M2	
A6M2	RP4M5	
RA63	RP52	
A7M1	RP54	
A7M2	RP56	
A7M3	RP6M6	
A7M4	RP6M9	
	RP7M2	

Gram-negative cocci

A41	P4M1	
A42	P5M2	
A4M5	P6M1	
A5M2	P6M3	
A61	P73	
A62	P7M1	
A63	RP73	
A6M1		
A6M3		
A71		

Gram-negative rods

A43	P42	RP5M1
A44	P43	RP5M2
A4M1	P45	RP5M4
RA41	P4M2	RP5M5
RA42	P4M3	RP5M6
RA4M1	RP42	RP5M7
A52	RP43	RP6M4
A53	P52	RP6M5
A55	P53	RP6M7
RA51	P5M4	RP6M8
RA52	RP51	P7M5
RA53	P62	RP71
RA5M1	RP62	RP74
RA5M2	RP65	RP77
RA5M3	RP6M1	RP78
RA62	RP6M3	RP7M1
RA6M1	P71	RP7M4
RA6M2	P72	RP7M6
A72	P74	
RA71	P75	
RA72	RP4M4	
RA7M1	RP53	
RA7M2	RP55	
	RP57	

Gram-positive cocci

A5M1	RP72
P5M1	RP7M3
P5M5	
P6M2	

capsule staining and motility, with positive results for catalase and oxidase tests. These characteristics are found throughout the *Actinomycetaceae*. Cover-slip cultures exhibited fine mycelia and some limited aerial growth. Certain cultures also possessed pale-staining ovoid cells which could be spores and sporangia.

It was also considered necessary to check for possible *Azotobacter* species. Colonies of all the groups described above grew on *Azotobacter* medium but the majority were negative rods. Of these, eight cultures (A7M4, RP43, RP44, RP53, RP6M1, RP77 and RP7M1) were found to have positive or negative ovoid cells and positive catalase reactions indicative of *Azotobacter* groups. Only RP6M1 showed any motility or flagellation; however, there are non-motile *Azotobacter* spp., (notably *A. beijeri*). Colonies varied from white to pink, beige or yellow. Further tests, including starch, mannitol and mannose utilization, could be used to confirm these cultures as *Azotobacter*.

As indicated for the possible *Azotobacter* cultures, medium type did not appear to restrict colonial development, with the percentages of cultures about equal for all pH values. The proportions of culture types derived from the supernatant and from the macerated tissue are also close; 48.7% are derived from the former and 51.3% from the latter. This tendency is repeated for each of the groups of cultures above; it is therefore unlikely that any groups are restricted to the surface or the root tissue. Results from further staining and test trials were insufficient to positively identify any groups but indicate that a variety of organisms

are present in the rhizosphere. Several groups of spore-bearing rods were isolated, both with and without capsules. Most of the cocci tested were capsulated forms and several were seen to consist of pairs of cocci. Fourteen cultures showed positive motility, most of these being rod-shaped with one to several polar flagellae. Two cultures displayed peritrichous flagellation. The majority of cultures were capsulated, as expected in soil microorganisms. Overall it appears that *R. chamaemorus* L. roots support a flourishing and varied microflora.

DISCUSSION

Observation of *Rubus chamaemorus* L. populations in Newfoundland indicates that they are similar in form and development to populations in other parts of the world. Observation of the species throughout Newfoundland indicates that its maximum vegetative success and fruit productivity are reached in nutrient-poor coastal bogs with poorly developed peat. Such areas are dominated by *Sphagnum* spp. with the occasional addition of *Polytrichum* spp., *Rhacomitrium* spp. and lichens such as *Cladonia* spp., or *Cetraria islandica* in more northern blanket bogs. *Empetrum nigrum* L. is the most frequent associate of *R. chamaemorus* L. in such habitats. This *Rubo-Empetretum nigri* association has been considered typical of the exposed, ombrotrophic blanket bogs of the northern coastal plains and is noted for the high density and low pH (3.81 ± 0.52) of its underlying peat (Pollett, 1972). Such peats generally have average total nutrient values comparable to other types; however, the available nutrient values are often lower than in any other type (Pollett, 1972). Competitive pressure in such bogs is considerably lower than in more nutrient-rich peatlands where the growth of sedges, grasses and ericacious shrubs is encouraged. Such a distribution is consistent with reports for other parts of the range. In North America, *R. chamaemorus* L. is described as growing in '*Sphagnum* bogs and mountain meadows' (Bailey, 1941), peaty soils throughout Northern Canada to the Arctic Ocean (Porsild, 1964) and in *Sphagnum* bogs in Newfoundland (Scott, 1975). In Britain it is reported

to be largely restricted to mountainous areas with a low potential water deficit (Taylor, 1971), although it can grow successfully in heathlands and fertilized soil (Taylor and Marks, 1971). The most common habitats of the species in Scandinavia are raised or open aapa type *Sphagnum* bogs and swamps with well-developed spruce cover (Resvoll, 1929; Mäkinen and Oikarinen, 1974).

However, as in these regions, *R. chamaemorus* L. in Newfoundland is able to colonize more marginal habitats if conditions permit. The species has been reported from a drying swamp association in northwest Mongolia (Simpson, 1912), on snowflushes (Resvoll, 1929), manured or grazed grounds (Taylor and Marks, 1971) and rocky or sandy coastal localities (Resvoll, 1929; Mäkinen and Oikarinen, 1974). The latter are of special interest in view of the discovery of *R. chamaemorus* L. in a sandy beach edge at Cape Freels. Resvoll (1929) considered that the high atmospheric humidity of such habitats supplies sufficient moisture for plant growth. The greater productivity of *R. chamaemorus* L. in coastal regions with a high frequency of foggy or rainy days during the growing season would appear to support this view.

Although many species, including *Carex* spp., *Ericaceae* and grasses, are noted as associates of *R. chamaemorus* L. throughout its range (Resvoll, 1929; Taylor, 1971; Mäkinen and Oikarinen, 1974), little information is available concerning their competitive effect on bakeapple growth. Observations throughout the programme, especially the results of greenhouse experiments in which competitors were selectively removed, indicate

that the competitive ability of the species is low against these taxa and that the distribution of *R. chamaemorus* L. can be linked to the absence of competition in poorer habitats. Environmental factors such as peat humification, available nutrients, moisture content and exposure act on the species only indirectly by discouraging growth of species with stricter physiological limits. The situation in Newfoundland appears to parallel the Scandinavian one, in which

> "In the present conditions, the physiological and ecological optima of the cloudberry hardly coincide; probably the biotic factors are much more important than the nutrients."
> (Makinen and Oikarinen, 1974).

Competition from these species could be enhanced by their rhizomatous growth which competes for bog space and available nutrients and their greater height and cover which can easily prevent available sunlight from reaching the low shoots of the bakeapple. However, where relatively open scrub forest is present around the bog surface *R. chamaemorus* L. is able to take advantage of increased shelter given by conifers and shrubs such as *Populus* spp. or *Alnus* spp. In shaded bogs, differences in leaf shape and degree of incision between male and female plants are more acute (Rosanova, 1928) and plants in spruce swamps and dwarf pine bogs possess larger shoots, larger leaves, more rapid vegetative growth and more successful fruiting (Lohi, 1974). The planting of low windbreaks in Norwegian bogs has proved effective in increasing vegetative growth, flowering and fruiting (Østgard, 1964).

This phenomenon also occurs in the Newfoundland population, for instance at L'anse-au-clair, and similar measures could be valuable.

The sensitivity of *R. chamaemorus* L. flowers to climate is well documented (Mäkinen and Oikarinen, 1979). Flowering times for the study populations were delayed by occurrence of severe winters or late frosts but, on average, agree with sources giving the flowering time as June (Ayre, 1935) in insular Newfoundland. Resvoll (1929) describes altitudinal and latitudinal variation in blooming dates. In the southern extremities of its range, the species flowers, in May and June but it can bloom as late as July 25th in arctic Norway. Populations in northern Newfoundland and Labrador show a similar trend. Accelerated floral development in greenhouse transplants supports the view that variations in flowering time are related to climatic conditions and that development cannot take place until thawing of the peat and raising of ambient temperatures has occurred.

Flower buds are especially sensitive to spring frosts, with temperatures of -2°C being sufficiently low to destroy female flowers (Mäkinen and Oikarinen, 1974). Male flowers are marginally more resistant to cold (Jaakola and Oikarinen, 1972) and appear earlier than the females (Resvoll, 1929). Cold-shock treatment had no effect on development, either because it was not administered at the appropriate period of growth for a sufficient length of time,

or because there is an additional requirement for changes in daylength which greenhouse plants do not experience.

R. chamaemorus L. is known to be insect pollinated (Dallman, 1932; Østgard, 1964; Taylor, 1971; Kortesharju et al., 1978; Hippa et al., 1978) predominantly by empedids, syrphids, anthomyids and bumblebees. Insect captures at Witless Bay indicate that similar groups act as pollinators for Newfoundland populations. In addition ants were found in male and female flowers and may be instrumental in pollination. Although ants and beetles have been found visiting bakeapple flowers (Havas, 1970) only cautious interpretation can be drawn from these observations. It is possible that these insects are taking advantage of the warmer surface of the flowers or are feeding on nectar but do not transfer pollen. Results indicate that measures such as the planting of windbreaks can also encourage pollination and thereby increase the success of fruiting as well as vegetative growth (Mäkinen and Oikarinen, 1974).

Berry development in Newfoundland parallels the Scandinavian situation where ripening occurs from August to September and is somewhat delayed at more northerly latitudes (Resvoll, 1929). Success of the crop is closely associated with flowering success and is therefore affected by much the same environmental factors. Wind and frosts can destroy immature carpels, as can excessively hot or dry weather as in 1979. Increases in the natural yield generally depend on increased numbers of shoots rather than on increased berry size (Mäkinen and Oikarinen 1974).

Scandinavian management and research procedures have concentrated on producing more dense stands of bakeapple by reducing competition and encouraging growth of new shoots.

In Newfoundland populations, vegetative development occurs primarily in the period between berry maturation and leaf senescence, with numerous aerial shoots produced during September in the wild. Seedlings spread rapidly when planted out in large peat-filled boxes due to their effective rhizome growth. It has been reported that 62.2% of the total biomass of seedlings consists of underground organs towards which most of the energy of growth is directed (Resvoll, 1929) and that mature plants have a high ratio of subterranean to aerial biomass (Shaver and Cutler, 1979). Shoot densities can vary considerably over a given bog and increase on hummocks and similarly dry areas, as in Scandinavia (Resvoll, 1929). It is therefore obvious that Newfoundland crop levels can be improved through the use of management techniques which increase vegetative growth. One such method, ploughing the bog surface so that rhizomes are cut, produced significant increases in shoot density at Bauline Line quadrats. The best results were obtained by ploughing at 0.25m intervals. New shoots develop at the edges of the cuts, as in Norwegian experiments (Østgard, 1964); however, an additional benefit of ploughing derives from the reduction of competitive species (Mäkinen and Oikarinen, 1974).

Greenhouse propagation of *R. chamaemorus* L. successfully provided accessible populations for experiments as previous work by Kortesharju

(1978) has indicated. Although floral development and floral induction were unsuccessful, vegetative growth occurred over a longer period of time each year and numerous rootlets were produced in the greenhouse plants. Scandinavian research indicates that propagation by rhizome fragments in the field is a viable means of spreading *R. chamaemorus* L. (Østgard, 1964; Mäkinen and Oikarinen, 1974).

Experiments on the effects of minimum length and apical dominance on regenerative capacity showed that the most rapid and vigorous growth is obtained from rhizomes at least 50cm in length with the apical bud intact. Although non-apical fragments were able to sprout, their growth was weak and they did not survive their first season. Dominance patterns follow those observed in *Agropyron repens* L. Beauv. (Leakey and Chancellor, 1977a, b; 1978a, b) and suggest that differential concentrations of various compounds in the apex and rhizome contribute to the success of regrowth. Rhizomes have been shown to accumulate both non-structural carbohydrates (Shaver and Billings, 1976) and growth substances (Shah and Raju, 1976) and to be rich in inhibitors and growth regulators in all seasons (Chenieux and Petit-Paly, 1975). The apex in particular is seen as an area of concentration for many growth-promoting substances including phytochromes (Duke and Williams, 1977) and amino acids (Nigam and Macintyre, 1977) which could be necessary for regeneration of rhizome fragments. Results from seed germination trials indicate that the many difficulties involved in preparing and germinating *R. chamaemorus* L. seed and the relatively low germination

percentages make vegetative propagation more practical.

Germination percentages can be improved by the use of gibberellic acid (GA_3) and kinetin (Warr et al., 1979), which have been implicated in dormancy breaking of related species (Lasheen and Blackhurst, 1956; Jennings and Tulloch, 1965). Attempts to utilize acid scarification (Moore et al., 1974; Rantala, 1976) as a substitute for mechanical removal of the seed coat to release excess cyanides (Mayer et al., 1957; Taylorson and Hendricks, 1973; Warr et al., 1979) were ineffective. Fungal mortality can be controlled using sterile techniques for preparation and incubation, since fungi do not appear to be present in seeds prior to germination. However, the slow development of seedlings limits their potential use as a management tool.

The pollination study could have potential value in increasing natural yields of berries. Scanning electron microscopy of female and male flowers confirms that considerable amounts of pollen are released from the anthers (Resvoll, 1929) and that successful germination occurs on the stigmatic surface. However, pollen viability is low (Mäkinen and Oikarinen, 1974) as is stigmatic specificity. The discovery of ericaceous pollen germinating on *R. chamaemorus* L. stigmas raises several questions. Stigma-pollen interactions have received considerable attention in recent years, with the attraction of pollen wall antigens for stigmatic glycoprotein binding sites seen as the key to specificity of pollen germination (Knox 1971; Heslop-Harrison, 1975 a and b; Knox et al., 1976; Heslop-Harrison, 1976). Bog

ericads have been shown to have low specificity related to their self-incompatibility (Reader, 1977). The flowering periods of several *Ericaceae* in Newfoundland overlap with that of *R. chamaemorus* L. and they share many insect pollinators, especially bee species such as *Bombus* and *Andrena* (Lee, 1958; Wood, 1961; Wood, 1865; Hippa et al., 1978). It seems likely that some of the high pe-centage of undeveloped female bakeapple flowers could be due to mechanical or chemical interference with pollination by 'foreign' ericad grains whenever the ericad population is high enough (Faegri and van der Pijl, 1979). Measures which reduce competitive species while encouraging pollinators could therefore increase the incidence of successful pollination. It is also notable that female flowers possess glandular trichomes which could produce nectar attractive to pollinating species.

Examinations of root and rhizome anatomy show that there are no significant structural differences between *R.chamaemorus* L. in Newfoundland and plants described by Resvoll in 1929. The structure of root and rhizome adapt these organs for growth in a peat matrix and for survivial during harsh winter conditions. (Resvoll, 1929).

Phenolic compounds were found in a variety of locations within the root and rhizome and varied in type. Dense cells, as discussed in previous work on *R. chamaemorus* L. (Bal, 1975; Bal and Sayory, 1980), were shown to possess phenolic deposits within the vacuoles, along the tonoplast membrane, throughout the cytoplasm, between the plasma membrane and cell wall and in the intercellular spaces and plasmodesmata. Tannins have been localized in membrane-bound cisternae of root cells

which form central vacuoles as the root tissues differentiate (Ginzberg, 1967). Terpenoid polyphenols are contained in spherule complexes in laticifers of *Gnetum gnemon* (Behnke and Herrmann, 1978). A model for the formation of C_6-C_3 phenolic compounds and the accumulation of esters and flavanoids in vacuoles and lignin in cell walls has been proposed by Stafford (1974). In this model, vesicles containing the necessary multienzyme complexes and subunits become attached to various organelles and eventually fuse, secreting flavanoids into the vacuoles. Other vesicles coming into contact with further enzyme systems at the dictyosomes eventually channel lignins across the plasma membrane via the microtubules and into the cell wall. The localization of ferric complexes in stained cells of *R. chamaemorus* L. is in keeping with these pathways, particularly the discovery of phenolics in the plasmodesmata.

Specific localization of various phenolic classes further supports this hypothesis, with flavanoid catachol tannins tending to be found in cell contents and walls, while terpenoid compounds are largely restricted to cell walls. The former show a scattered distribution within root and rhizome tissues, whereas the latter are more specific to bands of cells in the epidermis, xylem, outer pith parenchyma and outer cortex. Both catechins (Feucht and Nachit, 1977) and terpenoids (Mace et al., 1974) have been localized in young growing tissues, shoots and root tips respectively and have been implicated in growth and production of callus tissue in these organs. The groups of polyphenolic-containing cells described above clearly provide roots and

rhizomes with several protective "barrier" layers. Positive results with aniline-KIO_3 indicate that at least some of these phenolics, notably the terpenoids since the stained zones correspond, are derived from naturally occurring phenols which have been incorporated into the tissue.

The development of dense cells follows general trends discovered in the early stages of cotton fibre differentiation by Ramsay and Berlin (1976). Deposits of ferric-complexed material are diffused in dry radicles but become more concentrated and numerous after soaking. It appears that synthesis of polyphenolics is somehow triggered by soaking and increases rapidly after this stage. As development proceeds phenolic deposition occurs along the cell walls, in the plasmodesmata and in intracellular spaces. Accumulation in the vacuoles would seem to be delayed until the root is more mature.

The root and rhizome of greenhouse-grown specimens contain a variety of poly-phenolic compounds in clearly delineated zones, possibly enhanced by culture conditions. Although the functions of phenolic compounds are not as yet completely understood, there is considerable evidence for their role in defence against infection and disease. Terpenoids and other phenolic disinfectants have measurable bacteriostatic, bactericidal and fungicidal effects (Hegra, 1977; Liszka and Sendra, 1978). Resistance to disease has been linked to phenolic compounds found in cotton (Veech, 1977), sorghum (Woodhead and Bernays, 1978), eucalypts (Tippetts, 1976) and oak (Parker, 1977). Such compounds are often found in the epidermis, peridermis and

endodermis where they are able to prevent invasion of the vascular tissues by infective organisms.

Mature healthy *R. chamaemorus* L. roots have been shown to possess a rich and varied microflora in association with a thin mucigel layer outside the epidermis and in cells of the cortex. Bacteria are largely restricted to the former zone, although they are found in both macerated tissue and supernatants from washed roots. Fungal hyphae are found within cells and transversing cell walls throughout the root cortex as described by Lohi and Havas (1972). Comparison with established descriptions suggests that these are not vesicular-arbuscular forms. The bacteria within the mucilagenous sheath are surrounded by ferric-complexed material, suggesting that polyphenolics act to restrict and control their association with root tissues. Such sheaths or 'mucigel' layers have been described in members of the Ericaceae (Leiser, 1968) and other families (Greaves and Darbyshire, 1972). Their formation has been linked with directional secretion from the Golgi bodies of root cells (Juniper and Pask, 1973) and can apparently be stimulated by the presence of microorganisms.

Preliminary identification of root microflora cultures show a varied group of microorganisms, including possible members of the Actinomycetaceae, which have been implicated in enhanced nitrogen utilization by mycorrhizal roots (Williams, 1979; Rose, 1980). *Azobacter* spp. may also be present and additionally enhance the process as described for other non-symbiotic nitrogen-fixing bacteria (Evans et al., 1972; Mishustin

and Yemtsev, 1973). Other types of bacteria, especially proteolytic forms (Jaskiewicz, 1977, a, b, c), are considered to improve the nutrient utilization of associated vascular species.

Mycorrhizae can also have a beneficial effect on the growth of host organisms. Radiata pine seedlings with mycorrhizal associates show a greater resistance to the flow of water from the soil into plant tissues (Sands and Theadorou, 1978). However, the presence of mycorrhizae in halophytes, hydrophytes and xerophytes (Khan, 1974) suggests that they can enable plants to adjust to a low moisture content in the substrate, perhaps as at Cape Freels. The rate of uptake of phosphorous (Barrows, 1977; Azcin et al., 1978; Ho and Zak, 1978) and nitrogen (Stribley and Read, 1980) are increased in mycorrhizal roots. *R. chamaemorus* L. responds favourably to treatment with nitrogen and phosphorous (Østgard, 1964), with dry matter yield and flowering especially improved by phosphorous fertilization (Taylor, 1971). The rate of phosphorous uptake is temperature dependent and a phosphate leak occurs at low temperatures (Saebø, 1970).

Phenolic compounds and microflora therefore appear to interact and enable *R. chamaemorus* L. to utilize nutrient-poor habitats. Deposits of phenolics in root and rhizome prevent excessive infection by associated or undesirable microorganisms, while the microfloral associates provide enhanced capability in converting and incorporating nutrient elements in the peat substrate. By this means, *R. chamaemorus* L. compensates for its lack of competitive ability by being able to colonize

areas unsuitable for more nutrient-specific groups.

CONCLUSIONS

I. Field Observations

1. *Rubus chamaemorus* L. is found in a more restricted range of habitats in Newfoundland and Labrador than in Northern Europe and Asia, reaching its maximum productivity in nutrient-poor coastal bogs with low competitive pressure. However, it can succeed in marginal habitats, as at Cape Freels, provided that the atmospheric humidity is sufficiently high.

2. Its competitive ability is poor, especially against Gramineae, Cyperaceae and Ericaceae. These groups could dominate the substrate with their rhizomes and reduce available light to *R. chamaemorus* L. by their aerial growth.

3. *R. chamaemorus* L. responds well to increased shelter from wind and precipitation given by forest cover at the bog edge and displays enhanced size and productivity in such situations.

4. The phenology of both sexes is highly dependent on climate, with floral buds opening only after the bog surface had thawed. Severe springs and late frosts can destroy entire populations of male and female flowers, leading to poor berry yields. Transplantation to the greenhouse led to earlier flowering.

5. Insects, especially Formicidae, appear to be important pollinating agents.

6. Berry production is highest in bogs where *R. chamaemorous* L. can dominate. Populations in marginal habitats display a greater degree of failed berry development.

II. Growth Experiments

1. Cutting or ploughing the bog surface leads to significant increases in the production of aerial shoots in the field.

2. New plants from rhizomes can be successfully raised in the greenhouse, with cuttings of at least 50cm showing the best development after transplantation. However, floral development is inhibited by greenhouse conditions, possibly due to a requirement for winter chilling.

3. There is some evidence for apical dominance in rhizome cuttings and it appears that inclusion of the original apical shoot is necessary for continued growth.

III. Seed Germination

1. Mortality, due to fungal infection, is a limiting factor in the germination of *R. chamaemorus* L. seeds but can be considerably reduced by preparing seeds under sterile conditions. Fungi do not appear to be present in the seed prior to germination, although bacteria may be present.

2. Germination percentage is enhanced by incuration with gibberellic acid (GA_3) and kinetin, with 5.8×10^{-6}M and $\times 10^{-7}$M GA_3 showing best results.

3. Acid scarification is ineffective as a replacement for mechanical removal of the seed coat.

IV. Pollination Study

1. Glandular trichomes are present on the carpels and could produce nectar attractive to pollinating insects.

2. Production of numerous pollen grins and successful germination on the stigmatic surface have been demonstrated using scanning electron mieroscopy. However, stigmatic specificity appears to be low, as evidenced by the successful germination of a *Vaccinuim* pollen on a *R. chamaemous* L. stigma.

V. Root and Rhizome Anatomy

Anatomy of roots and rhizomes of Newfoundland plants are basically as described for Scandinavian plants.

VI. Phenolic Localization

1. Phenolic compounds are found in a variety of locations within the root and rhizome.

2. Phenolic deposits occur within the vacuoles, along the tonoplast membrane, throughout the cytoplasm between the plasma membrane and cell wall and in the intercellular spaces and plasmodesmata.

3. Several classes of phenolics can be distinguished, stained and localized. Catechol tannins and their flavonoid precursors are found within cells and cell walls of the epidermis, endodermis, peridermis, ray parenchyma and secondary xylem. Gossypol and related terpenoids are more predominant in cell walls and are found in the epidermis, xylem, vessels, pith parenchyma and outer cortex.

4. Positive reaction to the aniline-KIO_3 test indicates that at least some of the phenolics in the pith, endodermis, cortex and ray parenchyma are aniline addition products of quinones derived from naturally-occurring phenols incorporated into the root and rhizome tissues.

5. Phenolics appear to be present in dry seed radicles but their synthesis and accumulation increase rapidly after soaking. As development proceeds, phenolics are mainly laid down at the cell wall and in intercellular spaces.

VII. Root Microflora

1. Both bacterial and fungal structures are present in mature healthy roots.

2. Bacteria appear to be largely restricted to a thin mucigel layer outside the epidermis, although they are found in macerated root tissues as well as supernatant from washed roots.

3. Fungal hyphae are found within cells and transversing cell walls. Examination suggests that vesicular-arbuscular mycorrhizae are not present in this species.

4. The root microflora is rich and varied, including possible members of the Actinomycetaceae and *Azotobacter* spp. which could enhance the ability of the species to utilize nutrient-poor habitats.

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