THE BIOLOGY OF POPLAR RUST IN THE SOUTH WESTERN CAPE WITH SPECIAL REFERENCE TO THE CARBON PHYSIOLOGY OF THE HOST-BIOTROPH RELATIONSHIP

A thesis presented for the degree
of Doctor of Philosophy
at the University of Cape Town

by

Sharon Mary Roberts B.Sc. (Hons.)

Department of Botany,
University of Cape Town.

October, 1976.

TABLE OF CONTENTS

SUMMARY

An investigation of the host-obligate biotroph relationship of a commonly occurring rust fungus in the South Western Cape, Melampsora sp., infecting Populus canescens (Aiton) Sm., was stimulated by the apparently non-harmful effect of the pathogen on its host. The healthy appearance of the infected poplar tree and the lack of morphological changes in the leaves of infected shoots suggested that the rust was a benign obligate biotroph which, in the absence of its alternate hosts, appears to be adapted to a compatible existence with one host. The ready availability of poplar stands in the South Western Cape which are regularly infected with rust provided the opportunity to study the seasonal changes in carbohydrate levels and composition, total nitrogen levels and dry weight in the healthy and rust-infected poplar leaves. Further information was obtained by feeding entire shoots or single leaves of shoots with $^{14}CO_2$, then assessing levels of ^{14}C-assimilate and following the translocation of ^{14}C in the healthy and infected host.

The normal seasonal fluctuation of carbohydrates in the host was not drastically altered by infection, although small amounts of acyclic polyols and trace amounts of trehalose were detected. These compounds are characteristically synthesised by most biotrophs but the quantities found in the rust-infected poplar leaves formed a considerably lower proportion of the ethanol-soluble carbohydrates compared with other rust infections. Seasonal levels of polysaccharide in the pustule and in host tissues adjacent to the lesion did not differ greatly from the quantities in healthy host tissue, but a mannose-containing polymer constituted a greater proportion of the polysaccharides present in the pustule than in healthy tissues

Total nitrogen levels in the pustule region exceeded levels in healthy tissue only during the stages of infection when uredosori first appeared on the lamina surface, and then decreased as the uredosori aged. Dry weight levels were found to be increasingly greater in the pustule regions than in healthy host tissue.

^{14}C-assimilation studies indicated that the poplar rust constituted a weak "sink" for host assimilates, but revealed a slight stimulation of synthetic processes in the host tissue adjacent to the lesion. The rust did not appear to impede translocation of ^{14}C-assimilate from infected leaves nor restrict the flow of translocated assimilate to developing younger leaves of the shoot.

While the poplar rust shared several characteristics in common with other rust-host associations, it is evident that these symptoms were attenuated in the association described. The poplar rust is unusual in that it appears to coexist with its host with minimal disruption of the host's metabolism.

ACKNOWLEDGEMENTS

I am indebted to the following persons for help and cooperation during the course of this project and to the following institutions for financial support:

Dr. Derek Mitchell, under whose supervision the project was conducted, for his motivation and assistance;

Professor O.A.M. Lewis for facilitating the acquisition of the necessary equipment, for the use of the facilities of the amino acid analyser unit and for reading the final draft of the thesis;

Professor E.A.C.L.E. Schelpe for valuable suggestions during the preparation of the manuscript;

Mr. James Kaiser and Mr. Alex Fricke for their technical assistance and help with the photography;

Dr. S.C. Churms of the C.S.I.R. Carbohydrate Research Unit, Department of Chemistry, University of Cape Town, for her assistance and to Professor A.M. Stephen for the use of the facilities of the Carbohydrate Research Unit;

The University of Cape Town for the financial support from a Staff Research Grant (number 46-647) during 1972 and the Smartt Memorial Scholarship from 1973 to 1975.

The South African Council for Scientific and Industrial Research for financial assistance at the initiation of the project;

Miss Patricia Roberts for her valued help during the preparation of the manuscript.

Miss Jennifer Muller for typing the thesis;

My parents, for their constant support, encouragement and help throughout the duration of the project.

INDEX OF FIGURES

Page

Page

Page

INDEX OF TABLES

Page

Page

INDEX OF PLATES

CHAPTER 1.

INTRODUCTION

Research investigations of rust and mildew obligate biotrophs have been directed towards diseases of a wide range of economically important crop plants, especially wheat stem rust (12, 16, 22, 50, 105, 110, 136, 137, 140), flax rust (17, 18, 29, 30, 79, 80, 107, 128), clover rust (124, 141), and bean rust (65, 67, 90, 148). There is, however, little published information on diseases of horticultural crops such as carnation, pelargonium and hollyhock rusts and arboreal infections such as poplar rust. There are ultrastructural studies of *Populus tremula L.* leaves infected by *Melampsora pinitorqua* Braun Rostr. (81) and of blister rust (*Cronartium ribicola*) infecting tissue cultures of *Pinus monticola* (95). Most studies of host/rust pathogen interactions have been concerned with obligate biotrophs infecting susceptible hosts where a virulent reaction by the biotroph is observed. There is also a lack of information on the interaction between host and obligate biotroph in a natural population. An understanding of the mechanisms determining either the resistance or susceptibility of a plant to a particular pathogen is necessary (as stressed by Scott, 104) before adequate control measures can be evolved.

In most infections a fairly predictable pattern of host responses has been recorded. Initially the host tolerates the presence of the biotroph; the presence of the fungus may often be stimulatory (31) by enhancing respiration, initiating nuclear disturbances in the host and the production of green islands around the site of infection (9, 64). There is usually minimal tissue damage and a restricted host range. However, as infection progresses, depending on the virulence of the biotroph and the resistance of the host, a stage is attained where the photosynthetic capacity of the host is decreased and photosynthate is diverted and utilised by the biotroph to the host's detriment. A relationship of this type where movement of metabolites is essentially in one direction, from host to fungus, has been described as parasitic symbiosis by Lewis (63). There may be relationships, however, where host and obligate biotroph exist without obvious detriment to the host. These host/biotroph systems are regarded as essentially benign infections (145) where the degree of parasitism is low enough to permit the presence of the fungus on the host for an indefinite period.

Investigations of rust and mildew infections have produced some common trends in host/obligate biotroph interactions. The dependence of obligate biotrophs on the host carbohydrate supply was noted by Mains (73) and a changed nutrient status upon infection of maize leaves with Puccinia sorghi Schw. was observed where the starch levels in leaves decreased upon infection. Rust biotrophs have been demonstrated to mobilize nutrients to the site of infection. Short distance movement of translocate from uninfected to infected centres of the leaf

has been demonstrated with radioactive labelled compounds (35, 98, 111, 119, 125, 129, 147). The effect of the rust infection on translocation patterns in the host can only be evaluated when the pattern of translocation in the healthy host is understood. The work of Doodson et al. (26, 27), Livne and Daly (67), Thrower and Thrower (126) and Pozsár and Király (90) on several rust infections and the studies of Gaunt and Manners (32) on wheat infected with loose smut contribute significantly to understanding the effect of these obligate biotrophs on host translocation patterns. Translocation patterns in the host appeared to be affected in two ways after infection of the leaves with rust. Firstly, infection of the presentation leaf alone resulted in a strong retention of assimilate (27) and secondly, an infected leaf could influence the translocation patterns of other leaves and attract assimilate to the site of infection (67). In subsequent work, however, Siddiqui and Manners (116) modified their previous conclusions (27) on studies of movement of ^{14}C-assimilate in spring wheat heavily infected with yellow rust on all the leaves. These results will be discussed in more detail.

Doodson et al. (26) supplied $^{14}CO_2$ to the third leaf of a series of spring wheat plants (Jufy 1) at different stages of development. Each leaf in turn (except the first) was supported during its early development by the assimilates from the lower leaves. The amount of translocate to the leaf rose to a maximum, which coincided with the maximum rate of expansion of the leaf and then declined as the rate of growth decreased and its own photosynthetic system started to operate. Upon

infection of the presentation leaf with urediniospores of Puccinia striiformis West. the assimilation of $^{14}CO_2$ by the leaf decreased to 43,5% of that of the healthy leaf and translocation to 2% of that of the control leaf. Over a period of 3 hours translocation of ^{14}C assimilates from the 3rd leaf was only 0,87% of that of the control leaf. The rust infection had no effect on the distribution pattern of ^{14}C to other leaves from one which had taken up $^{14}CO_2$. An infected leaf could not distort normal distribution of translocate by attracting assimilate from other leaves. In addition, the percentage of assimilates moving to the roots in the infected plant was greatly decreased, which correlated with observed decreases in root dry weight (27).

In later studies Siddiqui and Manners (116) inoculated all the leaves of spring wheat in succession with urediniospores of yellow rust. It was demonstrated that when nearly the whole plant was infected, the distribution pattern of ^{14}C-assimilates was different to their previous results (27) when only the presentation leaf was infected. The proportion of assimilate going to the roots and the tillers was reduced while the proportion of assimilate moving to the leaves was increased. The effect of heavy infection on the percentage of assimilate leaving the presentation leaf in these experiments was less marked than when the presentation leaf was the only leaf infected. These results agree with those of Livne and Daly (67) and Thrower and Thrower (126) in related studies.

Livne (66) found similar effects of retention of carbohydrates

in leaves of bean infected with Uromyces phaseoli (Pers.) Wint. var. typica Arth. However, in later work Livne and Daly (67) showed that infection on one leaf could influence the pattern of translocation from other leaves, in contrast to the results of Doodson et al., (27). In normal healthy plants a single unifoliolate leaf exported approximately 50% of ^{14}C to the roots, stem and young trifoliate leaves. When $^{14}CO_2$ was supplied to the first trifoliate leaves of healthy plants, ^{14}C moved into the stem, roots and youngest trifoliate leaf, but none into the unifoliolate leaves. In contrast, when bean plants were inoculated with U. phaseoli on the unifoliolate leaves and then supplied with $^{14}CO_2$, less than 2% of the ^{14}C moved out of the infected leaf. When $^{14}CO_2$ was supplied to healthy trifoliate leaves of plants containing infected unifoliolate leaves there was a marked increase of ^{14}C movement out of the trifoliate leaves, and accumulation in the unifoliolate leaves at the expense of young growing leaves.

Similar results were obtained by Thrower and Thrower (126) who measured the import of labelled translocate from the expanded third leaf of Vicia faba L. to the expanding fourth and fifth leaves when they were healthy or infected with Uromyces fabae (Pers.) de Bary. They found that when the expanding fourth leaf was infected, the time of import was prolonged and further import was maintained at a higher level. Simultaneously with increased translocation of assimilate into the infected fourth leaf, there was decreased translocation into the smaller healthy expanding fifth leaf. Infection of one leaflet with U. fabae also increased movement of labelled

assimilate to this leaflet from the healthy leaflet of the pair, even when it was fully expanded.

There may be limitations on the movement of translocate depending on the morphological arrangement of the vascular bundles, as in healthy tobacco (53). Export of ^{14}C-labelled assimilates from the fully expanded presentation leaf was determined by the pattern of vascular interconnections, which caused only 3% of exported assimilate to reach the upper leaves and stem apex. No activity appeared in leaves below the presentation leaf so that the balance of exported assimilate was retained by the stem and roots. The pattern of distributed radioactivity in the leaves was found to conform to the 5/13 phyllotactic system of Linum (34.). The translocation of ^{14}C-assimilate in healthy plants of Populus deltoides Bartr. (Eastern Cottonwood) has been studied during maturation of young leaves (23, 52, 58, 59). The influence of phyllotaxy on the distribution of ^{14}C in the leaves was investigated and the location of ^{14}C in various chemical fractions of the leaves was studied. These results are discussed further in Chapter 6.

The movement of translocate may be greatly influenced by differences in vascular structure and the presence of intercalary meristems (126) and by concentration gradients in the plant. The role of plant growth hormones should be considered since higher concentrations of auxin were found in rusted wheat tissue eleven days after inoculation than in comparable healthy tissue (109). Rust colonies normally produce a sink by maintaining

a concentration gradient of sucrose and by producing substances which promote movement in adjacent sieve tubes (126).

The terms "source" and "sink" were applied by Canny and Askham (14) to the model system of the plant as described by Mason and Maskell (74, 75) which consisted of the "source", the assimilatory surface of the leaves, and a conducting pathway through which assimilates move to the many "sinks", which are the meristems, fruits, storage organs and roots. The model distinguishes between the processes of carbon assimilation (or dry weight increase) and of growth (85). These terms are basically derived from the thermodynamic description of energy flow in a system which may be split into three parts: the source, the sink and an intermediate system whereby energy moves from the source to the sink (83). When a plant is infected with rust there is the creation of another active sink for assimilate (67) which may compete for assimilate with the normal sinks and may alter the pattern of distribution of assimilate to these sinks. According to Livne and Daly (67) individual organs such as rust-infected leaves may form a source-sink system comparable to the entire plant system.

The spores of rust pustules were shown to accumulate ^{35}S, ^{32}P and ^{14}C to a greater degree than healthy bean leaves (146), and in further work on Pinto beans, inoculated leaves accumulated starch upon uptake of ^{14}C-sucrose (147). Around isolated pustules up to three zones of starch accumulation were observed, whereas crowded pustules showed very little accumulation. Selective accumulation of ^{14}C or ^{32}P as a result

of translocation was more effective in rusted bean leaves than direct application of radiochemicals on the lamina surface (147). Microautoradiographs of leaves of Trifolium subterraneum L. infected by Uromyces trifolii (Hedw. f.) Lév., showed considerable accumulation of ^{14}C-compounds in the uredinia after a three hour incubation in $^{14}CO_2$ in the light (125). After a further twelve hours a marked annulus of accumulation developed around the uredinia, in a position corresponding to that shown by starch accumulation in some infections, but the uredinia at this stage and after a further twelve hours showed a low ^{14}C labelling. However, after 2, 3 and 4 days after removal from $^{14}CO_2$ the uredinia became far more labelled than the host leaf. Similar results were achieved for other obligate biotrophs and their hosts (106, 111, 130, 131).

The higher respiratory rates in infected compared with healthy tissues suggest that infected leaves may act as "respiratory pumps" which actively remove assimilate from normal translocatory channels and drain carbon from adjacent areas by mass flow (67). The mechanism of accumulation of compounds at the site of infection was found to be inhibited by anaerobiosis and respiratory inhibitors (111) which indicated that accumulation is an active, ATP-dependent process. Although the respiratory levels of infected tissue have been observed to increase concomitantly with accumulation in many rust infections (2, 91, 141) there are records of infection of cereal leaves without increased respiration taking place (76).

A number of methods (145) have been employed to separate the

contributions of host and obligate biotroph to increased respiration. These methods include killing the parasite in situ with chemicals (91) and making measurements beyond the region occupied by the pathogen (1), as in Fusarium-infected tomato plants where the leaf blades were not directly invaded by the organism, and leaf respiration was observed to increase after infection (19). Both healthy and rusted bean leaves were heated at 45°C for 90 seconds to kill the pathogen without injuring the host (142). By comparing leaf temperatures of both treated healthy and rusted leaves it was shown that killing the pathogen did not greatly reduce infected leaf temperature. It was suggested that the increase in temperature of rusted leaves was primarily due to enhanced host repiration. Allen and Goddard (2) had previously drawn similar conclusions from experiments in which they mechanically removed ectoparasitic powdery mildew fungi from the host. However, in later work Bushnell and Allen (10) amended these results by reporting that the respiration of the mildew fungus alone was 7 to 10 times that of healthy tissue, whereas the respiration of the infected host was 2 to 3 times that of non-infected tissue (10, 100, 103).

There is not only a change in respiratory rate upon infection by an obligate biotroph since there appears to be a qualitative change in the pattern of respiration from a pathway mainly channelled through glycolysis and the Krebs cycle to one involving a pentose phosphate system (56, 62, 70, 71, 112). The participation of this pathway was suspected (5) when large increases in respiration rates at times of sporulation

were found to be characterized by a decline in C_6 / C_1 ratios. All the enzymes of this pathway were found in germinating spores of wheat rust, with the possible exception of transaldolase (115).

In green plants the reducing power is supplied by the production of reduced co-enzymes in photosynthesis. Since precursors of polyol synthesis may be intermediates in the pentose phosphate pathway (51) the production of polyols by the action of NADP-dependent polyol dehydrogenases may provide a source and store of reducing power for the obligate biotroph (21, 62). At the site of infection the presence of acyclic polyols and trehalose was first demonstrated in safflower and bean infected with Puccinia carthami L. and Uromyces phaseoli (Pers.) Wint. respectively by Daly et al. (21). In healthy tissues sucrose, glucose and fructose were the only soluble carbohydrates to become labelled whereas in diseased tissues three compounds identified as trehalose, mannitol and arabitol also became radioactive. Most rust infections have been shown subsequently to accumulate similar products at the site of infection. The existence of dehydrogenases which convert pentoses and heptuloses to corresponding alcohols has been demonstrated (139). Mitchell and Shaw (79) showed that flax infected with Melampsora lini (Ehrenb.) Lév. utilized the pentose phosphate pathway by obtaining low C_6 / C_1 ratios and labelling in mannitol, arabitol and trehalose.

The increased levels of phosphorus at the infection court and the stimulation of host cell growth by many obligate biotrophs

are evidence for an increased utilization of energy-rich phosphates, causing increased respiration. Greater hexokinase activity could have this effect and would lead to increased formation of glucose-6-phosphate (41). Lunderstädt (71) found a large post-infectional increase in the activity of hexokinase in wheat infected with Puccinia graminis f. sp. tritici Erikss. and Hen., race 5b. Sucrose dissimilation in the host/biotroph complex included mannitol and trehalose. The rapid appearance of label in these compounds in infected leaves soon after feeding with ^{14}C-sucrose indicates that the metabolic steps leading to these compounds may be related to hexokinase activity. There is also a great increase in invertase activity, which is of interest since the action of invertase provides the substrates for hexokinase activity. The participation of invertase in the provision of substrate for polysaccharide accumulation around the infection sites of the biotroph (69) has been described.

Changes in the metabolism of starch have been observed during the initial stages of pathogenesis (77, 101, 102). Using histo-chemical methods Schipper and Mirocha (101) showed that in Phaseolus vulgaris L. infected by Uromyces phaseoli var. typica two phases of starch metabolism occurred. Within 24 hours after inoculation there was a depletion of starch in tissues close to the site of penetration of the germ tube. Subsequently starch accumulated around the pustules prior to sporulation. While investigating mechanisms of starch depletion in the same host/pathogen combination, Schipper and Mirocha (102) detected a dialysable and relatively heat-stable activator of host β-amylase in aqueous extracts of disrupted

uredospores. Accumulation of starch around the site of infection is frequently accompanied by the formation of "green islands" in the same zone due to chlorophyll retention in chlorotic diseased tissue (11, 132). The "green islands" were shown to correspond to the region of accumulated radioactivity (124, 132). Although the region around the lesion was metabolically more active than uninfected tissue, the influence of the parasite did not become apparent until the surrounding tissue was senescent. "Green islands" appeared to prolong the life of the host in these regions by means of some local effect of the fungus on immediately adjacent host cells. This effect may be due to hormones such as auxins, for example indoleacetic acid (109). Cytokinins are also believed to be involved (90) since pre-sporulation changes in rust infected tissues are very similar to changes in leaf tissue produced by treatment with cytokinins (11). The formation of "green islands" is regarded by Lewis (63) as a tendency towards a mutualistic symbiotic state where the fungus contributes some factor to maintain the vitality of the host tissues.

Photosynthesis is usually reduced in diseased tissue (15, 57, 66). There are, however, several recorded cases of increased photosynthesis (3, 66, 143), although this is limited to the early stages of infection. Increased photosynthesis is most commonly found in plants infected with rust fungi, which may be further evidence for the benign nature of some rust infections (145).

The preceding review has shown that several characteristic symptoms are shared by most of the rust infections studied previously. The more detailed investigations have been concerned with pathogens of agriculturally important plants which usually elicit a strong reaction from their susceptible hosts, but a few comparative studies of host/parasite interactions with susceptible and resistant hosts are recorded (100, 101, 108, 112, Maclean (1972) in 104). During the initial stages of infection susceptible hosts tend to tolerate their respective obligate biotrophs and a certain degree of compatibility may exist until the pathogen becomes established in its host (124). There are no long-term studies of compatible relationships where the unharmed host controls the virulence of the pathogen by a degree of resistance. Although these relationships are known to exist in natural populations (97), they have been largely overlooked.

Investigation of a poplar rust association occurring abundantly in the South Western Cape was initiated by the observation that in spite of repeated seasonal infection, the rust did not cause any apparent damage to the host tree, apart from occasional premature leaf fall. Of further interest is the evident restriction of the rust to the urediniospore stage on poplar, since no other stages of the life cycle have been previously recorded in this area or discovered during this study. The interaction between the apparently avirulent rust fungus and its host will be studied and the results will be compared with the evidence of other rust infections showing a reaction between a susceptible host and virulent pathogen.

CHAPTER 2

MATERIALS

The host plant, Populus canescens (Aiton) Sm., is a deciduous tree of Northern European origin which is widely distributed in the South Western Cape. It is planted as an ornamental in gardens and parks and grows extensively in forests and along river banks in the Peninsula. Although the tree is deciduous, the mild climate permits the retention of leaves for a longer period than is normal in harsher temperate climates. In some poplar stands the leaves remain on the trees throughout winter and new shoots may be produced at frequent intervals. Other populations lose all their leaves in autumn (May/June) and regain their foliage in spring (September).

The rust which infects P. canescens is provisionally classified as Melampsora sp. and occurs in the uredinial stage only. The description of the uredinial stage (to be described in Chapter 3) agrees closely with the same characters described for the uredinial stage of Melampsora populnea (Pers.) Karst. (138). In Europe M. populnea occurs on both Populus alba L. and P. tremula L. in both uredinial and telial stages (138). M. populnea includes several races which do not vary to a great extent in morphological characters but possess different aecial hosts. Pycnial and aecial stages are found on Larix, Pinus

and Mercurialis. Since telial and aecial stages of the rust under investigation have not been found in the South Western Cape, further characterization was not possible.

In the South Western Cape area the rust has been recorded on various species of Populus and more specifically on Populus alba L. in Natal and the Transvaal (25). Doidge (25) suggested that this rust be named Melampsora aecidioides Schroet (sensu lato) as its life history was not completely understood. Material sent to Dr. John Gremmen, Wageningen, Netherlands, was identified as Melampsora aecidioides (D.C.) Schroeter. Specimens of rust-infected poplar have been deposited for future reference with the Commonwealth Mycological Institute, Kew, Surrey, U.K. and the National Herbarium, Plant Protection Research Institute, Pretoria.

During this investigation the uredinial stage of a Melampsora sp. has also been observed on Populus nigra L. var. italica Du Roi, a semi-deciduous form of this species which is a native of central and Southern Europe. This tree, known commonly as the Lombardy poplar, is widespread in the South Western Cape and the rust has a damaging effect on the host, eventually causing death. Another common host with the uredinial stage of Melampsora sp. is the Chilean poplar, Populus nigra L. var. chilensis.

Infected leaves of P. canescens have been observed throughout the year, since complete leaf fall may not always occur. Old leaves may still remain on the plant when new shoots are produced in spring and as a result these new leaves may become infected. However, some populations shed all their leaves

in winter and the new leaves produced in spring are reinfected in February. Reinfection is possibly dependent on the chance deposition of urediniospores on healthy leaves. M. populnea (Pers.) Karst. is known to over-winter in poplar buds in central Europe (138). The possibility of this occurring in the Melampsora sp./P. canescens association has not been investigated.

Three populations of P. canescens were sources of material. The localities of the populations were (1) Cecilia Forest, 3318 CD Cape Town, (2) a vacant lot, Stegman Road, Claremont, 3318 CD Cape Town and (3) a vacant lot, Hohenort Estate, 3418 AB Simonstown. The proximity of these sites to one another is shown in Figure 1. The Cecilia Forest population, consisting of mature trees and sprouting stumps, covered a steep mountain slope. The trees were derived from a nearby riverside stand, and were dominated and partially shaded by mature pine trees (Pinus pinaster Aiton). There was little ground cover due to the dense mat of pine needles. The vacant lot in Claremont contained P. canescens saplings situated adjacent to the railway line. The site was level, densely weed-covered and in a sunny aspect throughout the year. The vacant lot population in Hohenort Estate was dominated by mature oak trees (Quercus robur L.) and was consequently shaded most of the day. The site was overgrown with Vinca and Convolvulus species and was situated on a slight slope. Both vacant lot populations were derived from trees in gardens in the vicinity.

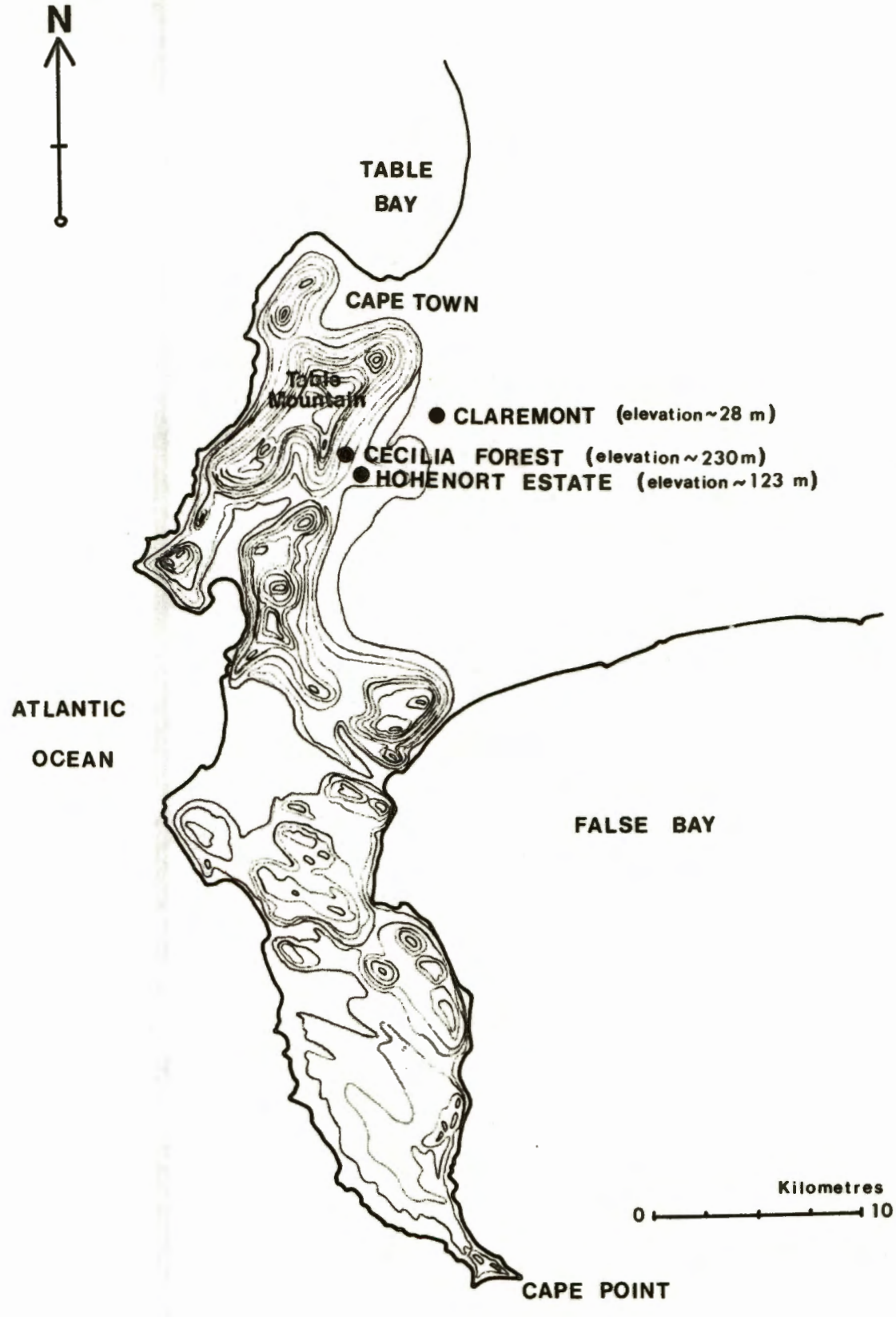


Figure 1:

Map of the Cape Peninsula showing the position of the three sampling localities.

Most of the experimental work was performed on material collected from the Hohenort Estate population which was the least disturbed of the three sites. Young poplar saplings (about 3 metres high) or sprouting stumps were chosen for study since material was collected more easily from these than from fully grown trees. The incidence of infection among stands of poplars is high, rising to a peak of infection during April and May.

CHAPTER 3.

ANATOMY

Anatomical studies of the pustule region and the region immediately adjacent to the lesions of rust infected poplar leaves were undertaken to determine the extent to which the mycelium ramifies in the host. Intercellular hyphae may either be confined to the pustule and tissue immediately underneath or ramify into the region adjacent to the pustule. An anatomical study of the uredium was made in an attempt to classify the rust.

Rust infected poplar leaves were cut into sections approximately 100 µm thick with a hand microtome. The sections were cleared with chloral hydrate and stained with cotton blue in lactophenol according to the method of Shipton and Brown (113). This method was applied to whole leaves and to leaf sections, but in both cases the stain was not retained by the fungal tissues. However, chloral hydrate cleared the tissue to such an extent that unstained hyphae and haustoria were clearly observed. The sections were viewed under oil immersion using phase contrast microscopy.

The uredinial characters are described as follows. The uredosori are yellowish-orange, pulvinate, pulverulent and hypophyllous, occurring under the dense mass of epidermal hairs on the under-surface of the leaf. The pustules are usually discrete, but may cluster densely when there is heavy infection (Plate 1) and they may cause chlorosis on the upper surface of the leaf. The diameters of the uredosori range from 1 - 3 mm. Uredoparaphyses occur, but these are not easily distinguished from developing urediniospores. The urediniospores (Plate 3 (a)) are ovoid to obovoid, 15 - 17 µm wide and 17 - 25 µm long with a wall 3 - 4 um thick. Small spines 2 - 3 um apart are distributed over the entire surface, as shown in Plate 3 (b).

Haustoria (Plate 4) occur in host tissue beneath the pustule, the number of haustoria decreasing from the lower to the upper leaf surface. There were usually up to two haustoria in the cells observed. Intercellular hyphae (Plate 4) were also seen in greater abundance near the pustule than the upper leaf surface.

No haustoria or intercellular hyphae were present in the region adjacent to the pustule, as shown in Plate 5.

In was concluded that the rust fungus was confined to the pustule and penetrated the spongy mesophyll of the leaf immediately under the pustule. Some penetration of

haustoria and hyphae into the upper palisade tissue was evident. The infection is superficial and localized, and most of the pustules appeared to be separate from the others on the leaf. After incubation in the dark, no green islands were observed at any stage of infection. There were no lesions on the petioles of infected leaves.

Plate 1:

Undersurface (L) of the leaf showing high density of uredosori. Yellow flecking on the upper surface (U) indicates the presence of uredosori on undersurface (x 7/10)

Plate 2:

Undersurface of poplar leaf showing low density of uredosori (us) (x 2).

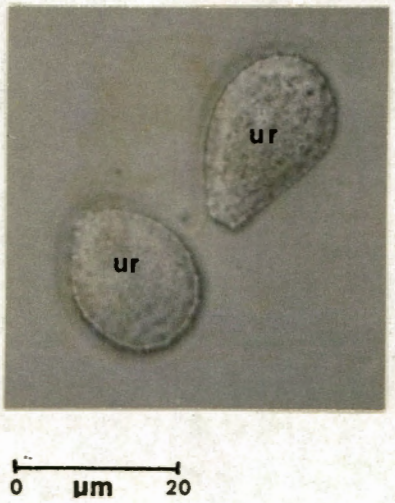


Plate 3 (a) : Structure of urediniospores.

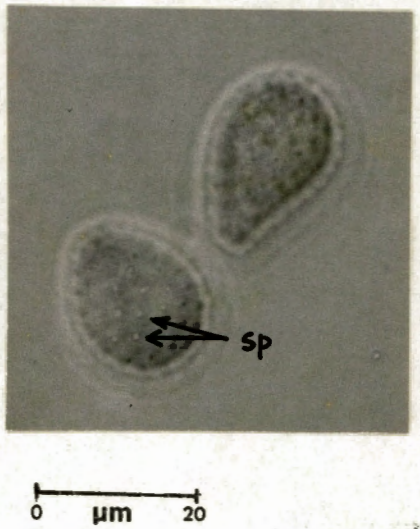


Plate 3 (b): Spines (sp) of urediniospores.

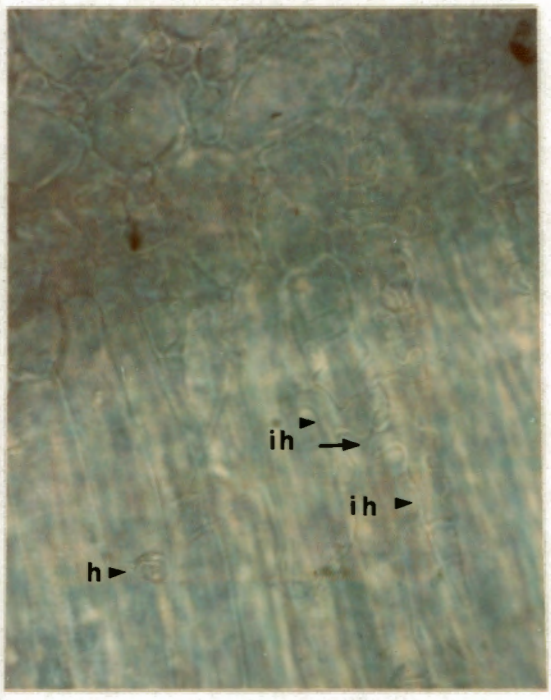


Plate 4:

T/S infected leaf showing intercellular hyphae (ih) and haustoria (h) (x 1000).

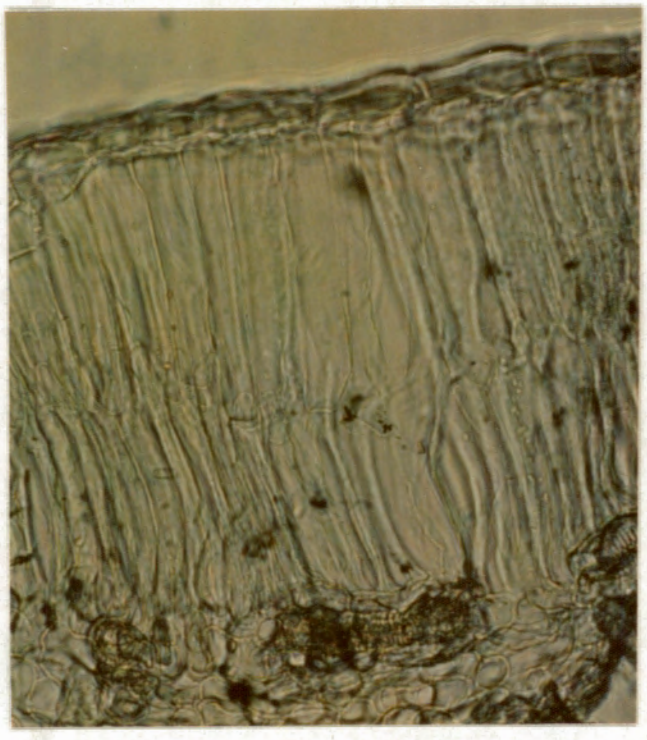


Plate 5:

T/S region adjacent to the pustule in the infected leaf showing the absence of haustoria (x 400).

CHAPTER 4.

SEASONAL VARIATION IN PUSTULE DENSITY, DRY WEIGHT AND TOTAL NITROGEN

4(1) INTRODUCTION

Infection of the host plant by rust fungi is known to cause increases in nitrogen content (13, 108) and dry weight of the infected regions (33, 100, 108, 141, 144) and the increased dry weight has been correlated with the development of pustules in the early stages of infection (44).

The effect of the poplar rust on dry weight and total nitrogen levels in the host was studied over two or more seasons and these results were compared with the increase in pustule density as the season progressed.

4(2) SAMPLING METHODS

A standard sampling procedure was employed in these investigations and those to be described in the following

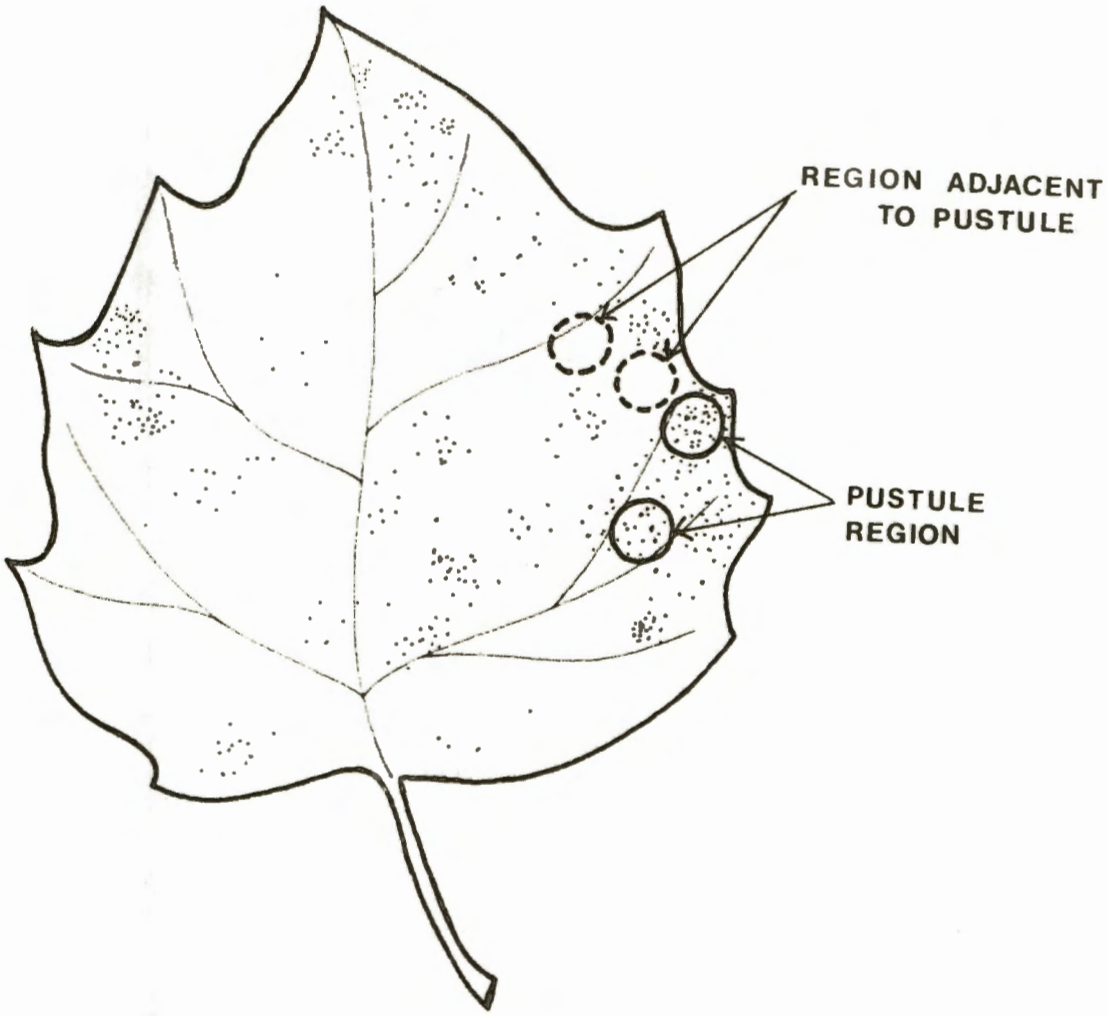


Figure 2:

Method of sampling the infected lamina showing the selection of pustule region and region adjacent to the pustule.

chapters. Mature, fully expanded healthy and rust-infected leaves of a similar age were collected from branches of saplings or sprouting stumps. The leaves sampled were usually the 6th, 7th and 8th from the shoot apex.

Leaves were divided into lamina and petiole samples and the healthy lamina was sampled from the areas between the main veins with a sharp 0,55 cm diameter cork borer. The lamina of infected leaves was divided further, as shown in Figure 2, into pustule region and the apparently uninfected regions adjacent to the pustule. The anatomical studies described in chapter 3 showed that the region immediately adjacent to the pustule was unoccupied by fungal hyphae. Samples from the region adjacent to the pustule were either selected from uninfected areas immediately adjacent to the lesions or from clear areas in the midst of dense groups of pustules. The pustule region was usually sampled from areas showing the highest pustule density.

4(3) PUSTULE DENSITY

The development of infection as reflected by the increase in pustule density was observed during two seasons, 1975 and 1976, on poplar leaves of the Hohenhort Estate population. Erupting uredosori were first observed in mid-January, 1975, and early January, 1976. Samples of 10 to 20 leaves were collected at weekly or fortnightly intervals until mid-May, 1975, and the end of March, 1976, by which time the highest density of infection had been attained. Infection density was low during the first three weeks of 1975 and 9 weeks of 1976 and infected leaves were non-randomly selected from various saplings in the population.

During the periods of low pustule density the level of infection was estimated by selecting up to ten 1 cm^2 areas of the leaves (depending on the size of each leaf) and counting the number of pustules in these areas. The mean number of pustules in 1 cm^2 leaf area was calculated and the standard error of the mean for each sample computed. The data for the periods of low infection density during 1975 and 1976 was plotted in Figures 3 (a) and (b).

The infection density had increased considerably after 5 weeks in 1975 and 9 weeks in 1976 and pustules became densely aggregated in patches (Plate 1). Since most of the mature leaves in the population were infected at this stage, it was possible to adopt a random sampling method. At each sampling period 15 to 20 leaves were collected randomly from the population. Since the infection density exceeded 30 or more pustules per 1 cm^2 of leaf area, it was not possible to count lesions in the densely infected patches. High infection density was represented by measuring the densely infected area of the leaf and expressing this value as a percentage of total leaf area. The method used was to outline the leaves on paper, indicating the extent of the infected area. The leaf outlines were cut out and weighed; the infected areas were then cut out and weighed, after which the % infected area was calculated. This method was not considered to be accurate, but was used in the absence of other methods for estimating high pustule density. The mean % leaf area was calculated for each sample, showing the standard error of the mean for each value, and these

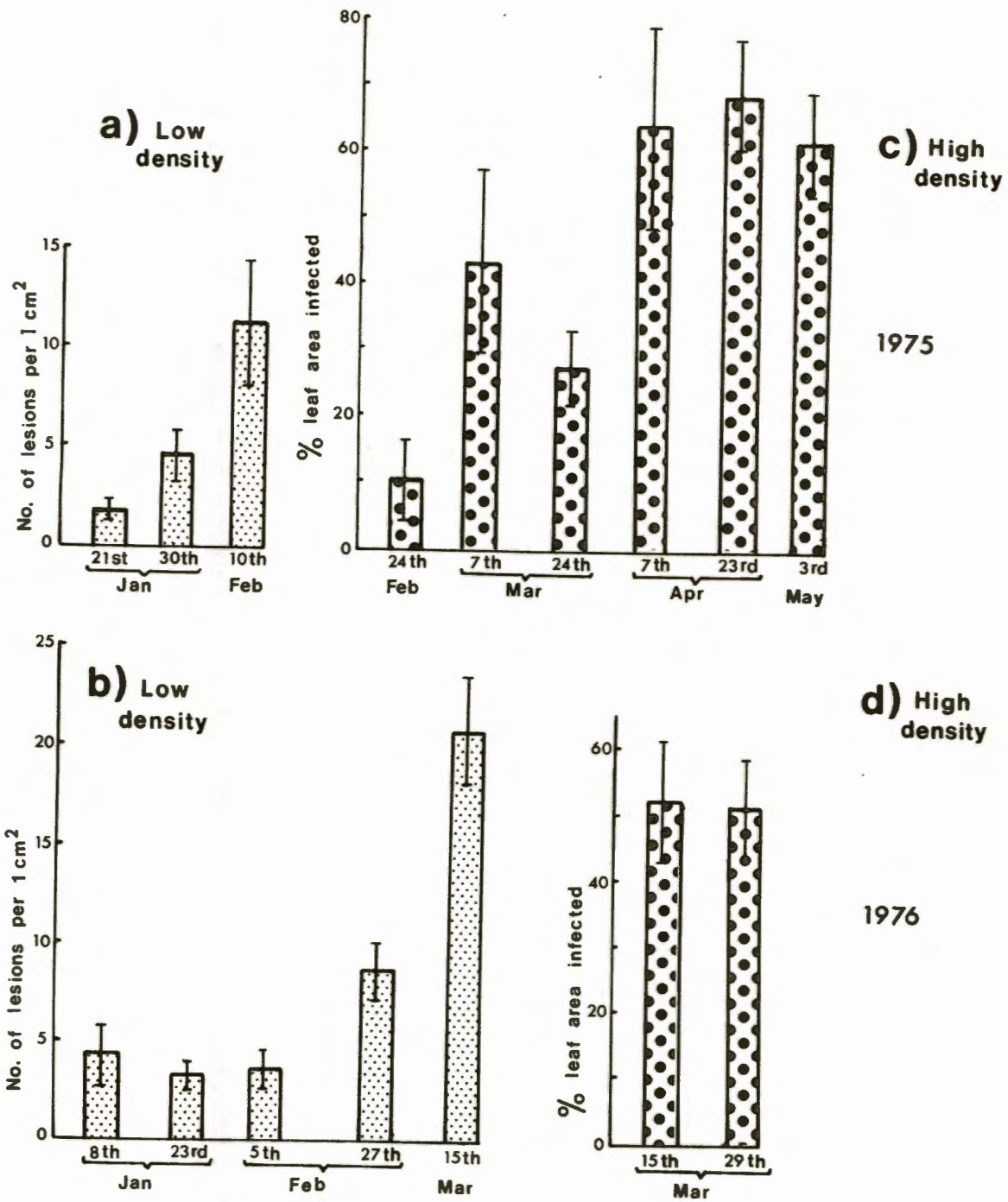


Figure 3:

Variation in pustule density during 1975 and 1976, showing low density of infection (a) and (b) expressed as the number of lesions per 1 cm^2 leaf area and high density of infection (c) and (d) expressed as % leaf area infected. Mean levels ± standard error of the mean are shown.

results were plotted in Figures 3 (c) and (d). The data shown in Figure 3 was tabulated in Appendix 1.

Results

Erupting uredosori were first observed during the first 3 weeks of January 1975 and 1976. Pustule density increased rapidly over a period of 3 weeks during 1975 whereas there was a more gradual development of infection over 9 weeks during 1976 (Figures 3(a) and (b)). During subsequent weeks lesions aggregated in patches or entirely covered the lamina (Plate 1).

During both seasons (Figures 3(c) and (d)) several of the values for % leaf area infected showed high standard error of the mean, demonstrating the variable occurrence of dense patches of infection in the population, where most leaves were infected to some degree.

While some leaves were completely covered with dense infection at the beginning of April, 1975, the average % area covered with dense infection increased to about 68% of the leaf area until mid-April (Figure 3 (c)). During May infection density decreased as senescence and leaf fall commenced. In this population most of the remaining leaves were yellow by the end of May and had fallen by early June. During March, 1976, the end of the 1976 study period, the average % area densely infected increased to about 59% of total leaf area, although many leaves were completely infected.

4(4) DRY WEIGHT

Leaves were collected at monthly or fortnightly intervals over the periods of October 1971 to May 1972, April 1973, to May 1974, and January to March 1976. The laminas were sampled as described in section 4(1), oven-dried at 80°C for 18-24 hours and then weighed. During 1976 the variation in dry weight within samples was estimated by taking 4 replicates and calculating the standard error of the means.

The dry weights of lamina samples from the 1971/1972 and 1973/1974 series were expressed in terms of unit fresh weight (Figures 4(a) and (b)). However, subsequent studies during 1976 (Figure 4(e)), showed that the fresh weight of healthy and infected tissues declined gradually towards the end of summer reflecting a water loss during this period prior to the start of senescence. The use of fresh weight as a parameter of comparison may therefore be misleading, since a decrease in fresh weight levels during the season may increase the calculated dry weight beyond the actual values. To test the validity of dry weight data expressed as a function of fresh weight, the dry weights of the 1976 samples were expressed in terms of both fresh weight and area (Figure 4(c) and (d)) and the two sets of data were compared. To test the significance of the difference between the three means of each fortnightly sample during 1976, one-way analysis of variance was performed and significance expressed to a 0,05 level. All data shown in Figure 4 are tabulated in Appendix 2.

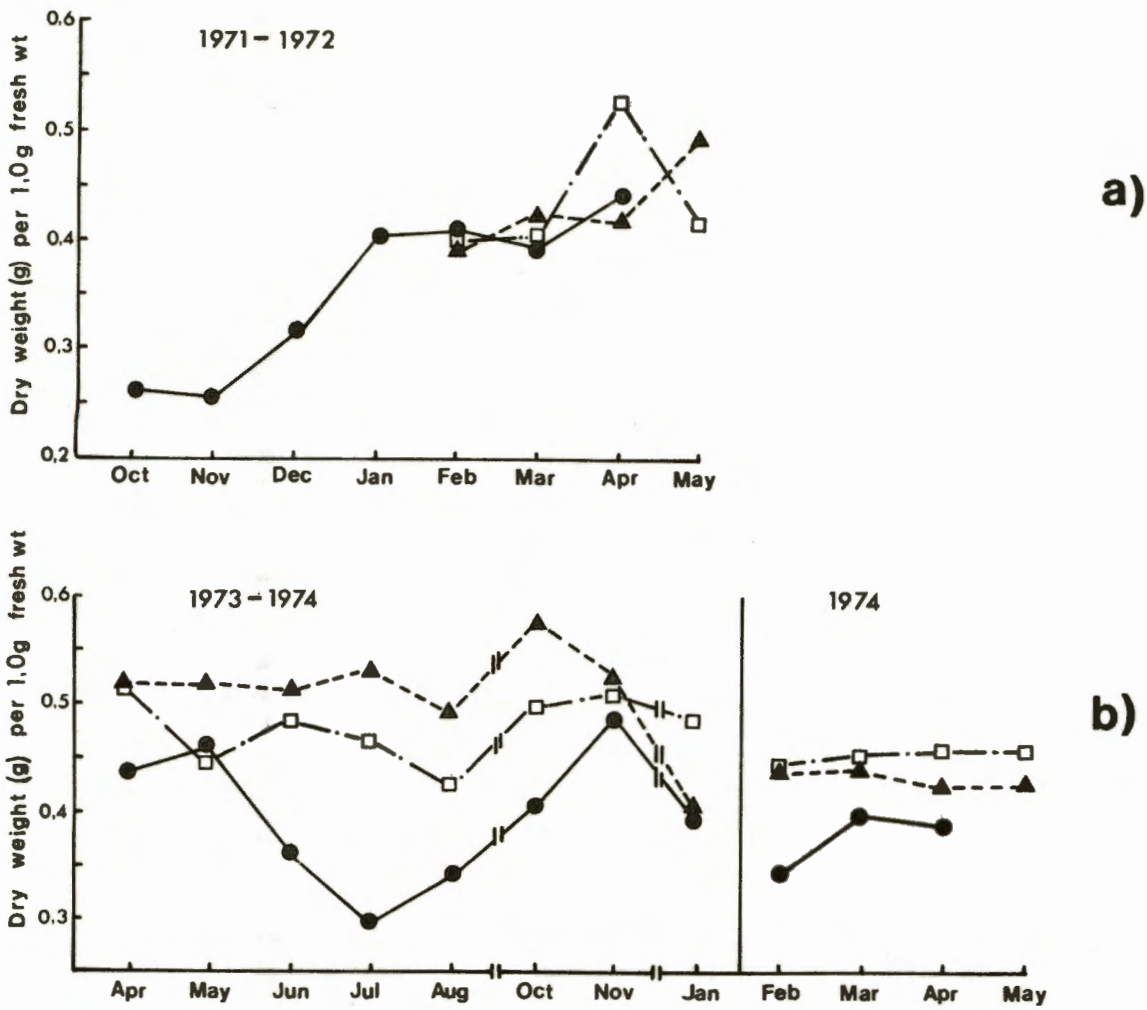


Figure 4:

Seasonal changes in dry weight in healthy and rust-infected poplar leaves during (a) 1971 - 1972 (Cecilia Forest Population) and (b) 1973 - 1974 (Claremont Population) and 1974 (Hohenort Estate Population). Dry weight shown in terms of 1,0 g fresh weight.

Key: ●——● healthy; ▲- - -▲ pustule; □—·—□ region adjacent to pustule.

CHANGES IN DRY WEIGHT DURING THE SEASONS 1971/1972 AND 1973/1974.

During 1971/1972 the healthy lamina (Figure 4(a)) showed distinct increases in dry matter, attaining a peak value in January as the leaves matured from spring (October) to mid-summer (January). After initiation of sporulation in February an apparent increase in dry weight was observed in the infected laminas during April and May.

During the 1973/1974 season (Figure 4(b)) the healthy lamina showed two peaks in dry weight values, the first in autumn (May) by which time leaves were senescing in most populations, and another peak in early summer (November). This population was unusual in that leaves were retained throughout the winter (June to August) and the infection was continuous for 7 months, possibly a result of the mild winter in 1973.

The dry weight increment in the pustule samples during this season was greater than during 1971/1972. During both seasons, however, the pustule and region adjacent contained greater dry weight than the healthy lamina.

CHANGES IN DRY WEIGHT DURING 1976.

When dry weight was expressed in terms of fresh weight (Figure 4(c)), both healthy and infected tissue showed a gradual increase in dry weight, the infected tissues having

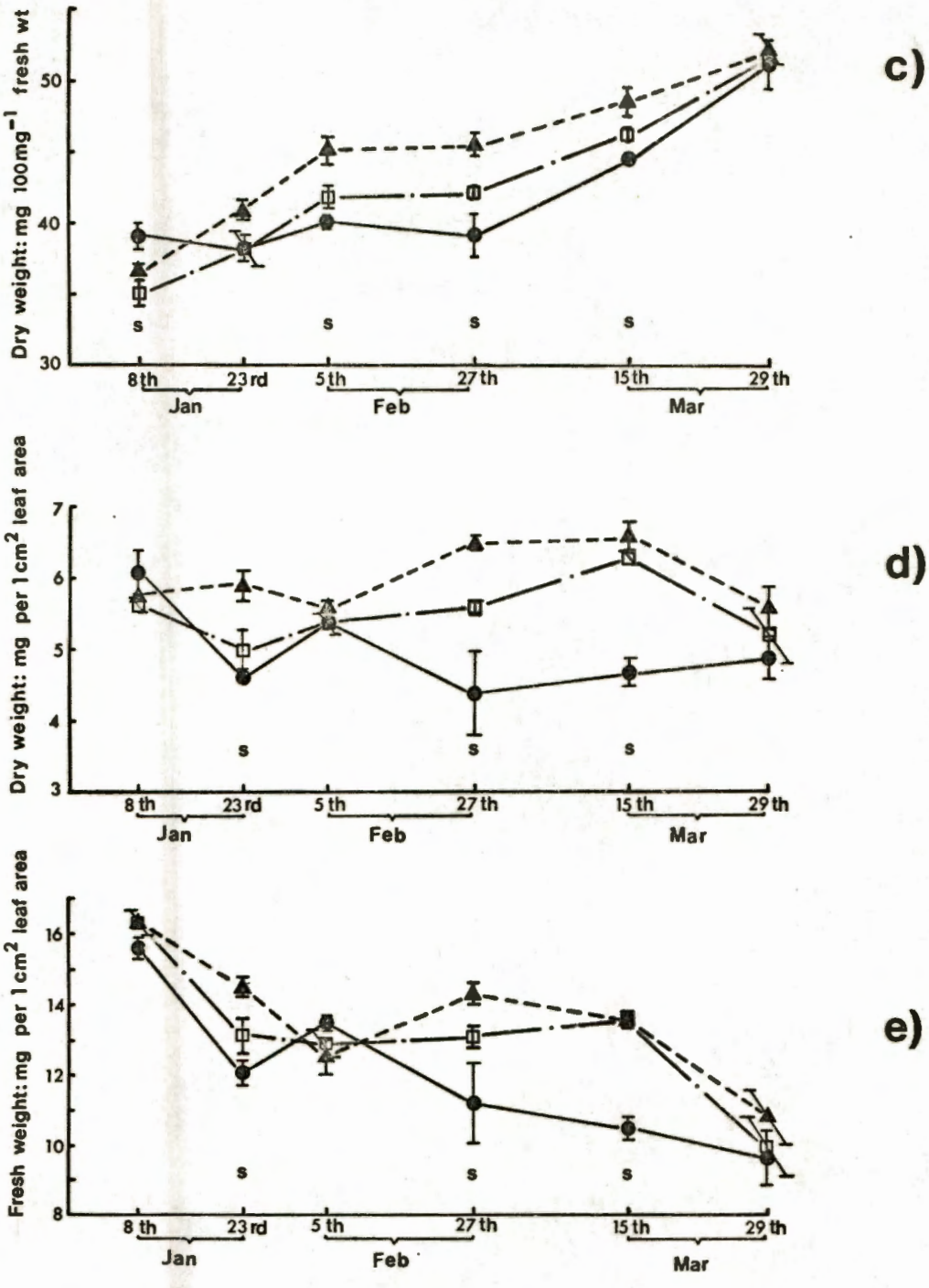


Figure 4: (Continued)

Seasonal variation in fresh and dry weight in healthy and rust-infected poplar leaves during 1976 in the Hohenort Estate population. Dry weight expressed as (c) mg 100 mg^{-1} fresh weight and (d) mg per 1 cm^2 leaf area. Fresh weight (e) expressed as mg per 1 cm^2 leaf area. Values shown are means of 4 replicates ± standard errors of the mean. Samples showing significant differences between the means at the 0,05 level are denoted by s.

Key: ●——● healthy; ▲----▲ pustule; □—·—□ region adjacent to pustule.

significantly greater dry weight than healthy tissues from 5th February to the 15th March, during the large increase in pustule density.

When expressed in terms of unit area (Figure 4(d)), the dry weight of healthy leaves showed no marked trends during the season, while the small increase in dry matter of infected tissues was more gradual than the trend shown on a fresh weight basis (Figure 4(c)). Corresponding with the period of increasing pustule density, infected tissues showed significantly higher dry weights per unit area than healthy tissues on 23rd January, 27th February, and 15th March.

The changes in fresh weight on an area basis during 1976 (Figure 4(e)) showed that the fresh weight of healthy tissues decreased, but that infected tissues retained a higher fresh weight which declined slowly during the season.

Dry weight data expressed on a fresh weight basis reflected the gradual decline in fresh weight which was due to lowering of the water content in all tissues. This trend was noted in a slight exaggeration of the increase in dry weight in healthy and infected tissues during March, 1976. Similarly, certain high dry weight values, April/May 1972, July to October 1973, and April/May, 1974, (Figure 4(a) and (b)), occurring at the end of the respective seasons, are clarified. Nevertheless, when dry weight changes are expressed in terms of both fresh weight and unit area,

it is evident that rust-infected tissues have higher dry weight levels than healthy tissues, and 1976 results show that the dry weight increment in infected tissues is significant mainly during periods of intense sporulation.

4(5) TOTAL NITROGEN.

The oven-dried lamina samples of the 1973/1974 and 1976 samples were analysed further for total nitrogen content by means of the micro-Kjehldahl method. Each sample (200-300 mg dry weight) was crushed with mortar and pestle and digested with 4 ml of concentrated sulphuric acid and half a pellet of selenium catalyst for 2 hours, by which time the digest had cleared. The digest was then diluted with about 5 ml of distilled water and was distilled into 4-12 ml of N/50 hydrochloric acid with 2 drops of Tshiro's indicator. The amount of nitrogen in each sample was determined by back-titrating with N/200 sodium hydroxide and calculated as µg N per 100 mg sample.
The levels of nitrogen in the healthy and infected lamina of the 1973/1974 season were plotted as µg N per 100 mg dry weight in Figure 5 (a). The samples from the 1976 season were plotted as µg N per 50 mg dry weight in Figure 5(b) and as µg N per unit area in Figure 5(c), showing the standard error of the mean for each sample. One-way analysis of variance was performed on the samples of the 1976 season, showing significance to the 0,05 level between the means within each fortnightly sample. The results were tabulated in Appendix 3.

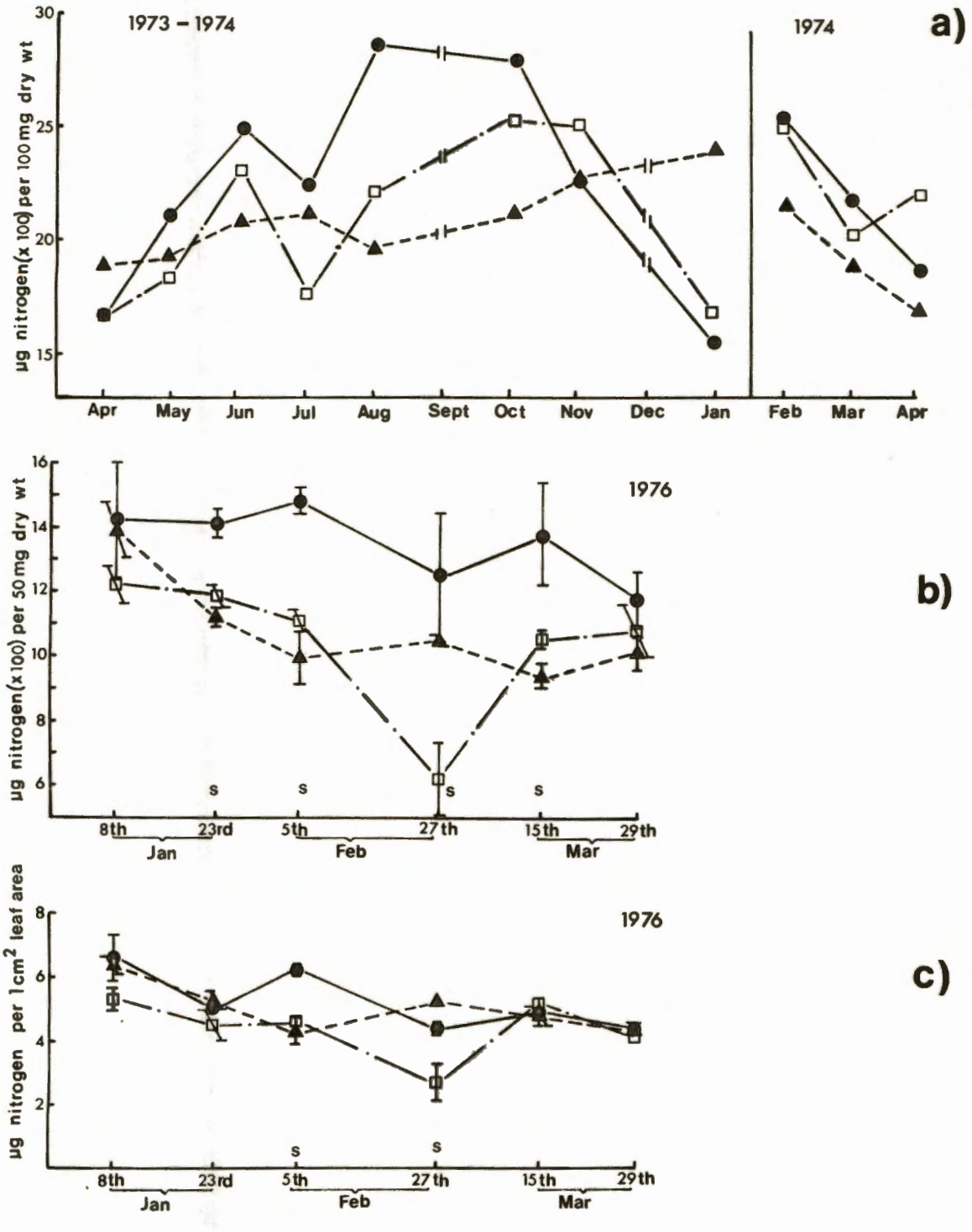


Figure 5:

Seasonal changes in total nitrogen.

(a) 1973-1974 (Claremont and Hohenort Estate populations), total N expressed as µg N per 100 mg dry weight.

(b) 1976 (Hohenort Estate population), total N expressed as µg N per 50 mg dry weight.

(c) 1976 (Hohenort Estate population), total N expressed as µg N per 1 cm^2 leaf area.

The values shown in (b) and (c) are the means of 4 replicates ± standard error of the means. Samples showing significant differences between the means at the 0,05 level are denoted by s.

Key: ●——● healthy; ▲- - -▲ pustule; □—·—□ region adjacent to pustule.

The results for the 1973/1974 season indicated that the total nitrogen levels in the pustule seldom exceeded the levels in the region adjacent or the healthy lamina. During April 1973, and January 1974, the pustule contained higher levels of total nitrogen, but during most of the 1973/1974 season the region adjacent and healthy lamina contained higher total nitrogen levels than the pustule. There did not appear to be a distinct seasonal pattern of fluctuation of total nitrogen levels in the healthy lamina.

Similarly during 1976 the healthy lamina contained higher total nitrogen levels than infected tissue when results were expressed on both a dry weight and unit area basis. When total nitrogen levels were expressed in terms of dry weight, the healthy lamina contained significantly greater amounts of nitrogen than infected tissue from 24th January to 15th March. Total nitrogen levels on a unit area basis in the healthy lamina were significantly different from levels in infected tissues only during February. Levels of nitrogen in all tissues appeared to decline gradually towards the end of the season, corresponding with decreasing fresh weight values. This trend was enhanced when results were expressed in terms of unit area.

4(6) CONCLUSIONS

Increased dry weight in poplar leaves was observed to coincide with the appearance of pustules. As the incidence of infection increased, levels of dry weight

greater than in healthy tissue were maintained in the lesion and the region adjacent throughout the period of infection. Similar results were obtained by Holligan et al. (44) who showed that as pustules developed there was a progressive accumulation of dry matter, particularly ethanol and water - soluble substances, in diseased areas. While dry weight increases were observed in infected tissues, total nitrogen levels were not correspondingly greater in these areas. Total nitrogen levels were nearly always higher in healthy lamina samples than in infected tissues. The low nitrogen levels in pustule samples may have been due to the continuous loss of nitrogen as urediniospores were dispersed. The highest levels of total nitrogen in pustule samples were observed during January, 1974, and January, 1976, as uredosori appeared on the leaf surface.

These results are in agreement with the data of Calonge (13) since barley seedlings infected with black rust (Puccinia hordei Otth.) showed higher levels of nitrogen in the lesion area up to 6 days after inoculation, after which levels declined below those in healthy tissue. Similarly the results of Shaw and Colotelo (108) for susceptible (Little Club) and resistant (Khapli) strains of wheat infected by Puccinia graminis tritici (Race 15B) showed that in the susceptible host (Little Club) total nitrogen levels and dry weight in the lesion area increased dramatically from 6 days after inoculation, whereas the resistant host (Khapli) showed an initial increase in nitrogen and dry weight very soon after infection, after which levels declined.

CHAPTER 5.

SEASONAL VARIATION OF CARBOHYDRATES

5(1) INTRODUCTION

In the South Western Cape healthy leaves of *P. canescens* remain on the branches for longer periods than in the northern hemisphere (see Chapter 2), but infection with the rust, *Melampsora sp.*, may cause premature leaf fall. As rusted leaves of *P. canescens* can be found around Cape Town during most of the year, a seasonal study of the carbohydrates of healthy and rusted leaves could be undertaken. Studies of this nature are lacking in the literature, although there is some important work on the effects of rust and smut infections on wheat during a complete growth cycle of the host (27, 32). There are no recorded investigations of natural populations of perennial plants which are regularly infected by rusts and apparently remain unharmed by the repeated infection.

Seasonal variation of carbohydrates in fruit trees and crop plants has been investigated but the literature is too extensive to be comprehensively reviewed in this thesis. Studies have been undertaken on the seasonal changes in the

carbohydrates of wheat leaves (6), subterranean clover leaves (38), alpine tundra plants (24, 82) and apple trees (37, 92, 99, 121, 134, 135).

Although sucrose is known to be the major carbohydrate which is translocated in most higher plants (120), a translocatory rôle for sugar alcohols in several fruit trees has been proposed. Much attention has been directed towards the carbohydrate composition and seasonal fluctuation in some apple cultivars of Malus domestica L. and Malus pumila and other members of the Rosaceae and sorbitol, as well as sucrose, was found to be the major component translocated in the sieve tubes (133, 135).

The seasonal study of P. canescens in a healthy and rusted condition was restricted to variation in carbohydrate levels in the leaves, since studies of sugar variation in other organs were beyond the scope of this investigation. Both qualitative and quantitative changes in carbohydrates were traced and populations were observed for morphological changes and discoloration in the leaves as infection density increased. It was intended that a single population should be sampled over more than one season, but this was prevented by deforestation and property development. The three populations of P. canescens which were used have been described in Chapter 2. The samples were collected from the following sites :-

1. Season 1 (1971-1972) : Cecilia Forest.
2. Season 2 (1973-1974) : Claremont.
3. Season 3 (1974) : Hohenort Estate.

5(2) SAMPLING PROCEDURES

Leaf samples were collected on sunny days at 11 00 to 12 00 hr at approximately monthly intervals and transferred as rapidly as possible to the laboratory where further sub-division of samples was performed. The leaves were divided into lamina and petiole samples and further sub-division of the infected lamina into pustule region and region adjacent to the pustule was performed as described in Chapter 4(2).

Since the rust fungus and its host could not be separated, a sample of purely fungal material was obtained by removing urediniospores with a pair of fine forceps.

Each sample of 1,0 - 2,0 g fresh weight (lamina) and 0,5 - 1,0 g fresh weight (petiole) was weighed and then immersed in boiling 80% ethanol to kill the tissues rapidly. In the gas liquid chromatography analyses of the carbohydrates, aliquots containing approximately 0,2 g fresh weight were used.

5(3) SAMPLE ANALYSIS

The carbohydrates were extracted from the plant tissues by means of three procedures which are outlined in Figures 6, 7 and 8. Some problems were encountered during analysis of ethanol-soluble samples and these are mentioned in Section 5 (6).

SAMPLE

Homogenised in 80% ethanol

|

Refluxed in 80% ethanol for 1½ hours

|

Centrifuged at 5000 x g for 20 minutes

|

Supernatant evaporated under reduced pressure

Sample taken up in distilled water

|

Partial deproteinisation

(by the modified method of Somogyi (118))

2 ml 5% $ZnSO_4$ and 2 ml 0,3 N $Ba(OH)_2$

Filtration

|

Deionised with mixed resins (Dowex-50W Hydrogen form, Strongly acid cation exchange resin and Dowex-1 basic anion exchange resin).

|

Freeze-dried

↓

ETHANOL-SOLUBLE CARBOHYDRATES

Figure 6:

Procedure of analysis of samples during Season 1 (1971-1972) from Cecilia Forest Station.

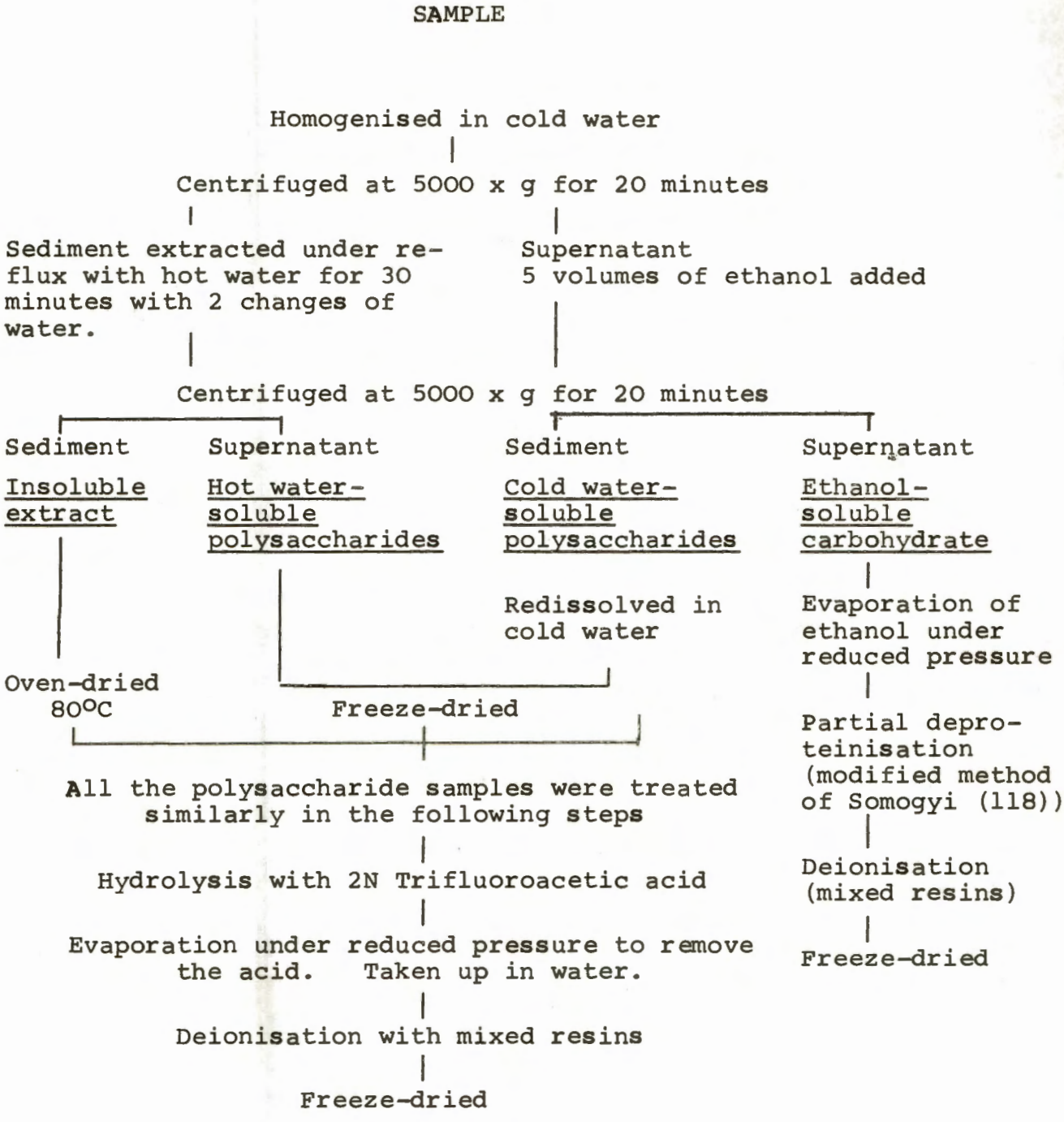


Figure 7:

Procedure of analysis of samples during Season 2 (1973) from Claremont, according to the method employed by Mitchell and Roberts (78).

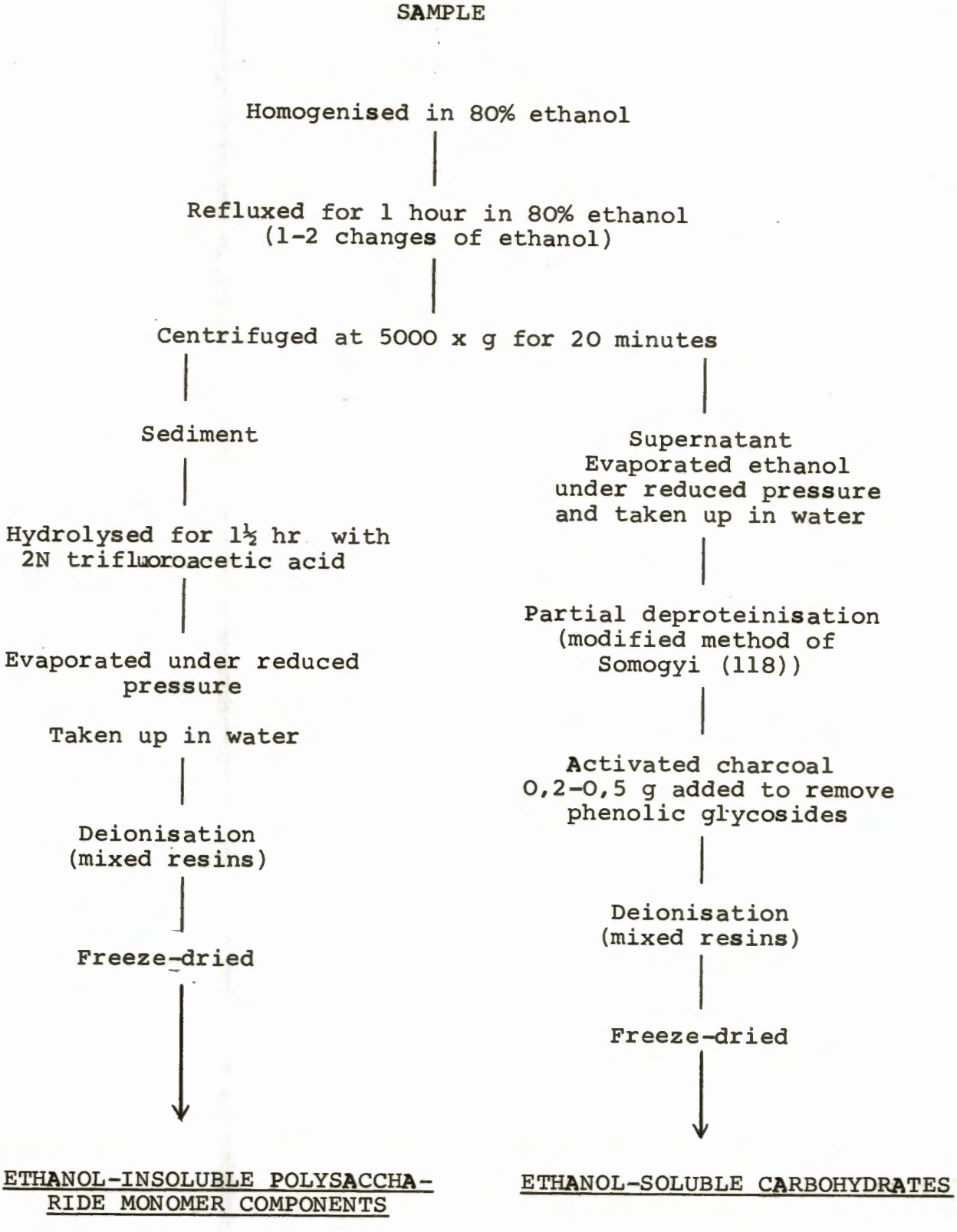


Figure 8:

Procedure of analysis of samples during Season 3 (1974) from Hohenort Estate.

Seasonal variation in the ethanol-soluble carbohydrates of lamina and petiole samples was studied during all three seasons. Levels of hot and cold water-soluble polysaccharides were studied during Season 2 and ethanol-insoluble polysaccharides during Season 3. All the polysaccharide fractions were hydrolysed in 2N trifluoroacetic acid in preference to 1,5N sulphuric acid. A comparison of the two methods of acid hydrolysis and reasons for the preference for trifluoroacetic acid are given in Section 5(4).

Samples were analysed for carbohydrate both quantitatively and qualitatively by GLC and further qualitative analysis was performed by means of paper chromatography.

5(4) METHODS OF ACID HYDROLYSIS

Sulphuric acid hydrolysis followed by neutralization which was used by Lewis and Harley (60) was considered to be cumbersome and time-consuming. Trifluoroacetic acid (TFA) was chosen as an alternative hydrolytic agent since this acid could be removed by evaporation under partial vacuum. The removal of sulphuric acid from samples by extraction with N,N-dioctyl-methylamine in chloroform (49) was not employed because the TFA method was considered to be less time-consuming. Both sulphuric acid and TFA are the least destructive of commonly used hydrolytic agents (49) and were equally satisfactory when applied to the samples under investigation. However, the suspected loss of some products of sulphuric acid hydrolysis during the subsequent

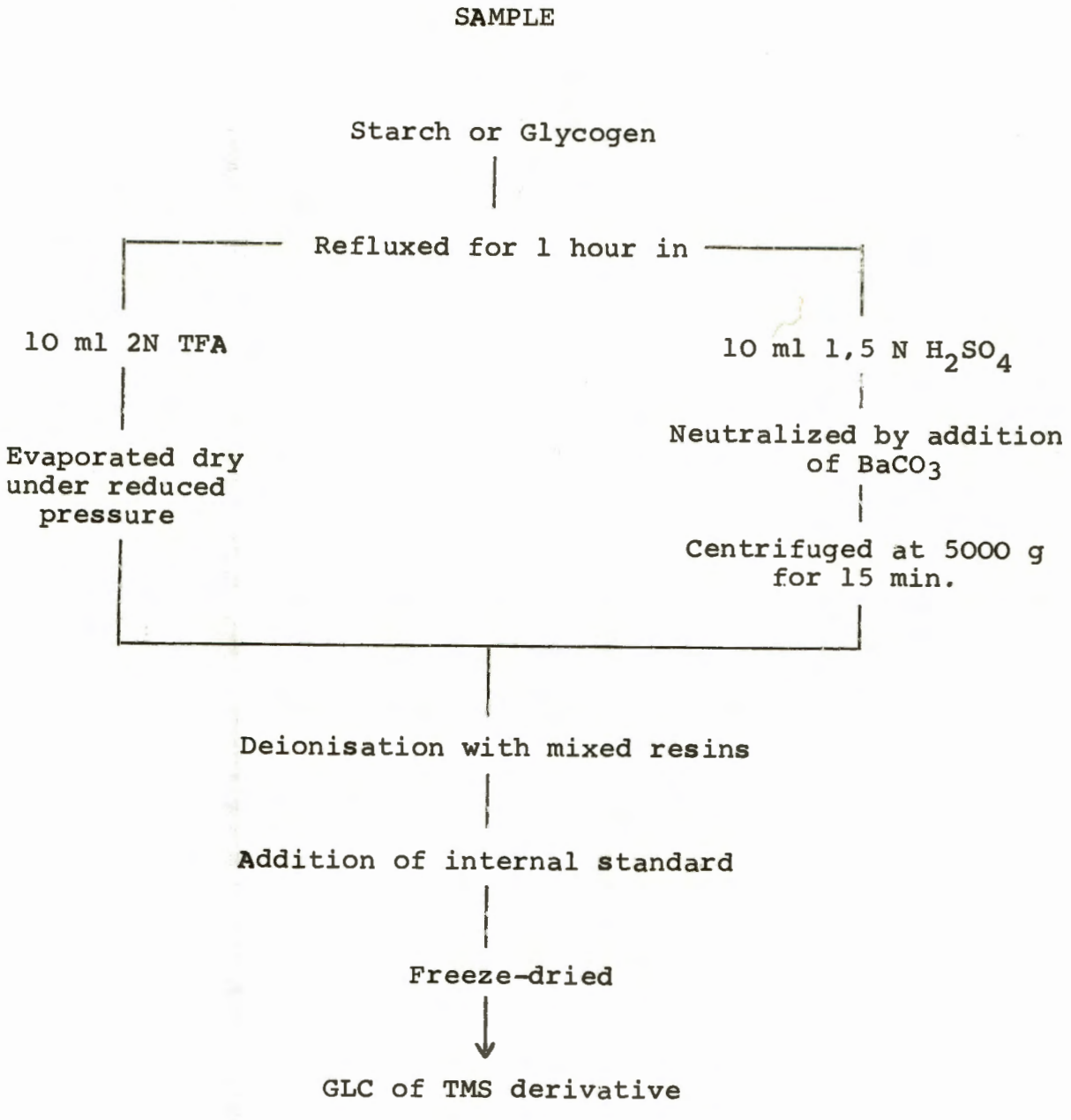


Figure 9:

Procedure for acid hydrolysis of samples followed by preparation for GLC.

Table 1:

Starch and glycogen levels expressed as glucose units, using the method of Dubois et al. (28),

Sample	Initial concentration of sample ($\mu g\ ml^{-1}$).	Sample concentration in glucose units: $\mu g\ ml^{-1} \pm$ standard error of the mean.	Ratio of polymer/ glucose units (correction factor)
Starch	67,70	70,87 ± 0,34	0,96
Glycogen	98,00	99,09 ± 1,42	0,99

Table 2:

Colorimetric determination of the estimated yield of hydrolysis products during preparation of samples. Values shown represent the means of 2 replicates.

Method of hydrolysis.	Sample	μg glucose ml^{-1} in initial sample	μg glucose ml^{-1} after hydrolysis	% recovery of initial sample
H_2SO_4 1,5 N	Glycogen	99,09	41,26	41,65
	Starch	70,87	36,87	52,03
TFA 2 N	Glycogen	99,09	43,28	43,84
	Starch	70,87	68,68	95,96

Table 3:

GLC determination of the estimated yield of hydrolysis products during preparation of samples. Values shown represent the means of 2 replicates.

Method of hydrolysis	Sample	Initial dry weight of sample (mg)	Calculated glucose content of sample* (mg)	Yield of glucose (mg)	% recovery of initial sample
H_2SO_4 1,5 N	Glycogen	31,2	31,5	3,7	11,7
	Starch	16,6	17,3	7,9	45,5
TFA 2 N	Glycogen	14,0	14,2	6,8	47,5
	Starch	15,2	15,8	9,5	61,3

* Calculated by using the correction factors derived in Table 1.

The colorimetric determination (Table 2) showed that sample loss by hydrolysis was considerably reduced by using TFA in preference to sulphuric acid as a hydrolytic agent. Recovery of glycogen was lower than that of starch, but was slightly improved by the use of TFA. However, recovery of starch hydrolysis products was greatly increased (from

52% to 96%) when TFA was used. The GLC analysis of hydrolysis products (Table 3) showed a similar improvement in yield after hydrolysis with TFA, especially of the hydrolysis products of glycogen.

The recovery of hydrolysis products of glycogen was low using sulphuric acid, possibly due to inefficient hydrolysis, but improved with the use of TFA. A better recovery of starch hydrolysis products was obtained when both methods were used, but recovery improved when TFA was the hydrolytic agent. GLC analysis of acid hydrolysis products revealed a predictable loss in yield due to the complexity of the analysis procedure.

5(5) PAPER CHROMATOGRAPHY OF ETHANOL-SOLUBLE CARBOHYDRATES

Although trimethyl silyl derivatives of ethanol-soluble carbohydrates have been detected by means of gas-liquid chromatography (section 5:6), it is known that other compounds can be derivatised using the standard silylating reagents (42). Further identification of ethanol-soluble carbohydrates was undertaken using one-dimensional descending chromatography. Standard carbohydrates in 80% ethanol and deionised, concentrated aqueous ethanol-soluble samples were spotted with capillary tubes on to Whatman No. 4 chromatography paper. The chromatograms were developed in a Shandon 500 Chromatank using ethyl

acetate:acetic acid:water (14:3:3) as the solvent system. The chromatograms were run for 12 - 16 hours at 20 - 22°C. The following detection reagents were used:

A. Silver nitrate-sodium ethoxide, the method of Trevelyan et al. (127) which detected mainly reducing sugars, some acyclic polyols and myo-inositol. The spots obtained were graded from grey to black.

B. Potassium permangenate (1%) containing 2% sodium carbonate, as described by Pacsu et al. (89), which detects some polyols, reducing sugars and some disaccharides. After spraying on the reagents the detected compounds were revealed as yellow spots against a purple background. They were marked immediately since the spots faded to grey regions on a brown background.

C. p-anisidine hydrochloride (1%) in n-butanol, the method employed by Hough et al. (48) to detect aldohexoses and ketohexoses, although it was also possible to distinguish methyl-aldopentoses and uronic acids with this method. Viewed under ultra-violet light aldohexoses were expected to appear as green-brown spots, but mainly brown spots were obtained.

D. Specific identification of myo-inositol was achieved by using Scherer's test (84), since myo-inositol reacted poorly with the silver nitrate-sodium ethoxide reagent. Myo-inositol was revealed as a rose-pink spot.

The Rg was calculated for all carbohydrates and the results are shown in Table 4.

Table 4:

Paper chromatography of sugars in healthy and rust-infected poplar leaves, showing mean Rg values for each compound, ± the standard error of the mean. The solvent system used was ethyl acetate: acetic acid: water (14:3:3). Mobility of glucose was 1,00 ± 0,03 cm/hr. The detection reagents used for identification were silver nitrate/sodium ethoxide reagent (S), potassium permanganate reagent (P), p-anisidine hydrochloride reagent (A) and Scherer's test for myo-inositol (ST).

Key: + - compound detected
t - trace amount

Sugar	Pustule	Region adjacent	Infected petiole	Healthy lamina	Healthy Petiole	Mean Rg ± S.E.M.	Detection reagent.
Trehalose	-	-	-	-	-	0,30	S
Maltose	t	t	t	t	t	0,32	P
Sucrose	+	+	+	+	+	0,48 ±0,00	P,A
Myo-inositol	+	+	+	+	+	0,33 ±0,01	S, ST
Glucose	+	+	+	+	+	1,00 ±0,00	S,P,A,
Mannitol	+	t	-	+	-	1,19 ±0,01	S
Fructose	+	+	+	+	+	1,41 ±0,01	S,P,A,
Arabitol	+	t	-	-	-	1,81 ±0,03	S
Compound A	-	-	+	+	+	1,78 ±0,04	S,P

The chromatograms confirmed the presence of glucose, fructose, sucrose and myo-inositol in healthy tissues, with the addition of mannitol and arabitol in infected tissues. An unidentified compound **A** was found in both petiole samples and in the healthy lamina, as shown in Table 4. The hexitol was shown to be mannitol and it occurred mainly in infected tissue. **A** compound with an Rg similar to that of mannitol was observed in the healthy lamina sample. The presence of an inositol was confirmed by the Scherer test which yielded pink spots that faded after two days.

Sucrose was detected by the p-anisidine hydrochloride and potassium permanganate reagents and appeared to be the main oligosaccharide. No reaction of sucrose with the silver nitrate-sodium ethoxide reagent was observed whereas it has been shown to react slightly with this reagent in other studies (60). Maltose was not clearly demonstrated because there was a limited reaction with only one of the detection reagents used (potassium permanganate reagent). The methods of GLC (section 5:5) did not verify the presence of this compound. Trehalose was not identified in any samples.

5(6) GAS-LIQUID CHROMATOGRAPHY

An internal standard, 0,5 mg erythritol, was added to each sample prior to the last freeze-drying step. i-Erythritol (meso-erythritol) was chosen because it does not occur in either healthy or infected poplar tissues. An internal standard was added to allow correction for variation in

detection response and carrier gas flow rate, although this may not be necessary if sample loss during derivative preparation is avoided and the detection response is shown to be constant (42). Trimethylsilyl (TMS) derivatives were made according to the methods of Holligan and Drew (43) by dissolving the freeze-dried sample in 0,85 ml anhydrous pyridine with the addition of 0,1 ml hexamethyl-disilazane and of 0,05 ml of trimethylchlorosilane. The sample was shaken for 30 seconds and allowed to stand for 1 to 3 hours.

Aliquots ranging from 2 - 10 µl of the TMS derivatives were injected with a 10 µl Scientific Glass Engineering syringe into a Pye series 104 gas chromatograph with a Philips PM 8000 pen recorder. A dual column system was used, consisting of 2,1 m x 6 mm glass columns containing Diatomite CQ as the solid support and 2% S.E. 52 (methyl phenyl silicone gum) as the non-polar liquid phase. The flow rate of the carrier gas (nitrogen) was 30 ml per minute. The temperature program was an initial isothermal run at 140^{o}C for 4 minutes, then a 6^{o}C/minute rise from 140 to 290^{o}C and a final isothermal run at 290^{o}C for 30 minutes. This program adequately separated the monosaccharides, polyols and disaccharides but did not clearly separate individual hexitols for quantitative analysis. There were no trisaccharides and for this reason the final isothermal run at 290^{o}C was reduced to 10 minutes.

Table 5:

Relative retention times of TMS carbohydrates from healthy and infected poplar leaves analysed on 2% S.E. 52 with a temperature program of 140°C to 290°C and an isothermal run at 290°C for 30 minutes.

Retention times expressed relative to i-erythritol which eluted after 8,74 ± 0,34 minutes, showing standard errors of the mean for each value.

TMS CARBOHYDRATE	RELATIVE RETENTION TIMES
Monosaccharides:	α : β
Glucose	2,01 ± 0,07 : 2,21 ± 0,08
Fructose	1,83 ± 0,06 : 1,94 ± 0,09
Acyclic Polyols:	
Arabitol	1,63 ± 0,05
Mannitol	2,10 ± 0,07
Alicyclic Polyol:	
Myo-inositol	2,43 ± 0,09
Other Compounds:	
Compound B	2,99 ± 0,11
Compound C	3,22 ± 0,13
Disaccharides:	
Sucrose	3,45 ± 0,14
Compound D	3,63 ± 0,15

Carbohydrates were characterised by comparison of their retention times relative to erythritol with those of standard compounds. The relative retention times of the ethanol-soluble carbohydrates found in healthy and infected poplar leaves are shown in Table 5. When the identity of a compound was not clear, standard sugars were co-chromatographed.

Representative chromatograms of the ethanol-soluble carbohydrates of infected leaves and urediniospores are illustrated in Figure 10 (a) and (b), while that of the healthy lamina is shown in Figure 10 (c).

Hexitols were observed in GLC chromatograms of infected and healthy tissues, as illustrated in Figures 10 (a) and (b). The identity of one hexitol, occurring mainly in infected tissue, was confirmed to be mannitol by co-chromatography of the standard compound and by paper chromatography analyses (Section 5:5). A putative second hexitol was observed in GLC chromatograms of healthy and infected tissues in trace amounts, but this compound was not identified. The three commonly occurring hexitols, mannitol, glucitol and galactitol, have not been separated on 2% S.E. 52 in previous studies (43) due to their similar retention times. However, it was found that a partially resolved peak of standard galactitol separated from mannitol, whereas mannitol and glucitol were not separated (Figure 11).

Disaccharides other than sucrose and trehalose were not adequately separated using 2% S.E. 52 non-polar liquid

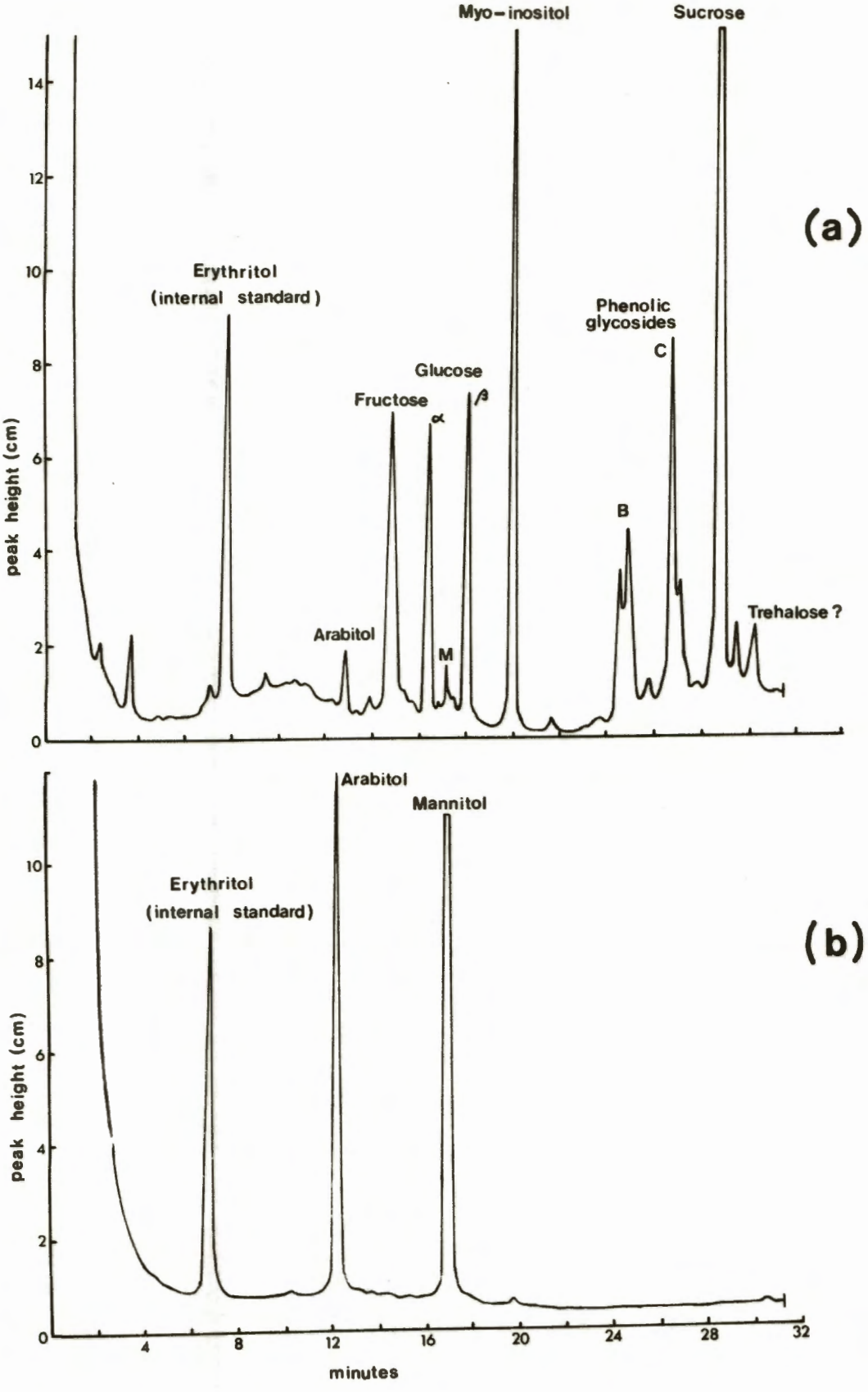


Figure 10:

GLC chromatograms of (a) TMS derivatives of ethanol-soluble carbohydrates of the pustule region of rust-infected poplar leaves and (b) TMS derivatives of a concentrated sample of the ethanol-soluble carbohydrates of the urediniospores. Erythritol was used as internal standard because it was not a component of the carbohydrates of poplar.

Notation: M = Mannitol; B and C = Phenolic glycoside peaks B and C.

Detector attenuation 32 x 10^3

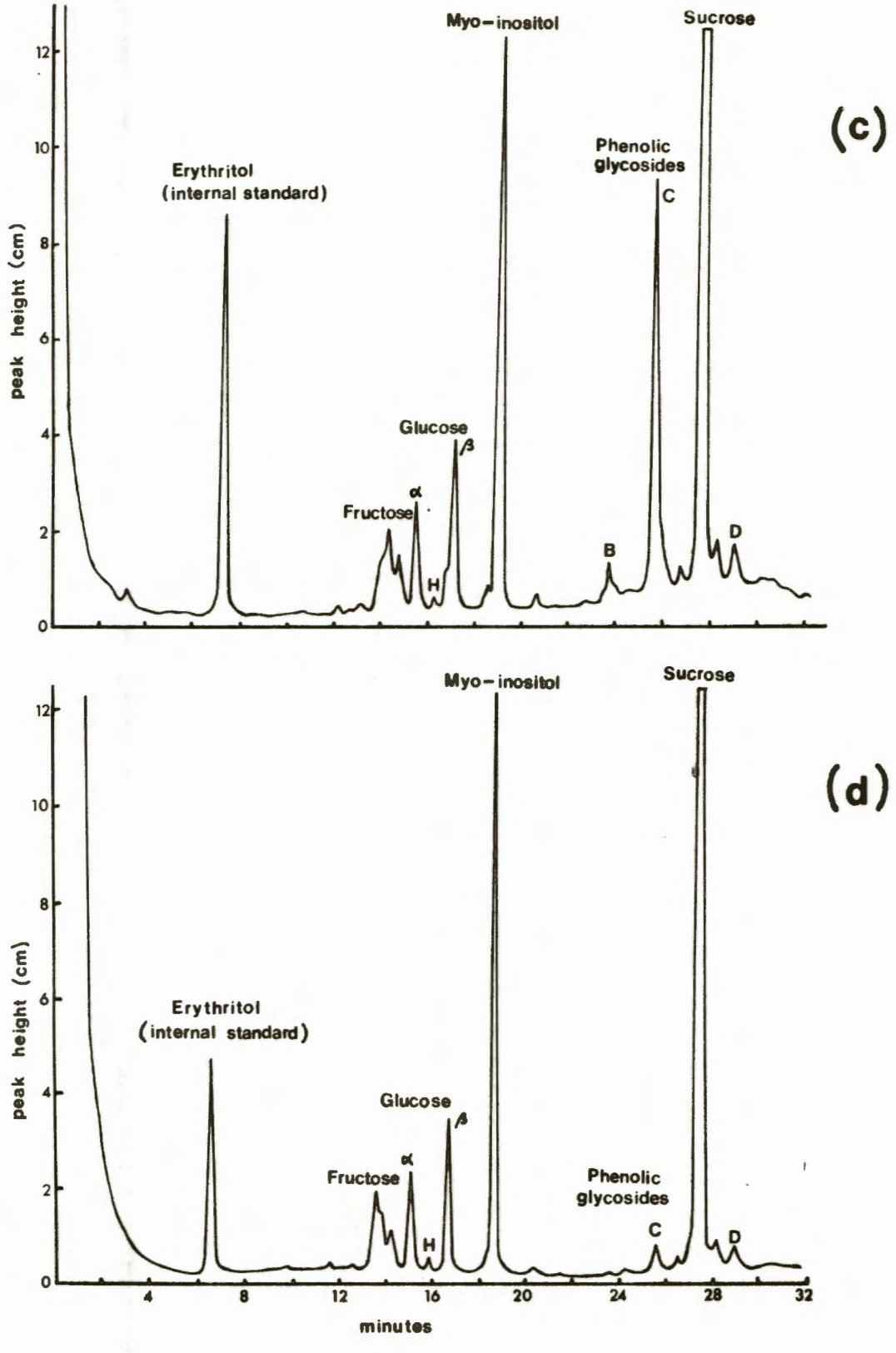


Figure 10: (continued)

GLC chromatograms of TMS derivatives of ethanol-soluble carbohydrates of healthy poplar leaves, showing the carbohydrate composition (c) before and (d) after treatment with activated charcoal. Erythritol was used as internal standard because it was not a component of the carbohydrates of poplar.

Notation: H = Hexitol; D = unknown compound;
B and C = phenolic glycoside peaks B and C;
Detector attenuation: 32 x 10^3

phase. α-Maltose coincided with trehalose and α-cellobiose. The compound D, suspected to be maltose, was present in most poplar samples and an attempt was made to identify this compound by using a different liquid phase. Haverkamp et al. (40) used a more sensitive method for separating the TMS derivatives of oligosaccharides using a medium polar liquid phase of 3% OV-17 on Diatomite CQ. Glass columns (2,7 m x 3,2 mm O.D.) were used and the flow rate of the carrier gas (nitrogen) was 20 ml per minute with an isothermal temperature program of 230°C. Figure 12 shows the separation of standard carbohydrates and those of poplar after using this method. The main disaccharide present was sucrose while trace amounts of a component (peak D) with a similar retention time as α-maltose were observed. However, the second maltose peak was not detected and the identity of compound D was not confirmed.

Analyses of Season 1 ethanol-soluble carbohydrate samples often produced confusing GLC chromatograms. This may have been due to either the use of an unsuitably large sample (0,5 - 1,5 g fresh weight), the result of prolonged refluxing in 80% ethanol or silylation may have been incomplete (42). The procedures for extracting the ethanol-soluble carbohydrates of Season 2 and 3 samples were slightly modified as illustrated in Figures 7 and 8 and final sample sizes were reduced to aliquots containing 0,2 g. Two peaks (compounds B and C, Table 5) in the disaccharide region of the ethanol-soluble carbohydrates, which impeded identification of the known disaccharides, were subsequently found to be phenolic glycosides (Figures 10 (a) and (c)). The details of the

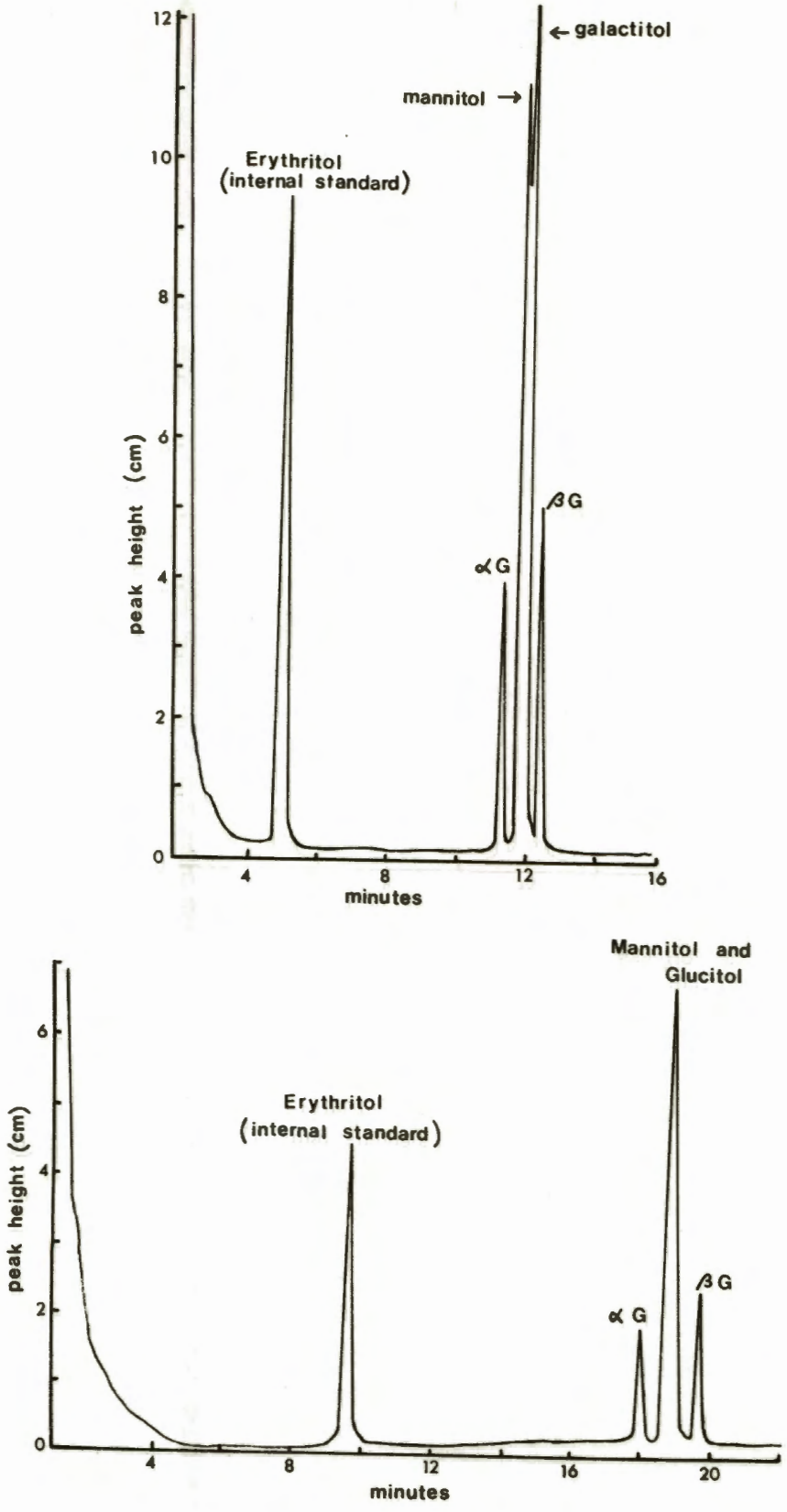


Figure 11:

GLC chromatograms of TMS derivatives of standard hexitols, mannitol, glucitol and galactitol, showing partial separation of mannitol and galactitol alone. i-Erythritol used as internal standard.

α,β G = α,β-glucose. Detector attenuation 32 X 10^3.

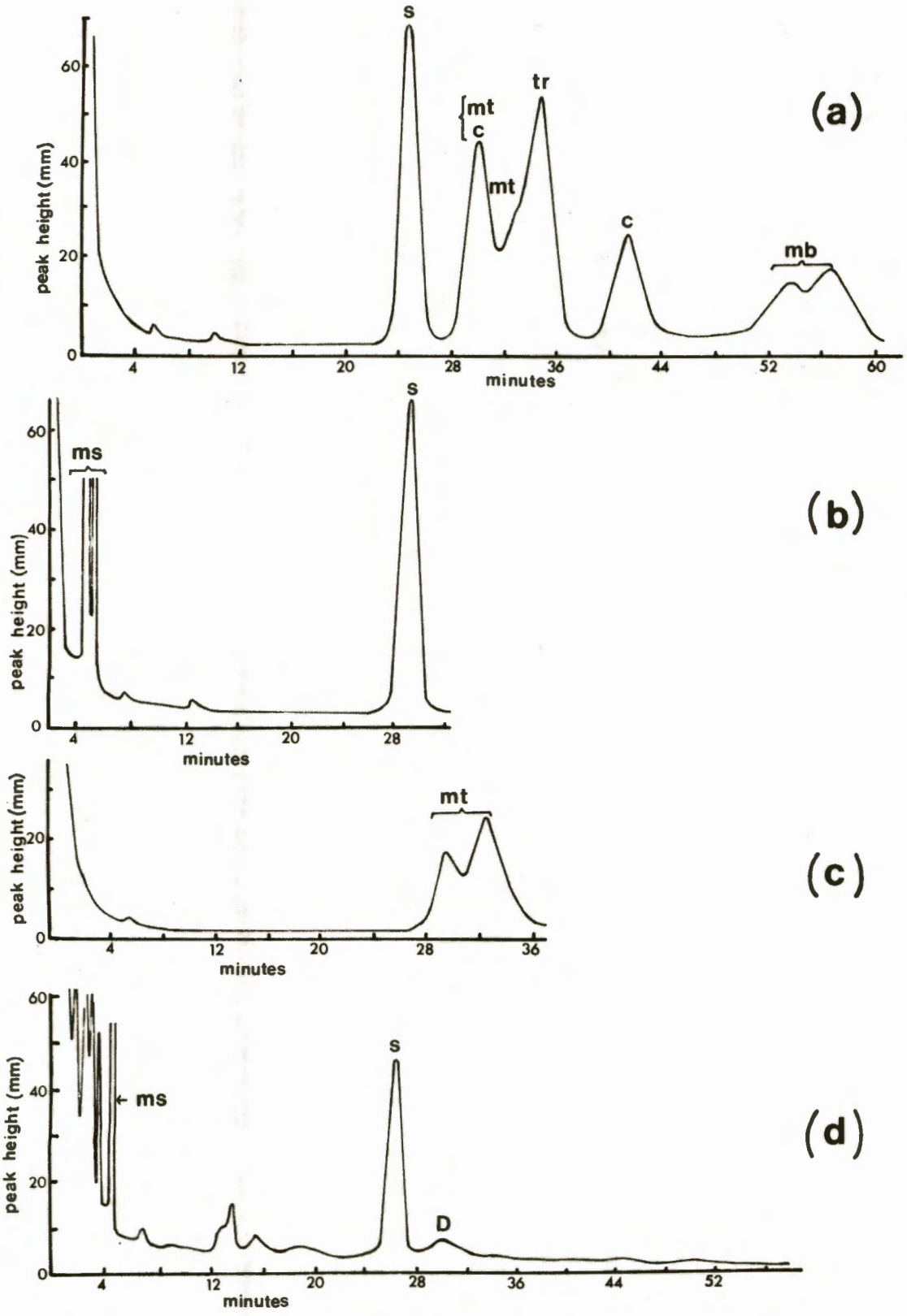


Figure 12:

GLC chromatograms of TMS derivatives of (a) a mixture of standard disaccharides, (b) standard sucrose, (c) standard maltose and (d) the disaccharides of healthy poplar leaves, separated by the method of Haverkamp et al. (52).

Notation: s = sucrose; mt = maltose; c = cellobiose; tr = trehalose; mb = melibiose; ms = monosaccharides D = unknown compound.

extraction and identification of phenolic glycosides are given in Section 5(7).

Levels of ethanol-soluble carbohydrate were calculated as mg g^{-1} dry weight of sample, after referring to the respective calibration curves for each sugar (Figure 13). The seasonal variation in ethanol-soluble carbohydrate is shown in Figures 16 to 19 and the carbohydrate composition of the urediniospores is shown in Table 10, Section 5(8).

Since the polysaccharide levels could not be determined accurately (discussed in Section 5:4), each monomer component was expressed as a mol-% of the total sugars in the fraction.

The levels of each monomer component were calculated as mg g^{-1} dry weight, as described for ethanol-soluble carbohydrates, to obtain a rough estimate of the polysaccharide levels. Another factor impeding accurate quantification of polysaccharides by means of GLC methods was the variable detection response of the internal standard (i-erythritol) in these samples. The levels of the most abundant individual monomer components and the combined levels of monomer components in each sample were plotted as mg g^{-1} dry weight in histograms (Figures 20 - 21). Polysaccharide levels expressed as mol-% are tabulated in Tables 12 - 14. Similar monomer components were obtained after acid-hydrolysis of hot-water soluble, cold water-soluble and ethanol-insoluble fractions. These components were arabinose, rhamnose, xylose, galactose, glucose and mannose and are illustrated for a hot water-soluble fraction in Figure 14.

The seasonal study of rust-infected poplar showed two main features when compared with healthy tissues. These were higher dry weight levels contrasting with low total nitrogen levels in the infected lamina during most of the infection period.

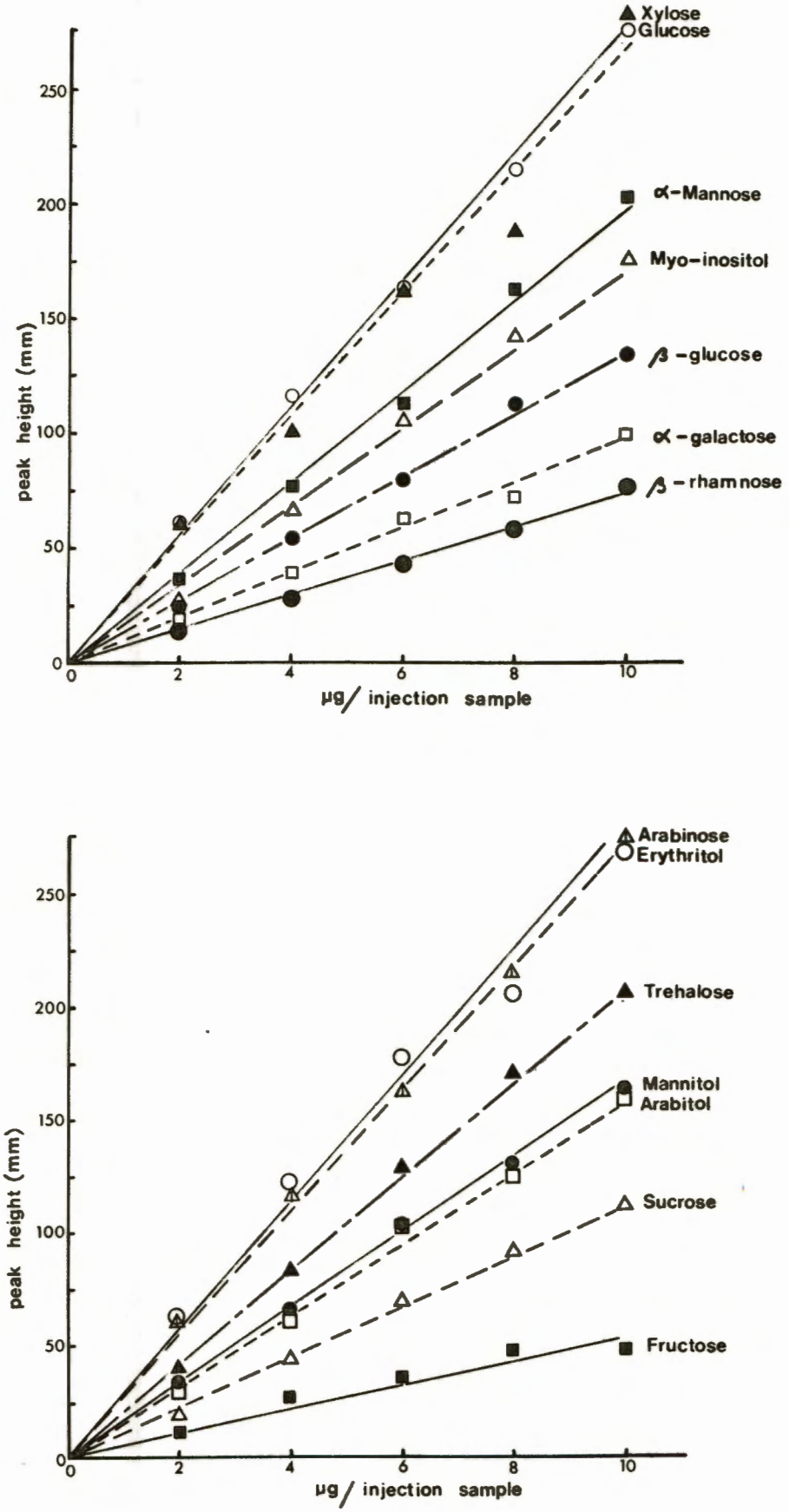


Figure 13:

Calibration curves for TMS carbohydrates occurring in healthy and rust-infected poplar leaves, showing the ethanol-soluble components and the monomers forming acid-hydrolysis products. The α or β anomer peaks of some components were used when one of the anomer peaks was only partially resolved.

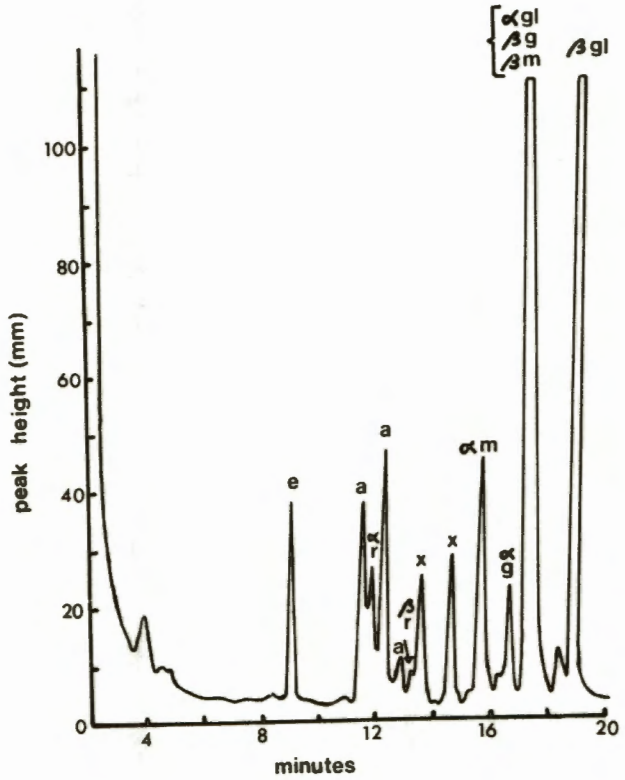


Figure 14:

GLC chromatograms of TMS derivatives of an acid-hydrolysed polysaccharide sample (hot water-soluble) of rusted poplar leaves showing the monomer components.

Notation: e - erythritol (int. st.); a - arabinose; α,β r - α,β rhamnose; x - xylose; α,β m - α,β mannose; α,β g - α,β galactose; α,β gl - α,β glucose.
Detector attenuation : 32 x 10^3.

The calculation of the molar proportions of arabinose and xylose was based on all the clearly resolved anomer peaks of these compounds. Proportions of rhamnose, mannose, galactose and glucose were estimated from only one clearly

resolved peak of each compound, i.e. β-rhamnose, α-mannose, α-galactose and β-glucose.

The mol -% of each component was calculated using the following equation :-

$$\text{Molar proportion} = \frac{\text{peak height (or area) of sugar}}{\text{molecular weight X K}}$$

where K is a constant for the respective sugar derived from GLC calibration curves such that

$$K = \frac{\text{peak height (or area) of sugar/weight of sugar}}{\text{peak height (or area) of internal standard/weight of internal standard}}$$

Each sugar component was expressed as a mol -% of the total molar proportion of the fraction.

5(7) ANALYSIS OF AN ETHANOL-SOLUBLE CARBOHYDRATE EXTRACT OF HEALTHY POPLAR LEAVES AND ISOLATION OF PHENOLIC GLYCOSIDES

A. Analysis of Fractions After Gel Filtration

During GLC analyses of seasonal samples of ethanol-soluble carbohydrates in the leaves of P. canescens, two peaks which consistently occurred in the disaccharide region of the chromatogram did not coincide with any standard oligosaccharides. Initially the

peak C in particular, shown in Figure 10 (a) and (c), caused confusion in the identification of disaccharides and it was necessary to identify the compound. The presence of one or more phenolic glycosides was suspected because of the yellow colour of aqueous samples after refluxing and deproteinization. Phenolic glycosides are known to occur in most Populus and Salix species, in bark and leaves. Several of these compounds have been chemically characterised, the most common in Populus species being salicin and tremuloidin (93), but many others are known to occur in bark of the Salicaceae (94). The object of this investigation was to discover whether the interfering peaks represented phenolic glycosides.

A sample of healthy poplar leaves (20 g fresh weight) was refluxed in three changes of 80% ethanol to extract the ethanol-soluble carbohydrates, then deproteinized and freeze-dried. A 1,0 g aliquot of this sample dissolved in 5 ml water was run through a column (112 cm x 4 cm) of Biogel P.2. at a flow rate of 50 ml per hour, with distilled water as eluting agent. Approximately 21 fractions (varying from 20 - 50 ml) were collected. Each fraction was assayed for total carbohydrate in terms of glucose units by the spectrophotometric method of Dubois et al. (28) as shown in Appendix 4.

The glucose levels were plotted for each fraction in Figure 15 and where peaks occurred a number of fractions were combined into single samples. Several fractions

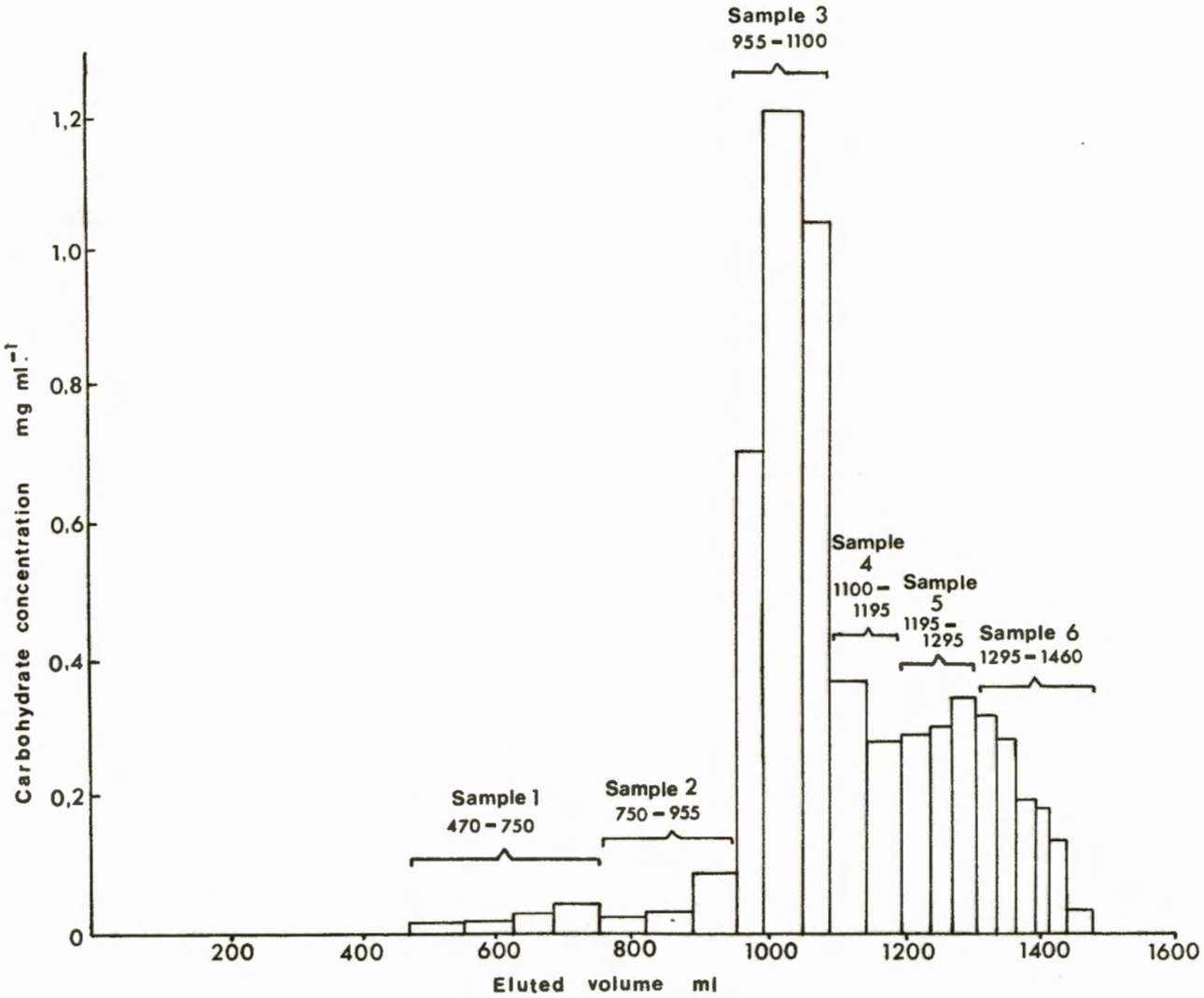


Figure 15:

Fractionation of extracted ethanol-soluble carbohydrates of healthy poplar leaves by gel filtration. The histogram shows levels of carbohydrate (mg per ml) in eluted fractions, grouped into six samples.

which were coloured yellow were grouped together into one sample. The samples were expected to be eluted in the following sequence, shown in Table 6.

Table 6:

Samples separated by gel filtration from an extract of ethanol-soluble carbohydrates of healthy poplar leaves, in order of elution.

Eluted volume (ml)	Expected Sample	Sample Number
470-750	High molecular weight soluble carbohydrate	1
750-955	High molecular weight soluble carbohydrate	2
955-1100	Disaccharides	3
1100-1195	Disaccharides and monosaccharides	4
1195-1295	Monosaccharides and phenolic glycosides	5
1295-1460	Phenolic glycosides	6

An aliquot of each sample was deionized and prepared for GLC as described in Chapter 5(5). Since sample 1 did not contain any soluble components, a further aliquot was hydrolysed under reflux into monomer components

with 2N trifluoroacetic acid for further GLC analysis.

The TMS derivatives of the carbohydrates in each fraction are shown in Tables 7 (a) and (b), showing peak heights and retention times relative to i-erythritol as the internal standard. However, there was some variation in the retention times of erythritol and since myo-inositol was detected in samples 2 to 6, it was possible to reconfirm the identity of each component with reference to myo-inositol.

The results in Table 7 (a) show that sample 1 consisted of high molecular weight polymers composed of glucose in the highest proportion, but also contained arabinose, rhamnose, xylose, galactose, and mannose. The other samples (Table 7 (b)) had the following composition :-

Sample 2: A high proportion of fructose and some traces of monosaccharides were present. Two unknown peaks f and g with retention times relative to i-erythritol of 3,38 and 3,97 respectively were observed.

Sample 3: Mainly disaccharides, especially high levels of sucrose. Small amounts of monosaccharides and peaks B and C with retention times relative to erythritol of 3,83 and 4,14 respectively were present. Peaks B and C were subsequently shown to be phenolic glycosides. The disaccharide peak D could not be identified although it

co-chromatographed with trehalose.

Sample 4: Mainly monosaccharides such as fructose, glucose, a component (peak e) with a similar retention time to mannitol and very large amounts of myo-inositol. Small amounts of sucrose were present.

Sample 5: Mainly large amounts of components B and C, later identified as phenolic glycosides. Myo-inositol was present.

Sample 6: Mainly components B and C, where peak C occurred in larger quantities than peak B.

An ultra-violet scan of fraction 6, containing mainly components B and C, revealed a peak at 270 nm with molar extinction coefficient $\varepsilon = 1450$, characteristic of phenolic glycosides (8).

An alternative method for the extraction of phenolic glycosides was employed using a procedure amended from that of Mabry et al. (72). In this method activated charcoal separates flavonoids, including flavonoid glycosides from most non-aromatic plant constituents such as the carbohydrates. The method is especially recommended for the removal of flavonoid glycosides which can be quantitatively removed from the charcoal with water containing 7% phenol (72).

Healthy poplar leaves (11 g fresh weight) were ground up in 200 ml 20% aqueous methanol with an MSE homogeniser and incubated at 4°C for 2 days (72). The homogenate was filtered. The filtrate was evaporated to dryness under reduced pressure and was dissolved in 125 ml hot methanol. About 5 g of activated charcoal (Merck) was added to the solution, stirred thoroughly and filtered. The flavonoid charcoal material was washed with two further 50 ml aliquots of boiling methanol.

The charcoal-flavonoid material was immersed in 3 x 100 ml aliquots of boiling phenol:water (7:93) to remove the flavonoids. The yellow liquid was reduced to a small volume (about 10 ml) under reduced pressure, diluted with distilled water (10 ml) and tranferred to a separating funnel, with several washes. The phenol was removed by extraction with diethylether. A small quantity (corresponding to 0,5 g fresh weight) of the aqueous flavonoid solution was deionised, freeze-dried and prepared for GLC using the TMS derivatives.

The results of GLC analysis of this sample, using the modified Mabry method (72) are shown in Table 8. A small fraction of the sample was monosaccharide, but the major peak was a phenolic glycoside, corresponding in retention time relative to myo-inositol with the phenolic glycoside peak C in other samples.

Table 7 (a):

GLC analysis of TMS derivatives of acid-hydrolysed high molecular weight compounds in Sample 1, showing peak heights and retention times relative to i-erythritol (eluted in 8,9 minutes).

* Both α and β peaks of rhamnose were clearly resolved.

SAMPLE	Sugar	Arabinose		Rhamnose*		Xylose		α Mannose	α Galactose	β Glucose
		α	β	α	β	α	β			
1	RT rel. to erythritol	1,30	1,39	1,34	1,51	1,55	1,67	1,80	1,91	2,18
	Peak ht. (mm)	14	17	15	10,5	11	9	12	14	70

Table 7 (b):

GLC analysis of TMS derivatives of carbohydrates in Sample 2 showing peak heights and relative retention times with either i-erythritol or myo-inositol. i-Erythritol eluted in 11,7 minutes and myo-inositol in 25,3 minutes.

SAMPLE	Sugar	Fructose		Glucose		Peak e	Myo-inositol	Phenolic Glycosides	Sucrose	Disaccharide D	Peak f	Peak g
		α	β	α	β							
2	RT rel to erythritol	1,59	1,68	1,72	1,86	1,79	2,16	-	-	-	3,38	3,97
	RT rel to myo-inositol	0,74	0,77	0,79	0,86	0,83	1,00	-	-	-	1,57	1,84
	Peak ht. (mm)	7,5	65,5	5,5	6	3	11,5	-	-	-	4	6

Table 7 (b) - continued:

TMS derivatives of carbohydrates in Samples 3 and 4, showing peak heights and retention times relative to i-erythritol or myo-inositol. i-Erythritol eluted in 6,9 and 10 minutes and myo-inositol in 21,2 and 22,1 minutes respectively (Samples 3 and 4).

Sample	Sugar	Fructose		Glucose		Peak e	Myo-inositol	Phenolic Glycosides		Sucrose	Disaccharide D	Peak f	Peak g
		α	β	α	β			B	C				
3	RT rel to erythritol	2,26	2,39	2,51	2,78	2,64	3,07	3,83	4,14	4,41	4,62	-	-
	RT rel to myo-insitol	0,74	0,78	0,82	0,91	0,86	1,00	1,25	1,35	1,43	1,50	-	-
	Peak ht (mm)	6	4	6	8	3	5,5	3,5	7,5	218	6	-	-

Sample	Sugar	Fructose		Glucose		Peak e	Myo-inositol	Phenolic Glycosides		Sucrose	Disaccharide D	Peak f	Peak g
		α	β	α	β			B	C				
4	RT rel to erythritol	1,69	1,77	1,85	2,01	1,93	2,21	-	-	3,11	-	-	-
	RT rel to myo-inositol	0,76	0,80	0,84	0,91	0,87	1,00	-	-	1,41	-	-	-
	Peak ht. (mm)	57	14	33	43	4	222	-	-	12,5	-	-	-

Table 7 (b) - continued:

TMS derivatives of carbohydrates in Samples 5 and 6 showing peak heights and retention times relative to i-erythritol and myo-inositol. i-Erythritol eluted in 6,9 and 8,9 minutes and myo-inositol in 21,0 and 21,6 minutes respectively (Samples 5 and 6).

Notation: t = trace amount.

Sample	Sugar	Fructose		Glucose		Peak e	Myo-inositol	Phenolic Glycosides		Sucrose	Disaccharide D	Peak f	Peak g
		α	β	α	β			B	C				
5	RT rel to erythritol	2,23	t	t	t	-	3,04	3,77	4,07	4,36	-	-	-
	RT rel to myo-inositol	0,73	t	t	t	-	1,00	1,24	1,34	1,43	-	-	-
	Peak ht. (mm)	13	t	t	t	-	148	3	206	8,5	-	-	-

Sample.	Sugar	Fructose		Glucose		Peak e	Myo-inositol	Phenolic Glycosides		Sucrose	Disaccharide D	Peak f	Peak g
		α	β	α	β			B	C				
6	RT rel to erythritol	-	-	-	-	-	2,43	t	3,19	3,42	-	-	-
	RT rel to myo-inositol	-	-	-	-	-	100	t	1,31	1,41	-	-	-
	Peak ht. (mm)	-	-	-	-	-	4	t	117	5	-	-	-

Table 8:

TMS derivatives of carbohydrate extracted from healthy poplar leaves according to the method of Mabry et al. (72), showing a high proportion of a phenolic glycoside.

Retention times are relative to myo-inositol in the sample with a retention time of 20,2 minutes.

Sugar	Fructose		Glucose		Myo-inositol	Phenolic Glycoside Peak
	α	β	α	β		
Relative RT	0,72	0,77	0,80	0,90	1,00	1,35
Peak ht. (mm)	5,5	5,0	7,5	9,5	10,0	74

B. The Application of the Charcoal Extraction Method for the Purification of Ethanol-Soluble Carbohydrate Samples.

Since the phenolic glycoside peaks consistently occurred in GLC chromatogram traces of all ethanol-soluble carbohydrate samples, it was considered necessary to include a step in the purification procedure which would remove these compounds.

A few assays were performed to determine the quantity of activated charcoal that could be added to the ethanol-

soluble samples, which were aliquots corresponding to 0,2 - 0,5 g fresh weight. A suitable amount of charcoal was found to be 0,5 - 0,7 g, which corresponds to the amount used by Bevenue (7). The addition of charcoal was included in the purification procedure as shown in section 5(3), Figure 8. The GLC chromatogram (Figure 10 (c)) illustrates the ethanol-soluble carbohydrate composition of a healthy poplar lamina sample without the inclusion of the charcoal purification step, showing the presence of the major phenolic glycoside C. The removal of most of Peak C, with only a small trace remaining, is shown in Figure 10 (d).

In order to estimate the loss of mono- and disaccharides using this procedure, a sample of standard sugars was treated with activated charcoal and a control sample left untreated. The samples were analysed by GLC and it was shown (Table 9) that 20 - 28% of sugars were removed.

Table 9:

Comparison of recovery of sugars with and without the inclusion of a charcoal-purification step in the preparation of samples for GLC analysis.

Sugar	Initial Sample (mg)	Yield after Charcoal Purification (mg)	Yield as % of initial sample	% Loss
Fructose	0,88	0,63	71,6	28,4
Glucose	0,77	0,58	75,3	24,7
Sucrose	0,64	0,51	79,7	20,3

5(8) THE SEASONAL VARIATION IN ETHANOL-SOLUBLE CARBOHYDRATES

The variation of ethanol-soluble carbohydrate in the leaves of healthy and infected poplar has been traced through three seasons which correspond to the following sample collection sites :-

A. Season 1, from February to May, 1972, at Cecilia Forest station;

B. Season 2, from March, 1973, to January, 1974, in Claremont;

C. Season 3, from February to May, 1974, in Hohenort Estate.

During Season 1 erupted uredosori were first observed in February, while during Seasons 2 and 3 leaves bearing erupted uredosori were available throughout the study periods.

Variations in levels of each carbohydrate component are discussed individually throughout the three seasons. The components studied were sucrose, glucose, fructose, a hexitol (shown to be mannitol in rust-infected tissues), arabitol, trehalose and myo-inositol. These components are plotted in histograms (Figures 18 and 19). To clarify the presentation of individual sugar levels, some monthly samples in each season were grouped into 3 to 4 month periods and a mean with standard error of the mean was calculated for each carbohydrate within each period. When two monthly samples were grouped together, a mean value is shown. Levels of combined carbohydrate in each sample, representing the summed values of ethanol-soluble sugars, were plotted as histograms for both lamina and petiole samples throughout

the 3 seasons. Phenolic glycoside levels (estimated in terms of sucrose) are shown for Seasons 1 and 2 in Table 11. On two occasions the ethanol-soluble carbohydrate composition in urediniospores was determined and these values are shown in Table 10. All carbohydrate values are tabulated in Appendix 5.

Combined Carbohydrate:

The levels of combined carbohydrate in lamina and petiole samples are shown in Figures 16 and 17.

During November to January of Season 1, before the appearance of erupted uredosori, combined carbohydrate levels in the healthy lamina declined. During Season 2 the healthy lamina contained exceptionally high levels of combined carbohydrate, possibly due to the sunny aspect of the population, in contrast with the partially shaded population studied during Seasons 1 and 3. Carbohydrate levels rose to a peak during June of Season 2 after which levels declined.

During Season 1 the pustule region of the infected lamina showed an increase in combined carbohydrate levels as infection density increased, whereas levels in the region adjacent declined. During Season 2 two peaks in levels of combined carbohydrate were observed, the first during June and the second during November, in both pustule and region adjacent to the pustule. The region adjacent had

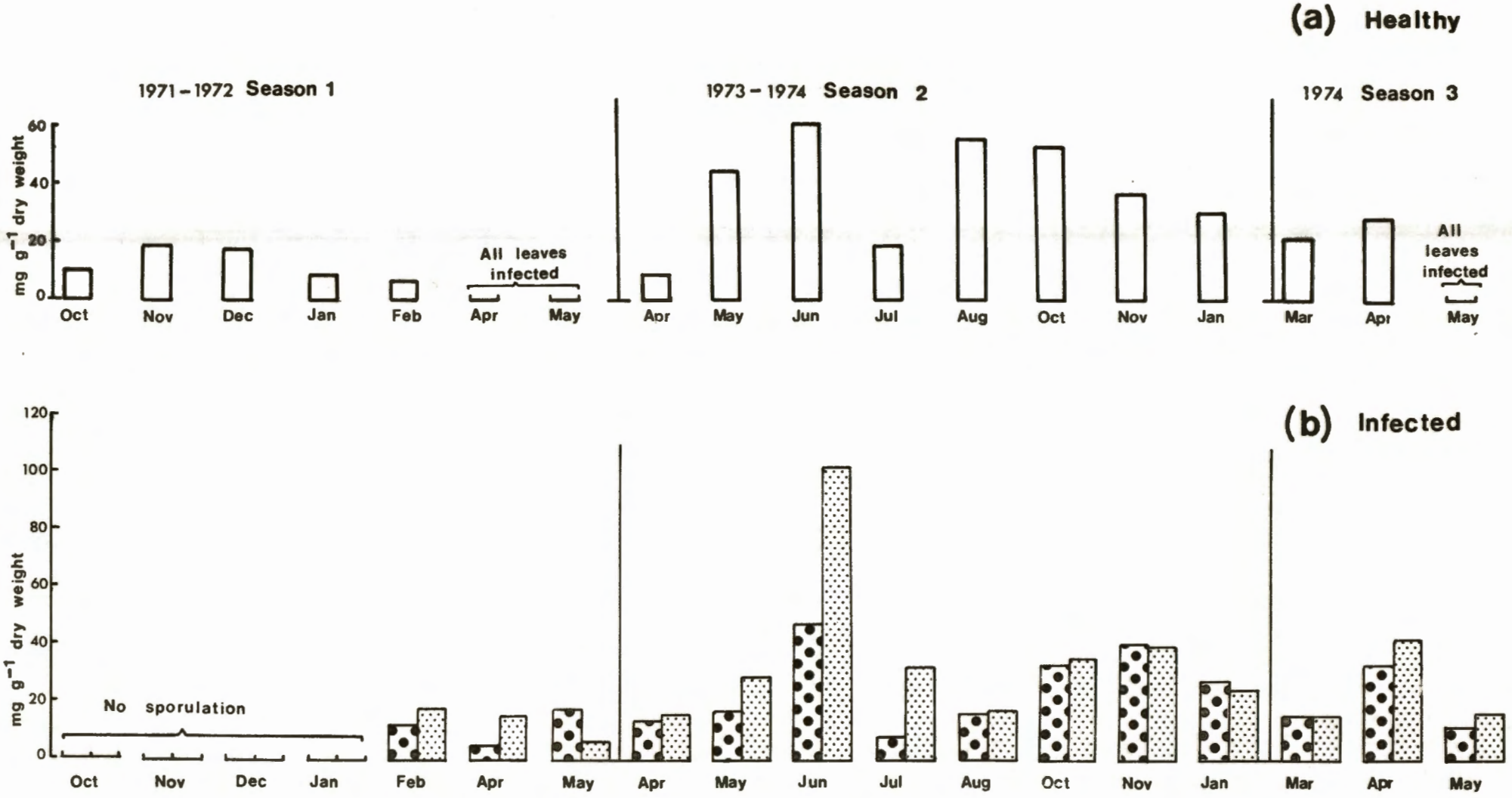


Figure 16:

Seasonal variation of combined carbohydrate (combined levels of ethanol-soluble components in each sample) in (a) healthy and (b) infected poplar leaves during 3 seasons. Levels shown as mg carbohydrate g^{-1} dry weight. Key: ☐ healthy lamina; ▣ pustule; ▨ region adjacent to pustule.

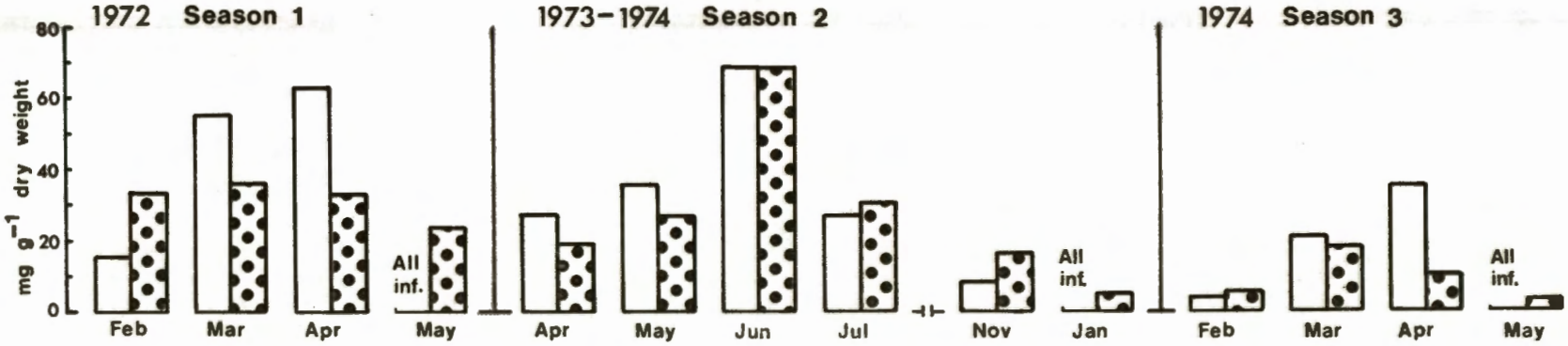


Figure 17:

Seasonal variation of combined carbohydrate (combined levels of ethanol-soluble components in each sample) in healthy and infected petioles during 3 seasons. Levels shown as mg carbohydrate g^{-1} dry weight.

Key: ☐ healthy petiole; ⊡ petiole from infected leaf.

mainly higher levels of combined carbohydrate than the pustule.

Levels of combined carbohydrate in the healthy and infected petioles showed some contrasts during Seasons 1 and 3. Levels rose to a peak in April in healthy petioles whereas there was a decline in levels in the infected petiole from February/March to May. During February combined carbohydrate levels were higher in the infected than in the healthy petiole, whereas during March and April higher levels were observed in the healthy petioles. During Season 2 both healthy and infected petioles showed a similar increase in combined carbohydrate to a peak in June, followed by a marked decline to the end of the season.

Sucrose:

Levels of sucrose in lamina samples are shown in Figure 18 (a). Sucrose formed the major component of ethanol-soluble carbohydrate in lamina samples. Levels of sucrose differed markedly during the three seasons, since exceptionally high levels were observed during Season 2 in both healthy and infected tissue. The pustule region appeared to contain lower levels of sucrose than the region adjacent or the healthy lamina.

During Season 1 sucrose levels decreased in the healthy lamina, whereas levels in the pustule increased to a small peak in May. During Season 2 the highest sucrose levels in the

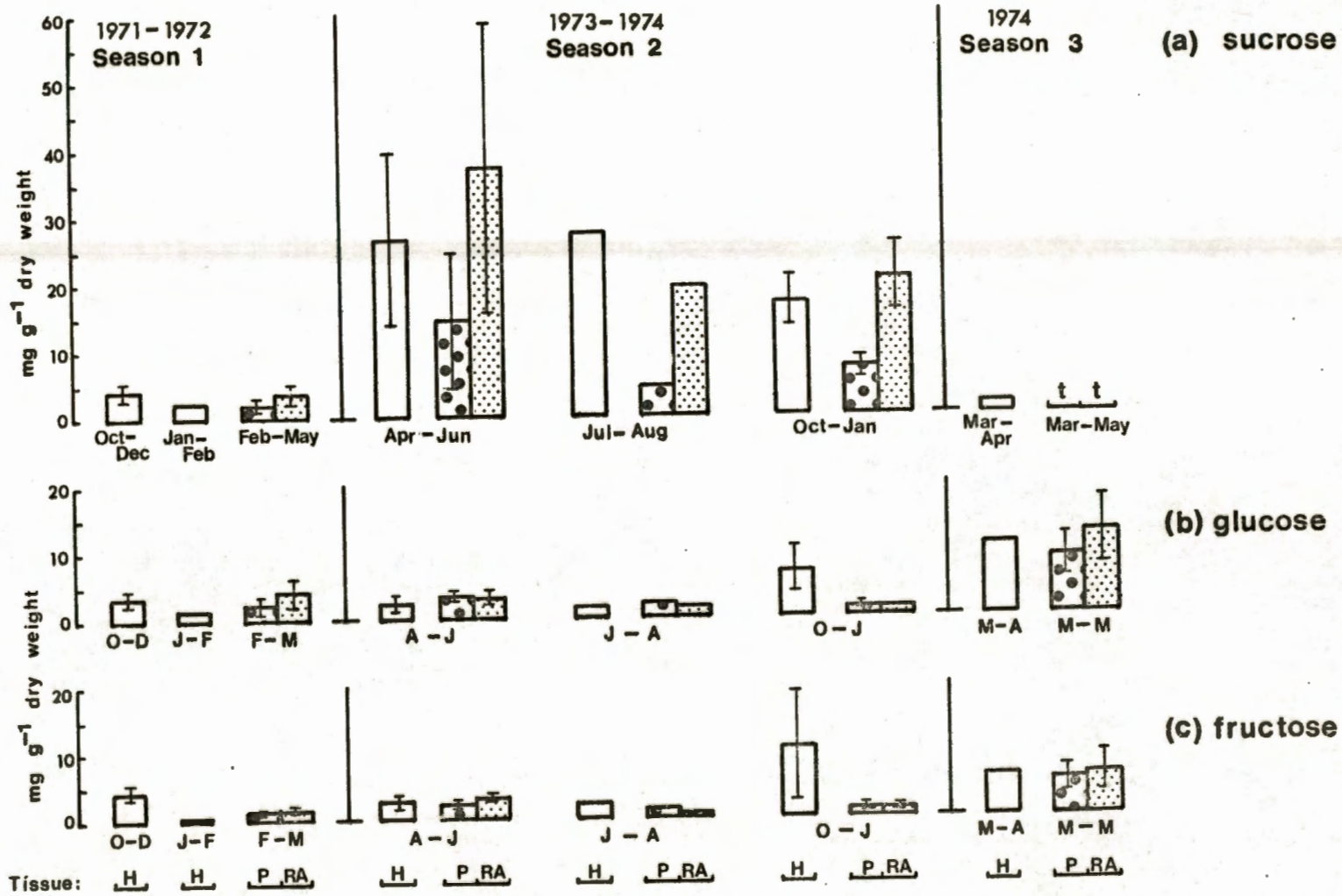


Figure 18:

Seasonal variation of (a) sucrose, (b) glucose and (c) fructose in healthy and rust-infected poplar leaves during 3 seasons, with the levels shown as mg g^{-1} dry weight. Carbohydrate levels shown are mean values of monthly samples grouped into 2 to 4 month periods within each season (see text). ± Standard error of the mean is shown for groups consisting of 3 or more monthly samples.

Key: □ healthy; ⁘ pustule; ▒ region adjacent to the pustule; t - trace amount.

pustule and region adjacent were observed during autumn (April-June), whereas there was little variation in the healthy tissue throughout the season. Levels of sucrose during Season 3 were very low in both healthy and infected tissue, possibly as a result of the shaded aspect of the population.

Glucose and Fructose:

Levels of glucose and fructose in lamina samples are shown in Figure 18(b) and (c). Both glucose and fructose levels in healthy tissue declined during the start of Season 1 to low levels in January-February, when sporulation was observed. In the infected lamina fructose levels were lower than glucose levels and the region adjacent contained higher levels of both compounds than the pustule.

During Season 2 slightly higher levels of both compounds were observed than during the preceding season. There was little variation in the levels of both compounds in healthy and infected tissue. An increase in levels of glucose and fructose was observed during October-January in healthy tissue, corresponding with the production of new leaves during spring.

During Season 3 larger amounts of glucose and fructose were observed in all samples in comparison with the amounts present during the preceding seasons. The high levels of glucose an

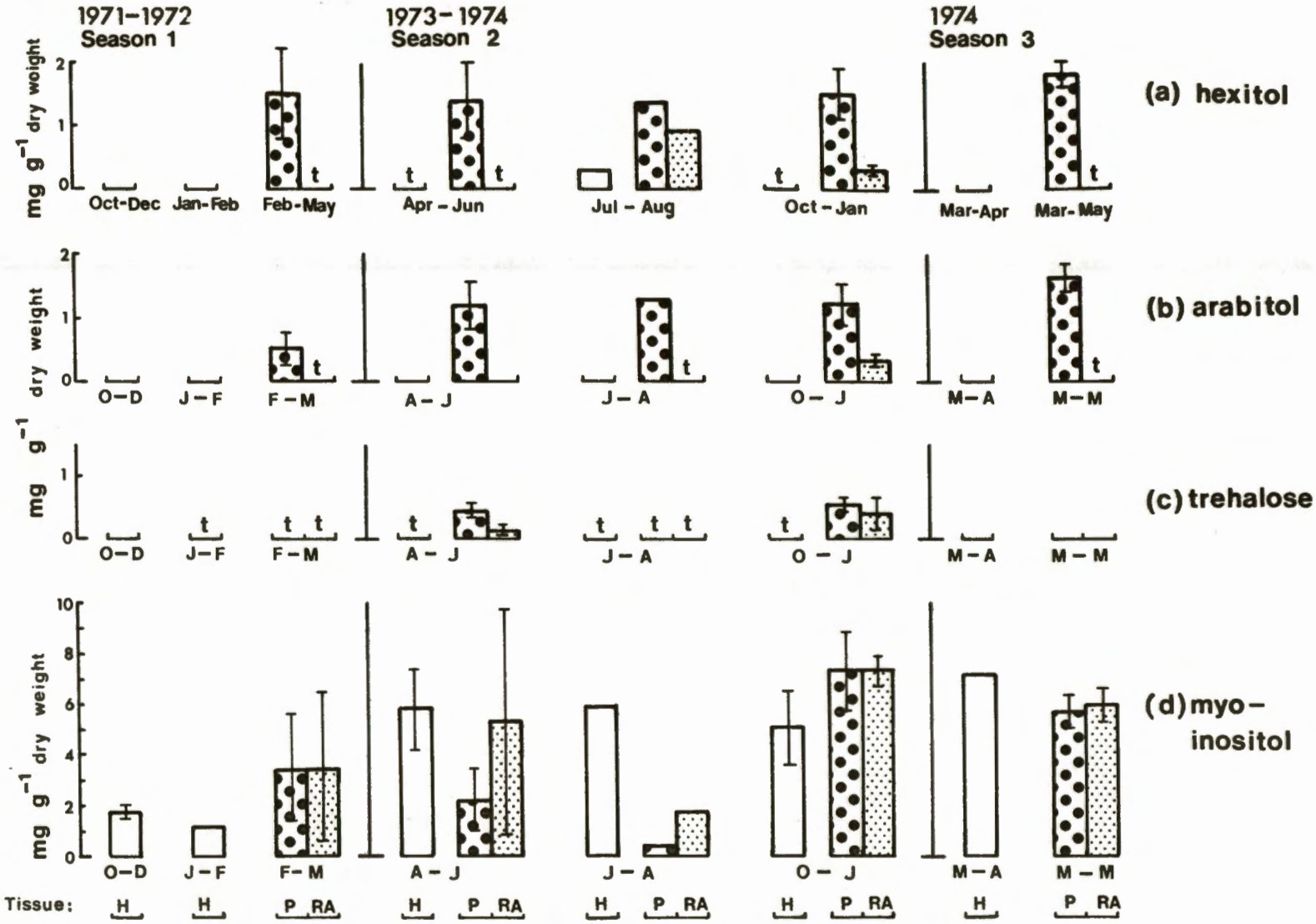


Figure 19:

Seasonal variation of (a) hexitol, (b) arabitol, (c) trahalose and (d) myo-inositol in healthy and rust-infected poplar leaves during 3 seasons, with the levels shown as mg g^{-1} dry weight. Carbohydrate levels shown are mean values of monthly samples grouped into 2 to 4 month periods within each season (see text). ±Standard error of the mean is shown for groups consisting of 3 or more monthly samples.
Key: ☐ healthy; ▣ pustule; ▨ region adjacent to pustule; t, trace amount; ⎵ compound absent.

fructose contrasted with the low amounts of sucrose during this season (Figure 18(a)).

Hexitol:

Hexitol levels are shown in Figure 19 (a). The pustule contained the largest amounts of a hexitol which was identified to be mannitol. During January-February of Season 1 small amounts of mannitol were found in the pustule samples, coinciding with the occurrence of erupted uredosori. As the incidence of infection increased, the levels of mannitol increased in the samples of all three seasons. Mannitol was the most abundant ethanol-soluble carbohydrate in the urediniospores (Table 10).

Small amounts of mannitol were detected in the region adjacent during Seasons 2 and 3. The healthy lamina of Season 2 contained small amounts of a hexitol with a similar retention time to mannitol, but the identity of this compound was not confirmed.

Arabitol:

Levels of arabitol are shown in Figure 19(b). Arabitol was found mainly in the pustule, where it formed the second ethanol-soluble carbohydrate component in the urediniospores (Table 10). Small amounts of arabitol were, however,

found in the region adjacent. Arabitol levels increased as the incidence of infection rose during February to May during Seasons 1 and 3, but remained at a fairly constant level during Season 2.

Table 10:

Levels of ethanol-soluble carbohydrate in the urediniospores, expressed as mg g^{-1} dry weight.

t - trace amount.

	Arabitol	Mannitol	Trehalose
April, 1972	9,07	9,48	t
August, 1973	16,29	23,60	0,32

Trehalose:

Trehalose levels are shown in Figure 19 (c). Trehalose was detected in the urediniospores (Table 10) and in the pustule samples of Seasons 1 and 2 in small amounts, but not in the pustule samples of Season 3. Small amounts were recorded intermittently in healthy lamina samples during Season 2, but may have been confused with the unknown disaccharide, compound D. The region adjacent also contained low levels of a compound identified as trehalose. There was no clear seasonal fluctuation of trehalose.

Myo-inositol:

Levels of myo-inositol are shown in Figure 19 (d). Myo-inositol formed a large proportion of the ethanol-soluble carbohydrates in healthy and infected lamina samples. While consistently high levels of myo-inositol were recorded in healthy lamina samples, there was no apparent seasonal fluctuation. In the pustule and region adjacent levels of myo-inositol fluctuated without a clear seasonal variation.

Phenolic Glycosides:

Levels of phenolic glycosides are shown for samples of Seasons 1 and 2. During Season 3 phenolic glycosides were removed, as described in Section 5 (7B). The levels of phenolic glycosides (peaks B and C) were estimated in relation to sucrose and these values are shown in Table 11. There appeared to be no seasonal fluctuation of these compounds in both healthy and infected tissue. Mean values of phenolic glycosides were calculated for Season 2 samples, with the standard error of the means, and the levels of phenolic glycosides in the pustule were compared with levels in the healthy lamina and region adjacent, using the t-test. It appeared that the pustule region contained lower levels of phenolic glycosides than the region adjacent and healthy lamina, but mean levels in the pustule did not differ significantly (at the 0,05 level) from mean levels in the healthy lamina and region adjacent.

Table 11:

Levels of phenolic glycosides in healthy and infected poplar leaves during Seasons 1 and 2. Levels were calculated in relation to sucrose in mg g^{-1} dry weight. Mean levels of phenolic glycosides were calculated for Season 2 samples, showing the standard error of the means (S.E.M.).

Key: t = trace amount.

Sample	Phenolic Glycoside Levels (mg g^{-1} dry weight, in terms of sucrose)		
	Healthy	Pustule	Region Adjacent
Season 1:			
Nov. 1971	4,95	no infection	no infection
Dec. 1971	3,65	" "	" "
Jan. 1972	3,08	" "	" "
Feb. 1972	4,17	0,60	2,55
Apr. 1972	t	3,10	14,90
May. 1972	all infected	4,04	2,57
Season 2:			
Apr. 1973	2,88	3,10	4,69
May 1973	14,86	5,82	9,43
June 1973	12,31	21,33	28,12
July 1973	7,82	7,23	10,34
Aug. 1973	19,20	7,88	18,53
Oct. 1973	14,44	0,97	1,49
Mean ± S.E.M. for samples of Season 2.	11,92 ± 2,36	7,12 ± 2,92	12,10 ± 3,98

5(9) SEASONAL VARIATION IN POLYSACCHARIDE LEVELS

Hot Water-Soluble Polysaccharide:

Levels of hot water-soluble polysaccharide were studied during Season 2 in the Claremont population. In the healthy and infected lamina (Figure 20 (a)) glucose constituted the greater proportion of hydrolysis products. Mannose levels were higher in the pustule than in the region adjacent and the healthy lamina. Arabinose, galactose, mannose and xylose were present in lower proportions, whereas rhamnose occurred mainly in small amounts (Table 12).

In the healthy lamina there were higher levels of combined carbohydrate during April-June than during July-October (Figure 20 (a)). In the pustule and region adjacent the combined levels of monomer components were lower than in the healthy tissue and declined during July-October. While glucose levels decreased in the pustule, mannose levels were similar throughout the season.

Cold Water-Soluble Polysaccharide:

Levels of cold water-soluble polysaccharide were studied during Season 2 in the Claremont population.

The major monomer component of the cold water-soluble polysaccharides (Figure 20 (b)) isolated from the poplar lamina was glucose. Lower proportions of galactose, mannose and arabinose occurred (Table 13), with rhamnose and xylose mainly in trace amounts.

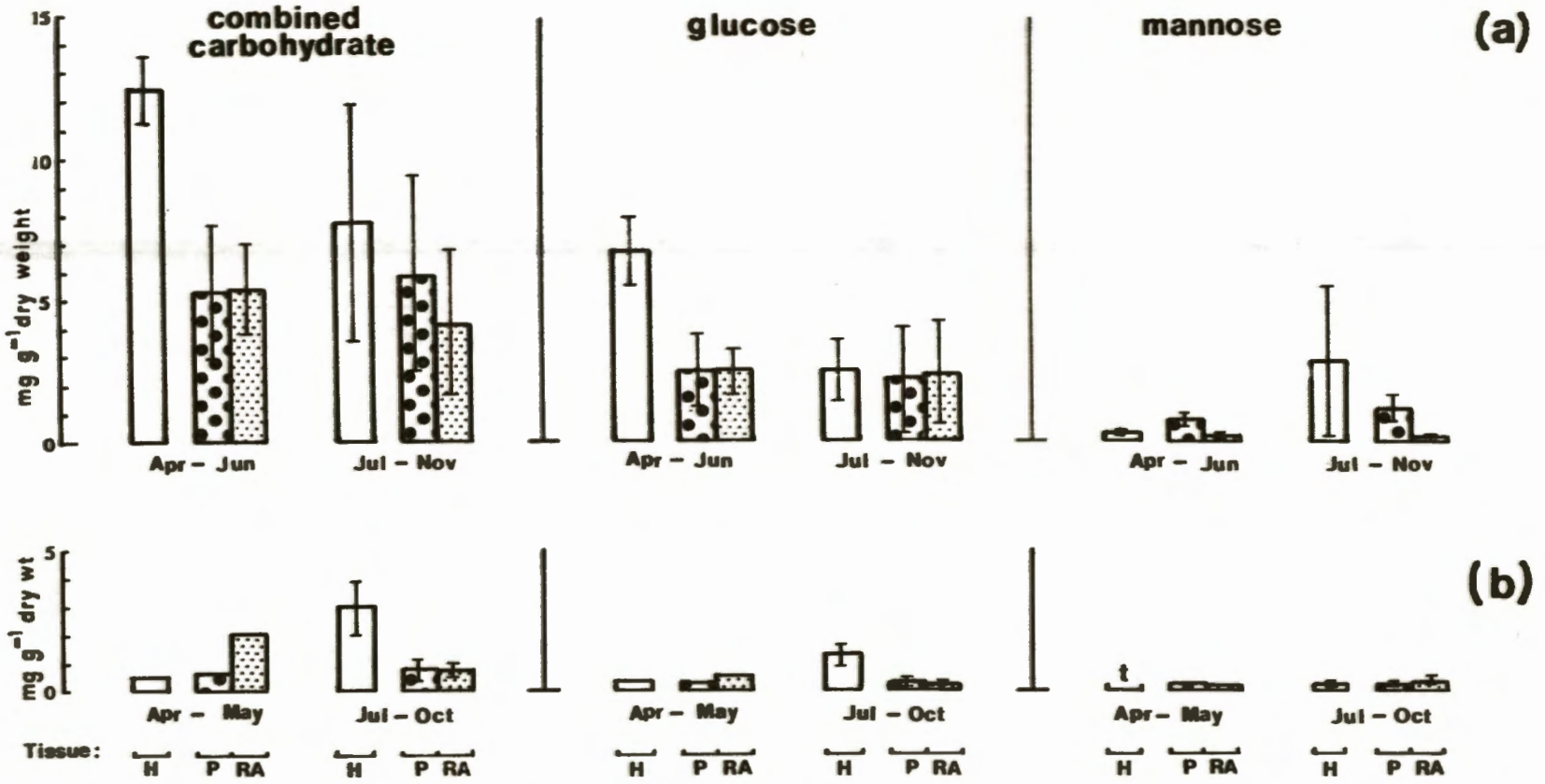


Figure 20:

Seasonal levels of acid-hydrolysed (a) hot water-soluble and (b) cold water-soluble polysaccharide extracted from lamina samples of healthy and rust-infected poplar leaves of Season 2, during April to October/November 1973. Carbohydrate levels (mg g^{-1} dry weight) shown are combined carbohydrate (totalled levels of glucose, mannose, xylose, arabinose, rhamnose and galactose) and individual levels of glucose and mannose. Hot water-soluble polysaccharide samples were divided into two groups, April-June and July to November. Cold water-soluble polysaccharide samples were divided into two groups, April -May and July-October. The carbohydrate levels of grouped samples are shown as mean values with ± standard error of the mean.

Key: ☐ healthy lamina; ⊠ pustule; ▨ region adjacent to pustule; t - trace amount.

Table 12:

Seasonal levels of acid-hydrolysed hot water-soluble polysaccharide extracted from lamina samples of healthy and rust-infected poplar during April to November, 1973.

Notation: t = trace amount.

	MOL - %					
	Arabinose	Rhamnose	Xylose	Mannose	Galactose	Glucose
HEALTHY LAMINA						
April	37,73	5,59	6,61	2,66	7,98	39,39
May	29,84	4,01	4,01	1,46	8,33	52,35
June	19,73	t	8,27	1,68	12,07	58,25
July	11,98	1,40	5,29	3,72	15,95	61,65
October	33,95	t	7,13	5,95	17,70	35,27
November	21,39	5,49	5,14	3,76	11,87	53,18
PUSTULE						
April	17,59	6,32	9,09	9,50	9,60	47,89
May	17,62	4,59	4,97	2,09	11,01	40,91
June	15,41	1,79	7,38	16,03	8,98	50,42
July	13,75	1,72	6,28	12,96	12,76	52,53
October	t	t	t	20,67	24,86	54,47
November	15,19	6,98	7,27	12,02	12,31	46,51
REGION ADJACENT PUSTULE						
April	32,56	6,63	12,99	2,44	13,36	32,01
May	20,78	5,37	11,64	3,96	14,13	44,12
June	12,45	t	7,34	3,73	14,34	62,14
July	22,16	2,03	9,72	3,95	13,51	48,79
October	29,96	8,95	6,09	3,89	13,49	37,61
November	13,38	5,92	5,06	3,01	10,08	62,56

Table 13:

Seasonal levels of acid-hydrolysed cold water-soluble polysaccharide extracted from lamina samples of healthy and rust-infected poplar during April to October, 1973.

Notation: t = trace amount.

	MOL - %					
	Arabinose	Rhamnose	Xylose	Mannose	Galactose	Glucose
HEALTHY LAMINA						
April	15,49	-	11,69	28,36	21,64	22,81
May	t	-	t	t	t	100,00
July	20,78	t	7,61	4,87	27,02	39,71
August	18,77	7,73	8,52	5,21	21,14	38,64
October	t	-	-	t	57,19	42,81
PUSTULE						
April	10,69	-	-	8,95	14,63	65,72
May	-	-	-	60,18	16,36	21,82
July	7,63	2,02	2,88	9,07	31,10	47,30
August	t	t	t	52,24	22,86	24,90
October	t	-	t	38,51	43,37	18,12
REGION ADJACENT PUSTULE						
April	13,68	6,83	8,70	4,01	15,44	51,34
May	-	-	-	61,82	16,36	21,82
July	19,54	5,19	5,03	3,38	15,91	50,95
August	t	t	t	54,52	19,71	25,77
October	t	-	t	65,26	24,03	10,71

When the absolute quantities of carbohydrates were considered, it was evident that the pustule and region adjacent to it contained very small amounts of polysaccharide in comparison with the healthy lamina. Cold water-soluble polysaccharide showed a similar decline in levels from April-June to July-October, as shown by hot water-soluble polysaccharide levels. Mannose-containing polymers occurred in variable proportions throughout the season in pustule and region adjacent samples, comprising up to 65% of the carbohydrate content of these samples (Table 13), whereas the absolute quantities were very low. Mannose occurred in lower proportions (Table 13) in the healthy lamina than in infected tissue, whereas arabinose proportions were higher than in infected leaves, but no apparent seasonal fluctuation was observed. Levels of cold water-soluble polysaccharide are tabulated in Appendix 6 (b).

Ethanol-insoluble Fraction:

Levels of ethanol-insoluble components were investigated during Season 3 in the Hohenort Estate population.

The ethanol-insoluble fraction (Table 14) yielded a high proportion of arabinose and xylose monomers in the lamina samples. Glucose and galactose occurred in considerably lower quantities while mannose constituted a larger proportion in the laminas, especially in the pustule region. Rhamnose was present in trace amounts in the healthy lamina but occurred in greater proportions in the pustule and region adjacent.

Table 14:

Seasonal levels of acid-hydrolysed ethanol-insoluble polysaccharide of lamina samples of healthy and rust-infected poplar during November, 1973, to May, 1974.

Notation: t = trace amount.

	MOL - %					
	Arab-inose	Rham-nose	Xy-lose	Man-nose	Galac-tose	Glu-cose
PUSTULE						
November	47,15	10,07	13,38	4,90	9,80	14,70
January	51,25	t	25,59	2,35	14,49	6,32
March	28,27	t	25,15	19,41	15,53	11,65
April	19,24	4,47	26,89	20.19	17,14	12,06
May	28,45	4,91	30,61	13,18	18,03	9,87
REGION ADJACENT PUSTULE						
November,	45,33	7,97	15,53	4,30	9,33	17,52
January	59,34	t	16,57	t	17,51	6,58
March	22,23	t	27,61	1,58	29,82	18,76
April	25,60	t	39,89	5,14	20,46	8,91
May	23,41	t	37,71	9,36	21,15	8,36
HEALTHY LAMINA						
November	50,46	t	3,09	t	20,68	25,77
January	63,56	t	13,25	3,56	12,76	6,87
March	35,74	t	37,09	3,27	12,63	11,27
April	26,04	t	40,21	10,03	19,38	4,34
May	56,09	t	20,43	1,25	13,26	8,96

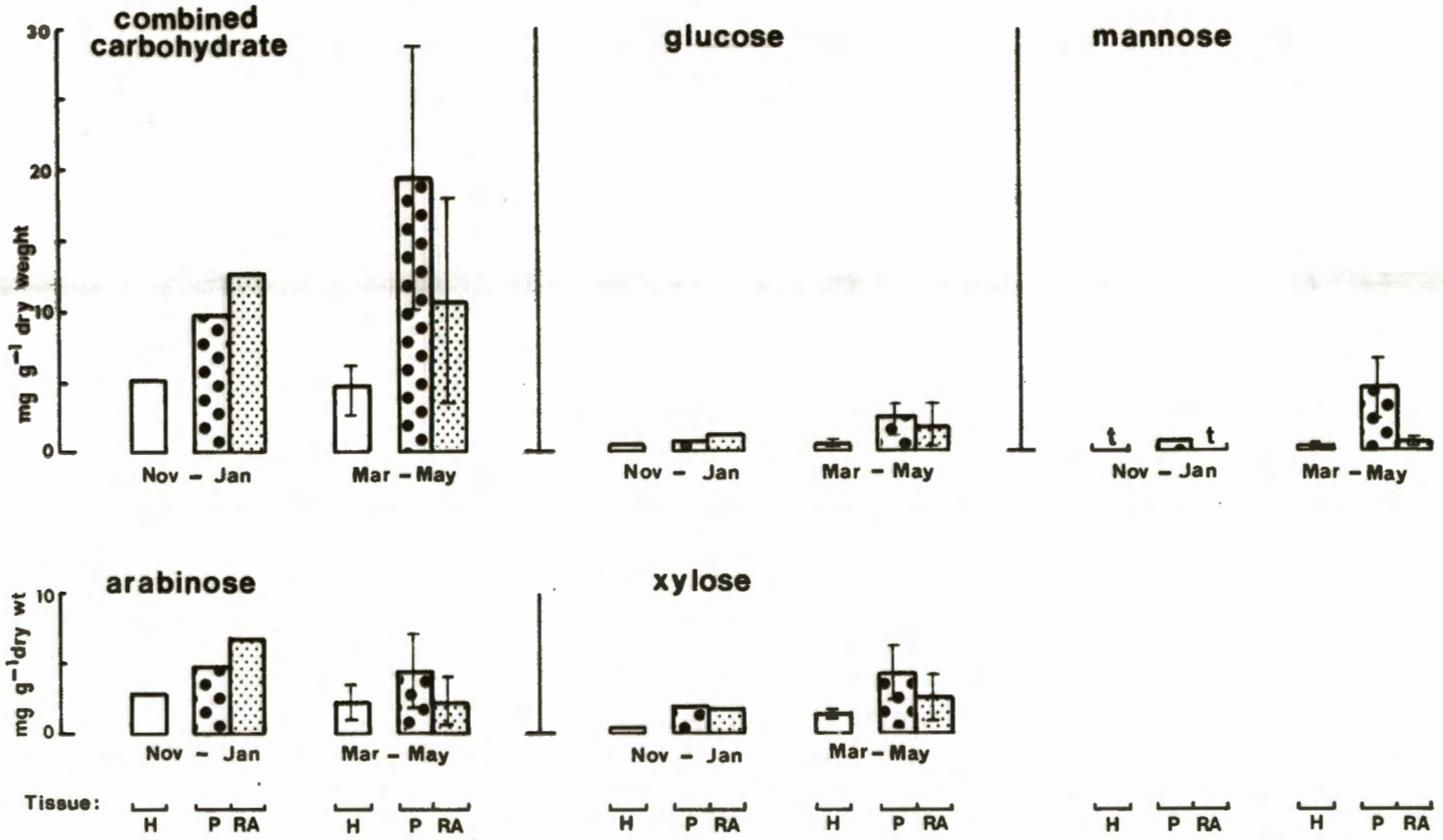


Figure 21:

Seasonal levels of acid-hydrolysed ethanol-insoluble polysaccharide extracted from lamina samples of healthy and rust-infected poplar leaves during November 1973 to May 1974. Carbohydrate levels (mg g^{-1} dry weight) shown are combined carbohydrate (totalled levels of glucose, mannose, xylose, arabinose, rhamnose and galactose) and individual levels of glucose, mannose, arabinose and xylose. Carbohydrate levels shown are mean values of monthly samples grouped into 2 to 3 month periods. ± Standard error of the mean is shown for periods consisting of 3 monthly samples.

Key: ☐ healthy lamina; ⊡ pustule; ▨ region adjacent to pustule; t - trace amount.

The ethanol-insoluble carbohydrate distribution (Figure 21) showed a similar decline from the start of the study period (November/January) to the end (March to May), when compared with hot and cold water-soluble levels (Figure 20). However, ethanol-insoluble combined carbohydrate levels were higher in the pustule region than in the healthy lamina, which contrasted with the higher combined carbohydrate levels in the hot and cold water-soluble polysaccharide fractions of the healthy lamina (Figure 20). The pustule region contained considerably higher levels of mannose than the region adjacent or the healthy lamina. The pustule and region adjacent contained slightly higher levels of glucose, arabinose and xylose than the healthy lamina. The low levels of glucose in the healthy lamina were interesting when compared with the greater quantities present in the hot and cold water-soluble polysaccharide fractions.

5(10) CONCLUSIONS

The most abundant ethanol-soluble carbohydrates in healthy and rust-infected poplar leaves were sucrose and myo-inositol. Glucose and fructose levels were lower than those of sucrose and myo-inositol. In infected tissue the polyols arabitol and mannitol occurred consistently in the pustule region and occasionally in the region adjacent to the pustule. Polyols were first detected when sporulation had commenced and it was shown that these compounds

were located mainly in the urediniospores. A very small amount of trehalose was found in the urediniospores. Polyol levels in the pustule were very low, the mean values for the 3 seasons being 1,19 ± 0,2 mg g^{-1} dry weight of arabitol and 1,64 ± 0,2 mg g^{-1} dry weight of mannitol. The proportion of polyols to the other ethanol-soluble carbohydrates in the pustule region did not exceed 24%. In contrast the pustules of Puccinia poarum Niels. on Tussilago farfara L. contained levels of mannitol and arabitol which constituted more than half of the non-polymeric carbohydrate in each disc containing mature aecia (44). Studies of the carbohydrates of other rusts occurring in the south western Cape showed that polyol levels in pustules of rust-infected pelargonium and hollyhock were 28,7% and 31,9% of the total ethanol-soluble carbohydrate respectively (78).

The very low levels of trehalose in the pustule samples were significant since trehalose is considered to be a common constituent of the soluble sugars of rust fungi. The apparent occurence of trehalose in healthy tissue was viewed with reservation because trehalose is not commonly believed to occur in Angiosperms, although evidence for its presence in cambial sap of Fagus sylvatica L. (88) and non-infected hypocotyls of cabbage (55) was published. However, subsequent work on hypocotyls of cabbage disproved this claim (36).

A potentially important carbon source in healthy and infected

poplar was myo-inositol which constituted up to 55% of total ethanol-soluble carbohydrate in the healthy lamina and up to 60% in the pustule, results which are similar to those of Holligan et al. (44).

The identification of disaccharides was complicated by the presence of phenolic glycosides which eluted with retention times similar to those of disaccharides on GLC chromatograms. The presence of phenolic glycosides in poplar stimulated interest in the possible rôle of these compounds. Hopkinson (47) suggested that the compounds performed a major rôle as a food reserve; however, the participation of phenolic compounds in the interaction between pathogen and host in many diseases has been known for some time. Phenolic glycosides have been found to accumulate during infection and there may be a relationship between resistance to the rust pathogen and the levels of the phenolic compounds. Khapli wheat, resistant to most rust races, was characterised by high phenol levels compared to the low endogenous phenol content of susceptible reaction types such as Little Club (56). In poplar, rust-infected tissues contained lower levels of phenolic glycoside than healthy tissues, but the differences were not significant. It was observed that the major phenolic glycoside peak was always present in all samples in quantities comparable to sucrose.

Levels of sucrose were the most variable during the three seasons studied. Sucrose levels appeared to be influenced by the degree of shading of the trees during Seasons 1 and 3,

since both populations were shaded to different degrees. The sunny aspect of population 2 which was not shaded by larger trees may have accounted for the high sucrose levels. The surprisingly low values in population 3 were probably due to the heavy shading during most of the day. The different extraction procedures employed should not have been responsible for the differences in sucrose levels, but the clearing procedures employed in Season 3, using activated charcoal, could have removed some sucrose, (see section 5:7).

There was a seasonal fluctuation of ethanol-soluble carbohydrate in healthy and infected poplar. Before the eruption of pustules was observed a decrease in sucrose occurred (Season 1), but after the pustules had erupted the levels of sucrose slowly rose in infected tissues to a second peak in late summer (April). Levels of hexoses in the pustule region did not appear to exceed the levels in the healthy lamina, but the region adjacent to the pustule frequently showed higher levels of hexoses and of sucrose. Polyol levels increased to a maximum in the pustule in May (Season 1) by which time all the leaves in the population were infected. At this time hexoses and sucrose had dropped to fairly low levels.

The population studied during Season 2 was infected at the beginning of the investigations, so that a study of healthy leaves before initiation of infection was not possible. The exceptionally high values for sucrose were observed in

all lamina samples, in particular in the region adjacent to the pustule. Hexose levels during Season 2 were low, with only one peak value in October in the new spring growth. When these values were compared with hexose values of Season 3 samples, it was noted that the pustule contained levels approximately similar to those in the region adjacent and the healthy lamina. The levels of polyols in the pustule were similar during the 3 seasons.

Water extraction of plant tissues is known to remove various cell wall polysaccharide components such as pectins (87), as well as some intracellular polysaccharides such as starch and glycogen. Therefore, the presence of starch in the hot water-soluble fraction could not be proved on the basis of the presence of glucose-containing polymers alone, since not all forms of starch, such as amylopectin, are necessarily soluble in hot water. The pectin polysaccharides consisted of mixtures of neutral (arabinogalactans) and mainly acid (galacturonorhamnans) polymers which after acid hydrolysis could account for the presence of arabinose, galactose and rhamnose in the water extracted fractions. Most of the uronic acids were assumed to have been removed by deionisation.

The major component in the hot and cold water-soluble polysaccharides was a glucose-containing polymer. In the hot water-soluble fraction an arabinose-containing polymer was commonly found and in the cold water-soluble fraction a galactose-containing polymer occurred in high proportions.

A mannose-containing polymer formed a significant proportion of the extracts of the pustule region in both hot and cold water-soluble fractions. Ethanol-insoluble mannose levels were particularly high during the early part of the season, coinciding with high infection density. The occurrence of mannose-containing polymers at the site of infection has been noted in other rust diseases (44, 78) and mannose-containing polymers in the beech mycorrhiza association (60) were shown to be of fungal origin. However, in these studies ethanol-insoluble mannose was detected in healthy tissue of the poplar lamina. Hot and cold water-soluble glucose-containing polymers were, however, more abundant in the healthy lamina and region adjacent to the pustule than in the lesion area itself. Ethanol-insoluble glucose-containing polymers occurred in greater amounts in the lesion area.

There was little seasonal variation in all the polysaccharide components apart from the gradual decline in levels during the study period. The higher levels of total ethanol-insoluble carbohydrate in the pustule than in the region adjacent to it or the healthy lamina were of considerable interest and were possibly linked with the dry weight increases observed in the pustule (Chapter 4).

During the course of the three seasons the populations exhibited no altered leaf morphology as a result of infection. The leaves remained green after infection, apart from chlorosis on the upper surface within the pustules (see

Plate 1), and usually retained a healthy appearance until the onset of senescence. When leaves were very heavily infected, a more rapid yellowing occurred and these leaves senesced more rapidly than healthy leaves.

CHAPTER 6.

STUDIES OF MOVEMENT OF ^{14}C-ASSIMILATE

6(1) INTRODUCTION

In view of the preceding evidence on the poplar rust association (Chapters 4 and 5), it was considered necessary to study the movement of ^{14}C-assimilate in the healthy and infected poplar before any conclusions could be made about the nature of the relationship. Since most ^{14}C-studies on other rust infections appeared to confirm the rôle of the pathogen as a strong "sink" for host assimilate (27, 67, 90, 126), these studies were planned to reveal whether the poplar rust behaved similarly. Three main trends in host/obligate biotroph interaction were to be verified. These were -

(a) the alteration in location and levels of assimilate in the immediate site of infection;

(b) the effect of the pathogen on translocation of assimilate from infected leaves to the rest of the shoot,

(c) the build-up of ethanol-soluble and ethanol-insoluble compounds in and around the pustule.

6(2) THE EFFECT OF THE PATHOGEN ON THE MOVEMENT OF ^{14}C-LABELLED ASSIMILATE

To find out whether poplar rust formed a "sink" for host assimilate, an artificial "sink" was created by covering the lower half of the infected lamina of each leaf on the shoot before supplying $^{14}CO_2$ in the light. The method used was similar to that employed by Wang (132) who covered sections of rusted bean leaves with pieces of cardboard. The artificial "sink" was anticipated to compete with the pathogen for assimilate from the exposed portion of the leaf and indicate the pathogen's strength as a "sink". The same procedure was carried out with comparable healthy leaves and these results were compared with similarly treated uncovered leaves. The level of β-radiation in the different areas was assessed by autoradiography and liquid scintillation spectrometry.

A. Autoradiography

Shoots (4 to 5 leaves each) of healthy and infected poplar were cut from saplings of the Claremont population during March and April, usually between 9 00 - 10 00 hours. The infected leaves had a high density

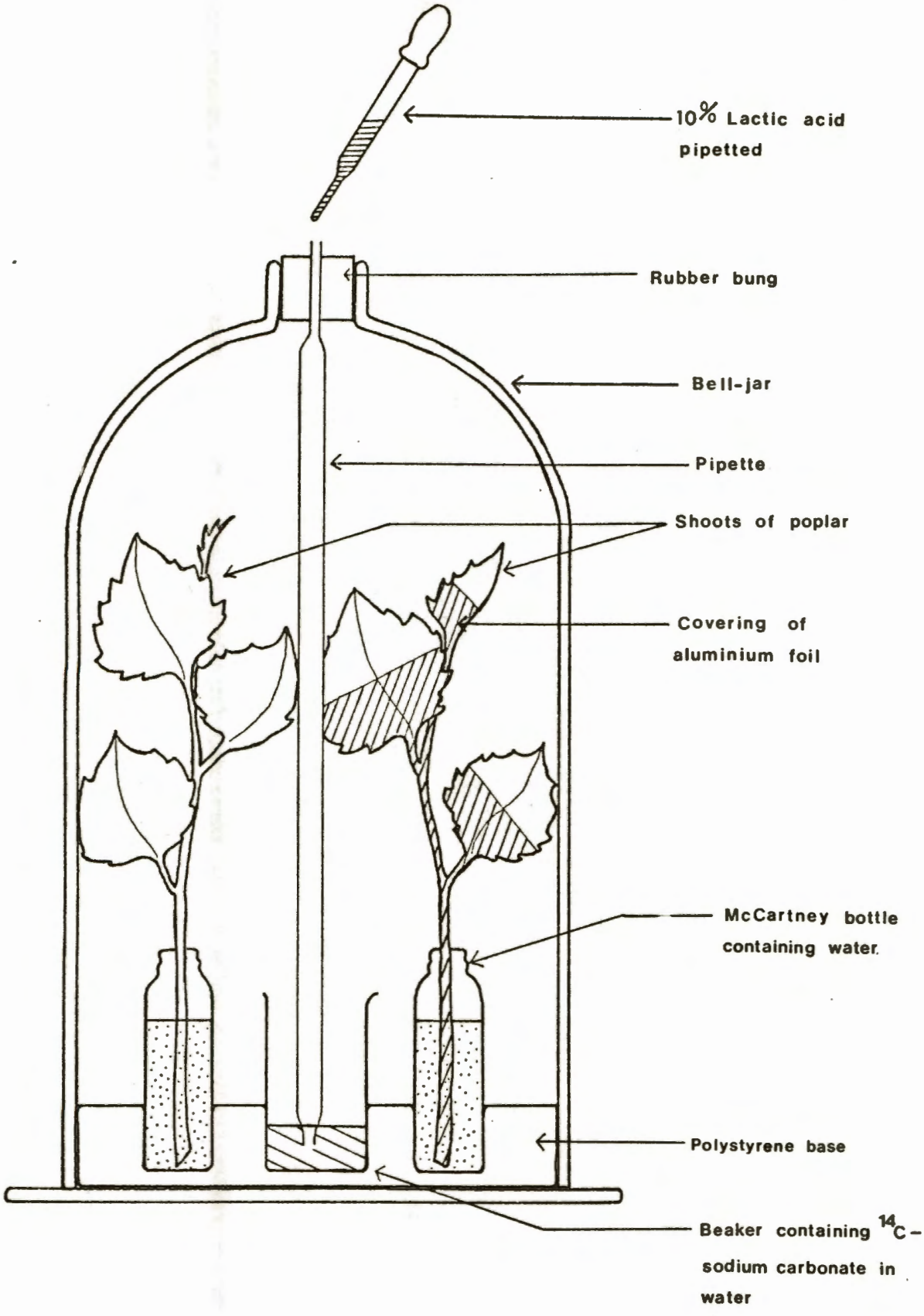


Figure 22:
Method of feeding $^{14}CO_2$ to poplar shoots in a bell jar.

of infection, ranging from 10 to 30 pustules per cm^2 over most of the lamina. The lower portion of the stems of the shoots were immersed in water in McCartney bottles (Figure 22). Using half the material, the lower halves of the laminas, the petioles and the stem were covered with aluminium foil. The central beaker (Figure 22) contained 50 µCi (25µl) ^{14}C-sodium carbonate in distilled water. The bell-jars were placed over the shoots and the shoots left for an hour to equilibrate in the new environment. Four replicate experiments were performed at a light intensity of 2 300 Lux (base of shoot) to 7 500 Lux (shoot apex) under a bank of Hitachi sun-line Ace lamps. After equilibration $^{14}CO_2$ was liberated in the bell-jars by introducing excess 10% lactic acid into the beaker containing ^{14}C-carbonate. During the 30 minute feed of $^{14}CO_2$ the bell-jar was sealed with plasticine. After the $^{14}CO_2$ feed samples were taken of infected and healthy leaves with intact petioles, both control samples and those covered with foil. The apparatus shown in Figure 22 was placed in a draught to remove residual $^{14}CO_2$. The remaining shoots were placed under illumination for a further 24 hours to enable the ^{12}C-fixation products to "chase" the ^{14}C-assimilate. After this period a final sample of leaves was taken.

The foil was removed from the leaves which were carefully drawn (Figures 23 (a) and (b)) to show the position

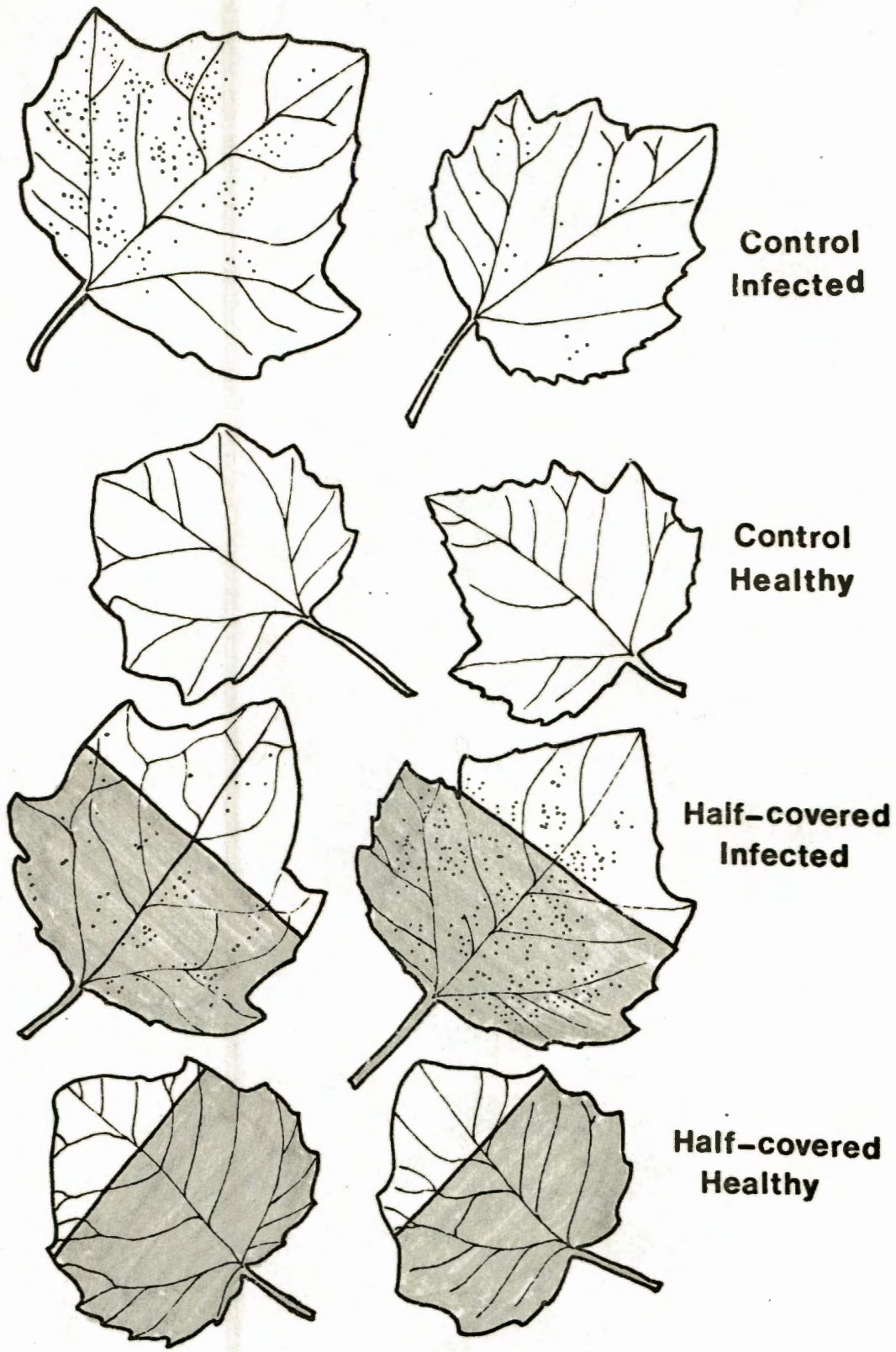


Figure 23(a)

Outlines of leaves which were exposed to $^{14}CO_2$ for 30 minutes, showing the position of pustules and the areas shaded with aluminium foil (▭). Autoradiographs are shown in Figure 24(a).

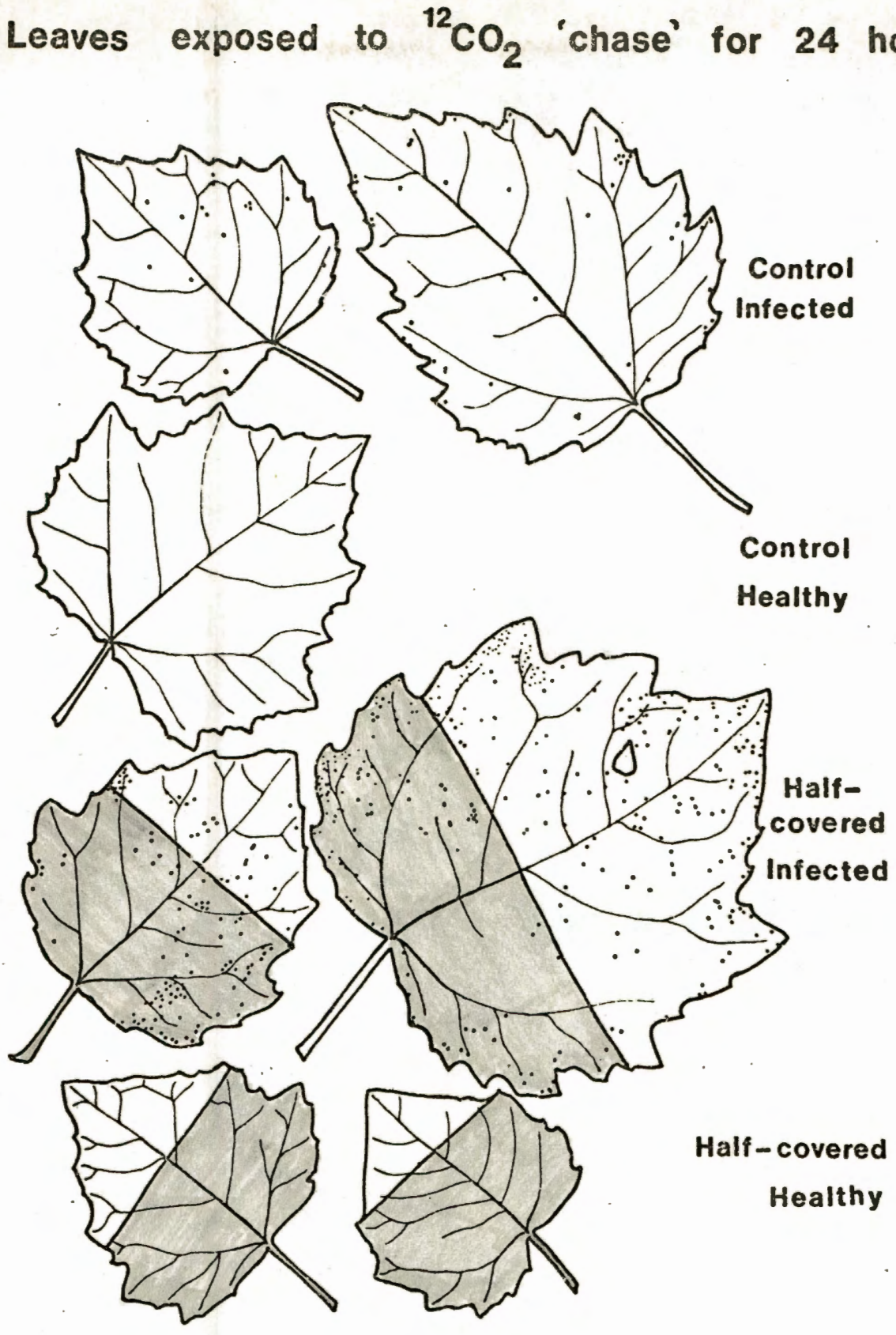


Figure 23(b):
Outlines of leaves incubated for 24 hours after the 30 minute feed of $^{14}CO_2$, showing the position of pustules and areas shaded with aluminium foil (▭). Autoradiographs are shown in Figure 24(b).

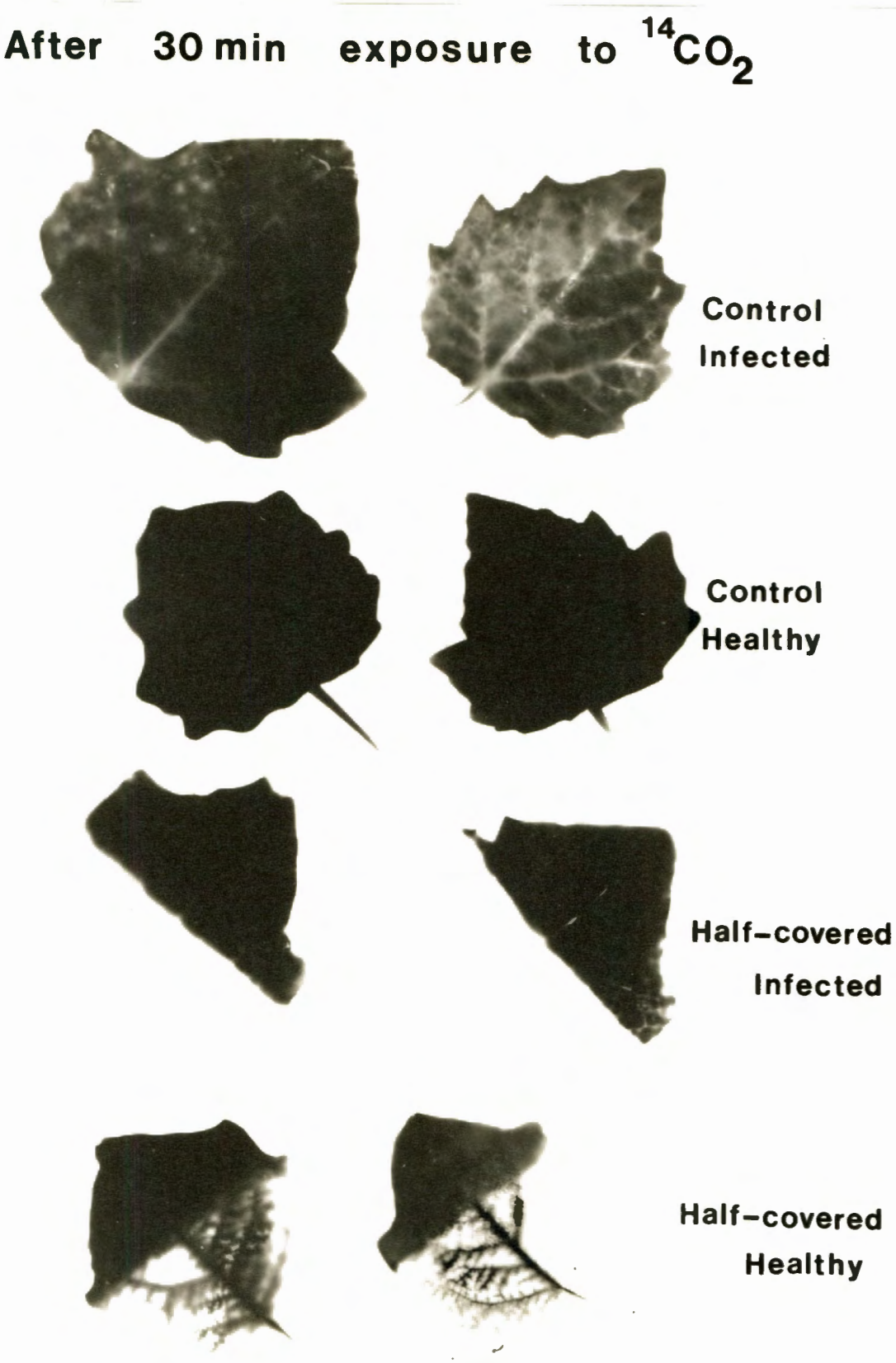


Figure 24 (a):
Autoradiographs of leaves exposed to $^{14}CO_2$ for 30 minutes.

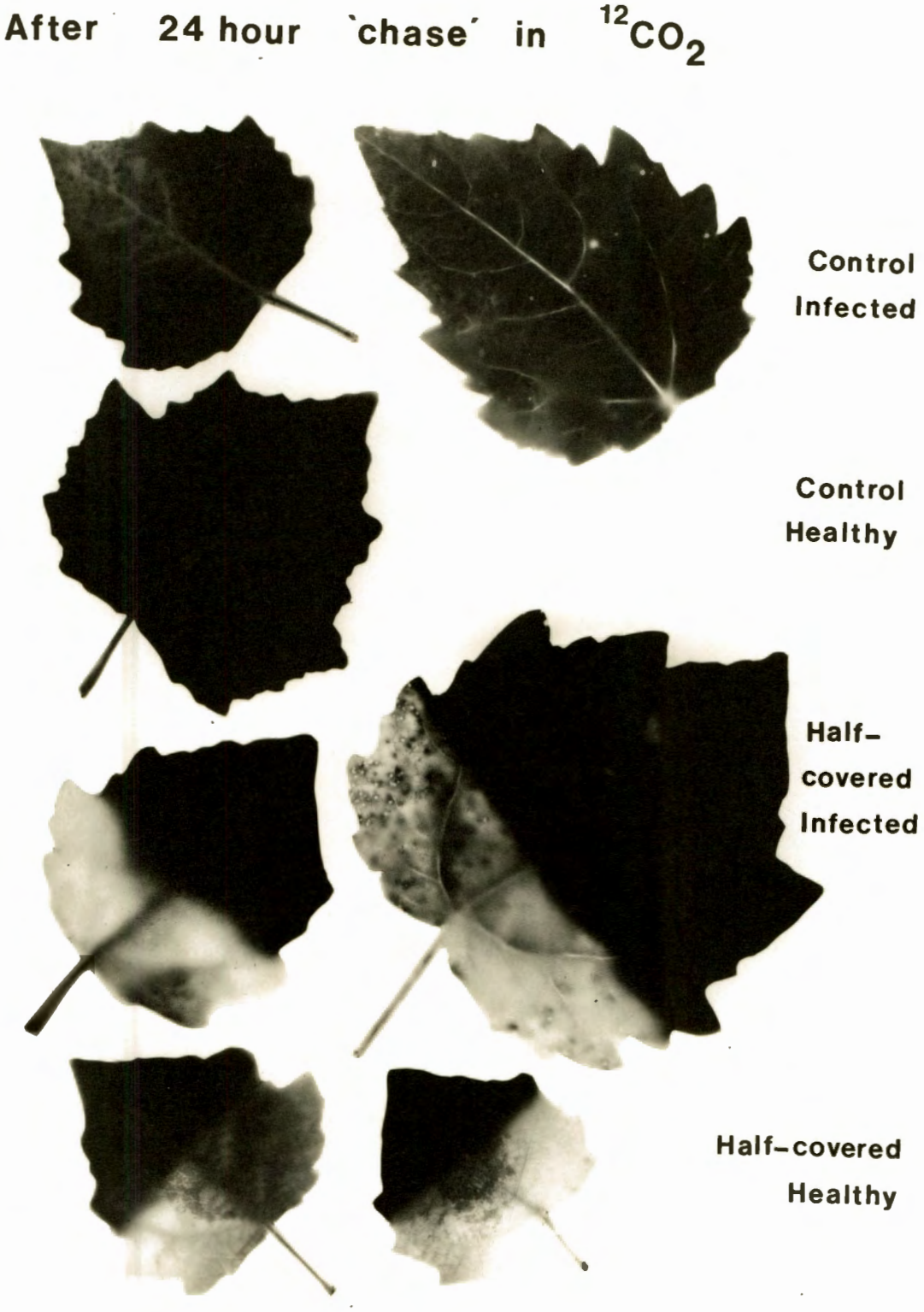


Figure 24 (b):

Autoradiographs of leaves incubated for 24 hours after a 30 minute exposure to $^{14}CO_2$.

of the pustules. The leaves were then placed between sheets of paper and pressed flat between large petri dishes. The leaves were either freeze-dried or oven-dried at 60 - 70°C, after which they were compressed against X-ray film (Kodak RP Xomat) in light-tight containers for 8 days. The plates were developed with Kodak rapid X-ray developer and Kodak X-ray fixer and the clearest autoradiographs were selected from the four replicates (Figures 24 (a) and (b)).

Results of the Autoradiography

The autoradiographs (Figure 24 (a)) showed that after 30 minutes exposure to $^{14}CO_2$ the covered halves of the infected leaves contained little radioactivity whereas in similar regions of healthy leaves ^{14}C accumulated mainly in the midrib, main veins and petioles. The density of radioactivity in the healthy control leaves indicated that healthy leaves assimilated far more $^{14}CO_2$ than the infected control leaves, which showed an apparently lower density of radioactivity. Pustule regions were revealed as faint, pale spots after 30 minutes exposure, in contrast to the dense labelling of pustules in rusted pelargonium in a similar experiment (Roberts and Mitchell (96)).

After the 24 hour "chase" in $^{12}CO_2$ (Figure 24 (b)), the infected control showed high levels of β-radiation in

the pustules, However, the covered portion of the infected lamina showed very little radioactivity in the areas corresponding to the pustules. The major localisation of radioactivity occurred in the midrib, main veins and petiole. Levels of β-radiation were higher in regions surrounding the pustules and in a few small young pustules. There was a similar degree of labelling in both healthy and infected covered petioles.

Autoradiography revealed that some deposition of radioactivity occurred in tissues surrounding the pustule, after the 24 hour "chase", but that there was no clear effect after 30 minutes exposure to $^{14}CO_2$. Levels of translocated assimilate did not vary greatly between healthy and infected petioles. It was concluded that the infected leaf did not retain labelled assimilate to a greater extent than comparable healthy tissue.

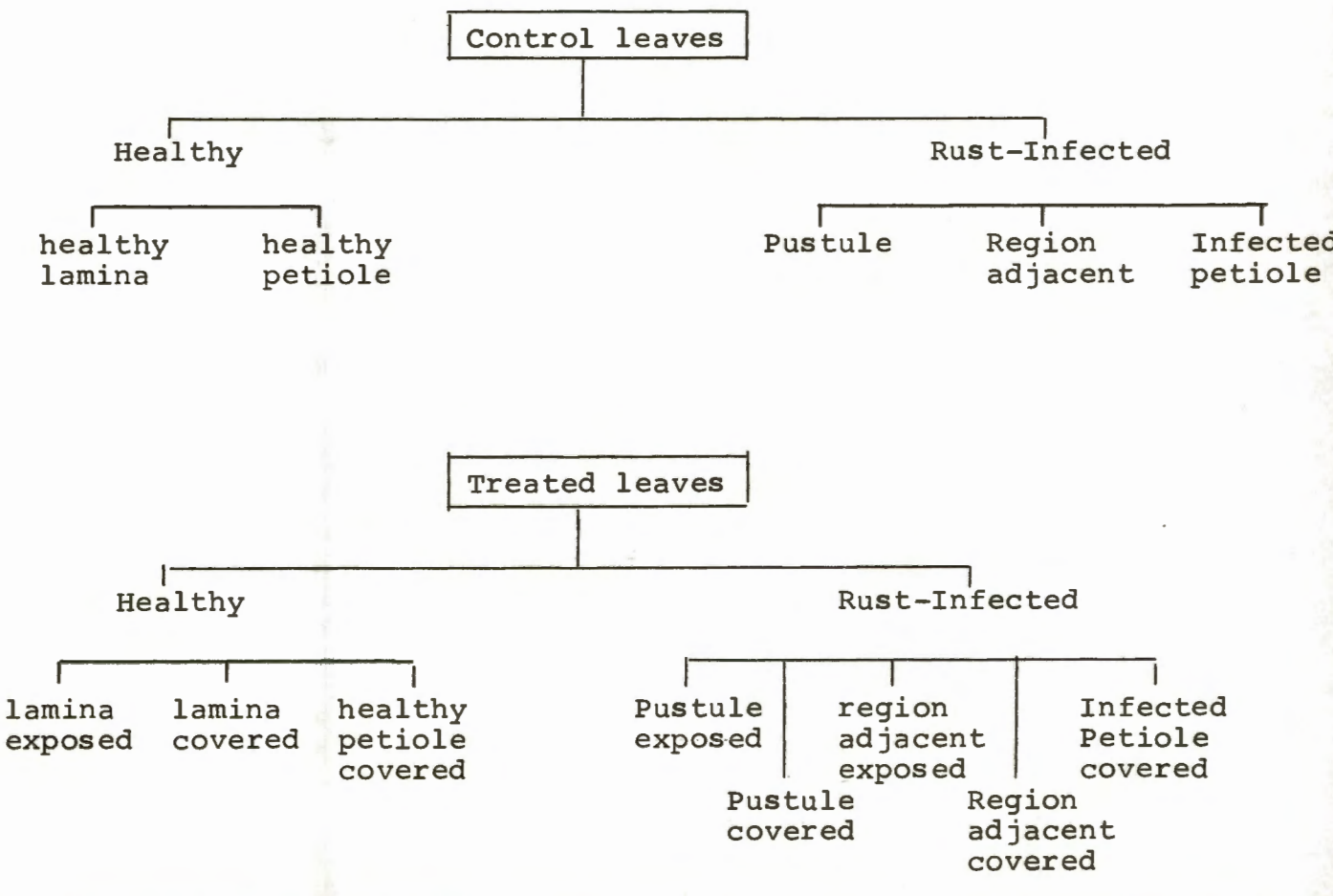
B. Liquid-Scintillation Spectrometry

Since the evidence provided by the autoradiographs was not conclusive without supporting quantitative data, the experiments described in Section A were repeated and ^{14}C-assimilation was analysed by means of liquid scintillation spectrometry using a Beckman LS 150 liquid scintillation counter.

Using procedures similar to those in the preceding section, infected and healthy shoots of poplar were fed $^{14}CO_2$ for 30 minutes and subjected to a 24 hour "chase" in $^{12}CO_2$. After each period of time leaves were removed from the shoots and sub-divided as shown in Figure 25. The pustule and region adjacent were sampled as described in Chapter 4 (2). The region adjacent to the pustule consisted of areas of the infected leaf not occupied by erupting pustules.

Figure 25:

The division of healthy and rust-infected control leaves and healthy and rust-infected leaves treated by partial covering with aluminium foil into samples for further analysis, after 30 minutes exposure to $^{14}CO_2$ and the 24 hour "chase" in $^{12}CO_2$.



Each sample (100 - 200 mg) was weighed and killed by immersion in boiling 80% ethanol. The samples were homogenised in 80% ethanol with an MSE homogeniser, refluxed for one hour and then centrifuged at 5 000 x g. The supernatant was transferred into scintillation vials, blown dry with a stream of air and redissolved in 1 ml distilled water. The pigment was removed in order to reduce colour quenching. An aliquot of diethyl-ether was added to each sample and the mixture was shaken vigorously. The aqueous phase was allowed to settle and was frozen at -40°C after which the diethyl-ether containing the pigment was poured off.

The ethanol-insoluble fractions were air dried and hydrolysed in 2 N trifluoroacetic acid under reflux for one hour. The acid was removed by means of a rotary evaporator, the hydrolysate dissolved in 5 ml distilled water and filtered into scintillation vials. The solution was reduced to dryness and redissolved in 1 ml of distilled water.

Both ethanol-soluble and acid hydrolysed ethanol-insoluble fractions were blended with 10 ml of scintillation cocktail, Instagel (Packard Instruments). Some of the ethanol-insoluble samples showed a colour quenching due to the reddish-brown hydrolysis products which were not removable. It was found that 1/10 dilution reduced quenching considerably without increasing the % error in counting, since the level of labelling was

sufficiently high.

In the preliminary experiments (Series 1), levels of radioactivity recovered from the leaves were expressed in terms of fresh weight. In a further experiment (Series 2), performed to confirm the results of Series 1, levels of radioactivity recovered were expressed in terms of fresh weight, leaf area and in terms of total radioactivity per leaf.

Series 1:

Four replicate experiments were performed, replicates 1 and 2 under light banks at 2 300 Lux (base of shoot) to 7500 Lux (shoot apex) at temperatures of 27°C and 27,5°C. Some control leaves of replicate 2 wilted at these temperatures so that lower temperatures were used in subsequent experiments to prolong the turgidity of the shoots. Replicate experiments 3 and 4 were conducted in a Conviron (controlled environment) growth chamber at 25°C (relative humidity 90 - 100%) with the light intensity varying from 18 000 Lux (base of shoot) to 20 000 Lux (shoot apex). The illumination was provided with incandescent Sylvania 100 W bulbs and fluorescent VHO Powertube 48" cool white (Sylvania, USA) strip lighting. The averaged results of replicates 1 and 2 are shown in Table 15 and of replicates 3 and 4 in Table 16. Results were expressed as disintegrations per minute (DPM) per 100 mg fresh weight of sample.

Table 15:

Mean levels of radioactivity (DPM x 10^3 per 100 mg fresh weight), for replicates 1 and 2 (Series 1), in lamina and petiole samples of healthy and rust-infected poplar.

Illumination: 2 500 - 7 500 Lux.

Key: Eth-sol = ethanol-soluble fraction
Eth-insol = ethanol-insoluble fraction.

SAMPLE		30 minutes exposure to $^{14}CO_2$		24 hour "chase"	
		DPM x 10^3 per 100 mg fresh weight			
		Eth-Sol	Eth-Insol	Eth-Sol	Eth-Insol
HEALTHY					
Control Leaves	Lamina	169,4	59,9	291,9	86,9
	Petiole	0,8	0,3	48,9	5,9
Treated Leaves	Exposed Lamina	214,2	91,2	140,6	51,3
	Covered Lamina	6,1	5,1	18,3	5,8
	Petiole	0,4	0,2	24,2	3,4
INFECTED					
Control Leaves	Pustule	99,2	40,8	193,9	78,4
	Region Adjacent	135,3	69,4	202,8	75,6
	Petiole	0,4	0,3	36,7	4,5
Treated Leaves					
Exposed	Pustule	132,0	103,9	204,9	109,8
	Region adjacent	185,6	121,2	198,3	92,8
Covered	Pustule	7,8	11,5	33,4	8,8
	Region Adjacent	10,2	8,4	25,9	10,2
	Petiole	0,2	0,2	30,7	2,5

Table 16:

Mean levels of radioactivity (DPM x 10^3 per 100 mg fresh weight), for replicates 3 and 4 (Series 1), in lamina and petiole samples of healthy and rust-infected poplar.
Illumination: 18 000 - 22 000 Lux.

Key: Eth-sol = ethanol-soluble fraction;
Eth-insol = ethanol-insoluble fraction.

		30 minutes exposure to $^{14}CO_2$		24 hour "chase"	
		DPM x 10^3 per 100 mg fresh weight			
		Eth-sol	Eth-insol	Eth-sol	Eth-insol
HEALTHY					
Control leaves	lamina	343,1	199,8	951,2	282,8
	petiole	4,9	3,6	46,0	6,6
Treated leaves	exposed lamina	2670,8	2423,2	2011,2	374,9
	covered lamina	410,5	226,7	318,6	39,4
	covered petiole	4,8	1,4	241,3	61,4
INFECTED					
Control leaves	pustule	849,0	484,9	476,9	472,5
	region adjacent	873,3	710,7	470,0	645,7
	petiole	1,6	1,3	195,0	17,9
Treated leaves					
Exposed	pustule	1097,9	767,4	1406,2	688,5
	region adjacent	1604,9	814,3	1933,1	605,9
Covered	Pustule	119,2	75,9	194,5	76,9
	region adjacent	124,2	87,8	151,1	237,5
	petiole	2,7	3,5	241,9	6,7

Series 2:

A similar experiment was performed under the following environmental conditions: a temperature program of 18 - 19°C (day) and 14 - 15°C (night), relative humidity 90 - 100% and light intensity of 20 000 (base of shoot) to 32 000 Lux (shoot apex).

The % area of healthy and infected laminas which was covered with aluminium foil had a mean value of 54,7% ± 2,0%. Erupting uredosori covered 46,7% ± 4,7% of the total area of infected leaves and the pustule density ranged between 10 - 30 pustules per cm^2. In covered regions of treated leaves, pustule areas formed 50,1% ± 14,1% of the covered region, while in exposed regions the pustule areas formed 43,2% ± 8,7% of the exposed lamina.

Each sample was replicated in triplicate and the results were expressed as (a) DPM per 100 mg fresh weight; (b) DPM per 10 cm^2 leaf area; and (c) DPM in each region expressed as a % of the total DPM in the respective leaf. The triplicated results were expressed as means with standard errors of the means and are shown in Tables 17, 18 and 19. The differences between the means of selected pairs of samples were tested for significance at a 0,05 level with the t-test and those pairs showing significant differences are shown in Table 20.

Discussion of Results

Series 1:

The trends shown in the preliminary experiments are discussed briefly and then compared with the additional data of Series 2. When the two sets of averages (Tables 15 and 16) were compared, it was evident that at the lower light intensity far less ^{14}C-fixation occurred than at the higher levels of illumination. However, the proportion of radioactivity in the different regions in both sets of data was comparable.

The region adjacent in control and treated leaves showed higher ^{14}C-ethanol-soluble and ^{14}C-ethanol-insoluble levels than the pustule after 30 minutes exposure to $^{14}CO_2$. In the covered regions the pustule accumulated less ^{14}C-assimilate than the region adjacent. However, after 24 hours the covered pustules showed greater ^{14}C-ethanol-soluble levels than the region adjacent, while ^{14}C-ethanol-insoluble levels were greater in the covered region adjacent.

It appeared that covering the lower portion of the lamina and petiole caused a drain of ethanol-soluble ^{14}C-assimilate into the petiole, as was revealed by the autoradiographs (Figures 24 (a) and (b)) in both healthy and infected leaves after 24 hours. Levels of

^{14}C-ethanol-soluble components in the covered infected petiole were similar to the ^{14}C-ethanol-soluble components in covered healthy petioles, at both light intensities. This indicated that the rust infection was not impeding translocation in the infected leaf.

After the 24 hour "chase" there were increased levels of radioactivity in some regions of both healthy and infected leaves, particularly in the control lamina and the exposed and covered pustule and region adjacent. Although extreme care was taken to remove $^{14}CO_2$ from the bell jars at the end of each feed, the increased radioactivity after the 24 hour "chase" may be due to assimilation of residual $^{14}CO_2$.

Series 2:

The levels of radioactivity were expressed in terms of fresh weight (Table 17), leaf area (Table 18) and the whole leaf (Table 19). The results confirmed the main trends shown in the experiments of Series 1 and are discussed below.

Since the ^{14}C-levels on a fresh weight and area basis were similar, these results (Tables 17 and 18) were discussed together and were then compared with data on a whole-leaf basis (Table 19). The merits of the three parameters of data comparison were evaluated.

Table 17:

Mean levels of radioactivity (DPM x 10^3 per 100 mg fresh weight) in lamina and petiole samples (Series 2) of healthy and rust-infected poplar. Values show the means of 3 replicates of each sample with standard error of the mean (S.E.M.). Illumination: 20 000 - 32 000 Lux.

Key: Eth-sol = ethanol-soluble fraction;
Eth-insol = ethanol-insoluble fraction.

SAMPLE		30 minutes exposure to $^{14}CO_2$		24 hour "chase"	
		DPM x 10^3 per 100 mg fresh weight			
		Eth-sol ± S.E.M.	Eth-insol ± S.E.M.	Eth-sol ± S.E.M.	Eth-insol ± S.E.M.
HEALTHY					
Control leaves	lamina	177,5 ± 35,9	46,6 ± 5,9	2,2 ± 1,6	23,6 ± 14,0
	petiole	0,5 ± 0,0	0,4 ± 0,0	60,5 ± 19,4	1,8 ± 0,6
Treated leaves	exposed lamina	53,9 ± 11,0	10,2 ± 2,4	91,3 ± 43,8	26,3 ± 1,6
Covered	lamina	7,8 ± 2,7	2,6 ± 1,1	12,0 ± 11,4	2,2 ± 0,6
	petiole	0,2 ± 0,0	0,2 ± 0,0	16,6 ± 5,7	0,7 ± 0,1
INFECTED					
Control leaves	pustule	284,6 ± 99,5	133,3 ± 51,9	94,5 ± 18,6	30,1 ± 7,4
	region adjacent	179,7 ± 60,8	49,5 ± 13,8	59,9 ± 27,6	28,6 ± 9,7
	petiole	0,4 ± 0,1	0,4 ± 0,0	25,5 ± 6,3	2,1 ± 0,5
Treated leaves					
Exposed	pustule	304,3 ± 59,8	94,2 ± 24,1	254,1 ± 45,8	51,9 ± 18,2
	region adjacent	216,7 ± 21,3	107,8 ± 19,4	284,9 ± 84,8	71,9 ± 11,7
Covered	Pustule	32,9 ± 5,4	13,7 ± 2,2	33,5 ± 7,9	4,3 ± 0,6
	region adjacent	65,7 ± 23,4	22,1 ± 7,4	32,7 ± 6,0	6,2 ± 3,5
	petiole	0,5 ± 0,0	0,2 ± 0,0	38,9 ± 3,2	0,8 ± 0,2

Table 18:

Mean levels of radioactivity (DPM x 10^3 per 10 cm^2 leaf area) in lamina samples (Series 2) of healthy and rust-infected poplar. Values show the means of 3 replicates of each sample with standard error of the mean (S.E.M.).
Illumination : 20 000 - 32 000 Lux.

Key: Eth-sol = ethanol-soluble fraction;
Eth-insol = ethanol-insoluble fraction.

SAMPLE		30 minutes exposure to $^{14}CO_2$		24 hour "chase"	
		DPM x 10^3 per 10 cm^2 leaf area			
		Eth-sol ± S.E.M.	Eth-insol ± S.E.M.	Eth-sol ± S.E.M.	Eth-insol ± S.E.M.
HEALTHY					
Control leaves	lamina	250,1 ± 49,3	66,2 ± 8,9	3,3 ± 2,4	35,0 ± 20,6
Treated Leaves	Exposed lamina	81,2 ± 15,9	15,6 ± 3,9	119,7 ± 45,8	37,3 ± 1,4
	Covered lamina	13,9 ± 5,7	4,8 ± 2,1	19,8 ± 18,7	3,6 ± 0,9
INFECTED					
Control Leaves	Pustule	435,1 ± 163,6	201,9 ± 79,7	101,6 ± 22,1	31,6 ± 7,2
	region adjacent	250,5 ± 76,9	318,4 ± 269,3	52,8 ± 11,4	34,8 ± 15,1
Treated leaves					
Exposed	pustule	382,4 ± 51,9	125,5 ± 37,2	305,9 ± 44,5	63,7 ± 23,3
	region adjacent	272,3 ± 16,4	137,8 ± 28,8	170,1 ± 66,3	76,3 ± 9,9
Covered	pustule	43,4 ± 7,5	17,9 ± 2,7	46,7 ± 9,3	6,1 ± 0,7
	region adjacent	90,0 ± 27,4	30,5 ± 8,7	194,3 ± 157,1	7,9 ± 4,3

Table 19:

Mean levels of radioactivity in each region, expressed as a % of total radioactivity per leaf, in lamina and petiole samples (Series 2) of healthy and rust-infected poplar. Values show the means of 3 replicates of each sample with standard error of the mean (S.E.M.).
Illumination: 20 000 - 32 000 Lux.

Key: Eth-sol = ethanol-soluble fraction;
Eth-insol = ethanol-insoluble fraction.

SAMPLE		30 minute exposure to $^{14}CO_2$		24 hour "chase"	
		% DPM per whole leaf			
		Eth-sol ± S.E.M.	Eth-insol ± S.E.M.	Eth-sol ± S.E.M.	Eth-insol ± S.E.M.
HEALTHY					
Control leaves	lamina	77,60 ± 5,69	22,35 ± 5,69	11,28 ± 8,77	71,75 ± 8,31
	petiole	0,02 ± 0,00	0,02 ± 0,00	16,56 ± 7,78	0,42 ± 0,09
Treated leaves					
Exposed	lamina	68,44 ± 6,19	12,81 ± 1,06	62,05 ± 2,87	24,87 ± 7,29
Covered	lamina	13,95 ± 4,33	4,69 ± 1,76	7,65 ± 6,43	2,90 ± 0,85
	petiole	0,06 ± 0,01	0,06 ± 0,01	2,40 ± 0,52	0,14 ± 0,06
INFECTED					
Control leaves	pustule	31,40 ± 3,10	13,58 ± 0,73	34,30 ±12,70	9,70 ± 1,95
	region adjacent	41,94 ± 4,19	13,03 ± 2,52	28,56 ± 2,48	24,02 ±10,95
	petiole	0,02 ± 0,01	0,02 ± 0,01	3,18 ± 1,37	0,24 ± 0,08
Treated leaves					
Exposed	pustule	21,70 ± 2,51	8,16 ± 3,32	40,56 ±12,74	6,81 ± 1,69
	region adjacent	33,60 ± 3,37	16,72 ± 2,84	30,04 ± 8,24	8,95 ± 3,32
Covered	pustule	4,37 ± 0,05	1,83 ± 0,17	7,72 ± 3,42	0,96 ± 0,32
	region adjacent	10,15 ± 4,42	3,41 ± 1,39	6,33 ± 1,85	1,40 ± 0,96
	petiole	0,03 ± 0,01	0,01 ± 0,00	1,99 ± 0,33	0,04 ± 0,01

Table 20:

Pairs of samples showing significant differences between the means of 3 replicates to a 0,05 level, when compared by means of the t-test.

Pairs of Regions Compared	Time Interval	^{14}C-fraction
Fresh Weight Basis		
Control healthy lamina/ exposed healthy lamina	24 hours	ethanol-soluble
Control healthy lamina/ exposed healthy lamina	24 hours	ethanol-insoluble.
Whole-leaf basis		
Exposed pustule/ exposed region adjacent	30 min.	ethanol-soluble
Covered infected petiole/ covered healthy petiole	30 min.	ethanol-soluble
Covered infected petiole/ covered healthy petiole	30 min.	ethanol-insoluble

Mean ^{14}C-levels in pairs of samples were compared with the t-test and those showing significance to the 0,05 level are shown in Table 20.

Levels of ^{14}C-ethanol-soluble components in the pustule of control leaves were slightly greater than in the region adjacent, on both a fresh weight and area basis, but the differences were not significant. Levels of ^{14}C-ethanol-insoluble components in the control pustule and region adjacent samples were not significantly different after both time periods, on a fresh weight and area basis. On a whole-leaf basis, the region adjacent had greater ^{14}C-ethanol-soluble levels after 30 minutes, while after 24 hours levels in the pustule were higher, but the differences between the two regions were not significant. ^{14}C-ethanol-insoluble levels, on a whole-leaf basis, while initially similar in both regions, were greater in the region adjacent after 24 hours, but not significantly different from levels in the pustule.

In healthy control leaves, most of the radioactivity on a fresh weight and area basis was located in the ethanol-soluble components after 30 minutes, but after 24 hours ^{14}C-ethanol-insoluble levels were higher. On a whole-leaf basis similar trends were observed. The healthy petiole contained high levels of ^{14}C-ethanol-soluble components after 24 hours, when compared on a fresh weight basis. On a whole-leaf basis, ^{14}C-ethanol-soluble levels in the control healthy control petiole

exceeded levels in the lamina, but were not significantly different.

In treated leaves, the exposed pustule region had greater ^{14}C-ethanol-soluble levels than the region adjacent after 30 minutes on a fresh weight and area basis. After 24 hours, levels of radioactivity on a fresh weight basis differed slightly from those on an area basis, but no significant differences were found between the levels in the pustule and region adjacent. ^{14}C-ethanol-insoluble components in the exposed region adjacent exceeded, but were not significantly different from levels in the pustule after both periods of time. On a whole-leaf basis, however, the region adjacent had significantly higher levels of ^{14}C-ethanol-soluble components than the pustule after 30 minutes. After 24 hours, levels of ^{14}C-ethanol-soluble components were higher in the region adjacent after both periods of time, but were not significantly different from levels in the pustule.

In treated infected leaves the covered regions adjacent had mainly higher levels of both components compared with the pustule on a fresh weight and area basis, but the differences between ^{14}C-levels in the two regions were not significant. On a whole-leaf basis, levels of both components were not significantly different in the covered pustule or region adjacent, although the region adjacent had a higher proportion of ^{14}C-components

than the pustule.

In healthy treated leaves levels of ^{14}C-ethanol-soluble components on a whole-leaf basis differed from those in healthy control leaves after 24 hours. ^{14}C-ethanol-soluble levels were significantly higher (62,05%) in the exposed healthy lamina than in the healthy control lamina (11,28%). A significantly lower proportion (24,87%) of ^{14}C-ethanol-insoluble components was found in the exposed lamina than in the healthy control lamina (71,75%). The ^{14}C-ethanol-soluble levels in the healthy covered petiole were reduced when compared with levels in the healthy control petiole, but the differences were not significant.

In healthy control and infected control petioles, levels of ^{14}C-ethanol-soluble components on a whole-leaf basis were similar after 30 minutes, but after 24 hours the healthy petiole had higher, but not significantly different levels than the infected petiole.

In the infected covered petiole, however, levels of both components on a whole-leaf basis were significantly lower than in the healthy petiole after 30 minutes, but after 24 hours no significant differences in both components were observed.

The "sink" effect of covered regions of healthy and infected leaves was evaluated by comparing their respective percentages

of / ....

radioactivity on a whole-leaf basis in the covered regions. In the infected covered leaves, if ^{14}C-levels in the pustule and region adjacent were combined, ^{14}C-levels in the covered regions of infected leaves were not significantly different from ^{14}C-levels in covered healthy leaves, after both time periods. It was concluded that the presence of pustules did not enhance the "sink" effect of the covered regions of infected leaves.

Levels of radioactivity expressed in terms of fresh weight and leaf area yielded similar results. Fresh weight as a basis of comparison was more useful than leaf area because ^{14}C-levels in the petioles could be assessed on a fresh weight basis, but not on an area basis. However, the most useful expression of ^{14}C-levels was on a whole-leaf basis since it greatly clarified the location of radioactivity in the various regions and facilitated evaluation of the "sink" effect.

The results of the autoradiography were clarified by liquid scintillation spectrometry. The region adjacent to the pustule appeared to mobilize assimilate more actively than the pustule region and up to a quarter of the radioactivity on a whole-leaf basis was incorporated into ethanol-insoluble material in the region adjacent. However, levels of radioactivity in the two regions were not consistently significantly different. The total "sink" effect of the darkened

infected lamina was not significantly greater than that of the darkened healthy lamina when the levels of radioactivity in the covered regions were compared on a whole-leaf basis. The covered infected petiole was shown to contain significantly lower levels of both ^{14}C-components after 30 minutes than the covered healthy petiole, but after 24 hours there was no significant difference between ^{14}C-levels in healthy and infected petioles. Infection by the pathogen did not appear to influence translocation of assimilate from the infected leaf.

The work of Wang (132) on a mature infection of Uromyces phaseoli on Pinto beans (Phaseolus vulgaris)provided interesting parallels and contrasts to the results of similar work on poplar rust. Although the bean rust showed a clear-cut interaction with its host by forming "green islands" and showing distinct starch accumulation in areas corresponding to the "green islands", it was considered to be a fairly benign pathogen and could provide a comparison with the behaviour of poplar rust.

The autoradiographs of rusted bean leaves (132) showed that the highest level of radioactivity was always found in the lesion area in tissue exposed to light whereas in poplar rust levels of radioactivity in the lesion areas did not differ greatly from the levels in regions adjacent and healthy tissue under similar

conditions, as shown by both autoradiography and scintillation counting.

However, when rusted poplar and bean leaves were covered, a similar pattern of ^{14}C-distribution was observed in autoradiographs, although the bean leaves were fed $^{14}CO_2$ for up to 2 hours. Little or no accumulation of radioactivity occurred in the covered lesion areas in bean leaves at the end of 5 hours, nor in poplar after the initial feed of $^{14}CO_2$. After this period a slow increase in radioactivity of the bean rust lesions was noted and this was also observed in poplar rust lesions. ^{14}C-levels increased slightly after 24 hours as shown by scintillation counting.

In rust-infected bean leaves most of the radioactivity was incorporated into starch in tissues exposed to the light whereas in rust-infected poplar the ethanol-insoluble fraction (possibly including starch) contained up to a quarter of the radioactivity in tissues exposed to light. In healthy poplar, however, up to three-quarters of the radioactivity present was located in the ethanol-insoluble fraction after the 24 hour "chase". In contrast in shaded bean tissues most of the radioactivity was located in ethanol-soluble components, while rust-infected poplar leaves showed considerable ^{14}C-levels in the ethanol-insoluble fraction as well as in ethanol-soluble components.

6(3) TIME-LAPSE STUDIES OF LEVELS OF ETHANOL-SOLUBLE AND ETHANOL-INSOLUBLE COMPONENTS IN HEALTHY AND RUST-INFECTED POPLAR SHOOTS.

In the preceding section an accumulation of ^{14}C-ethanol-insoluble compounds was reported in the regions adjacent to the pustule, after the 24 hour "chase" period. The identity of the accumulated ethanol-insoluble compounds was not known, but the presence of starch was considered likely since the major polymeric carbohydrates in healthy and infected tissue were found to be glucose-containing polymers (Chapter 5).

The effect of the pathogen on location and levels of ^{14}C-ethanol-soluble and ethanol-insoluble components in the host was studied further by extending the "chase" for a longer period than 24 hours. Ethanol-insoluble components were extracted with perchloric acid (86), which dissolves starch and dextrin (54, 77). In addition to ^{14}C-components, total sugar levels were estimated during this period.

Much of the previous work on starch accumulation around rust lesions (77, 111, 124, 132, 147), was based on the procedure of subjecting infected leaves to dark incubation, extracting the pigments and then staining the tissues with iodine/potassium iodide to reveal the presence of starch around the pustules. When rust-infected poplar leaves were treated in a similar manner, the results were inconspicuous because the lesions were so small, and for this

reason any localization of ^{14}C-components (including starch) around the lesions was not clearly evident. The region of starch accumulation described by other workers would have been included in the pustule sample of poplar leaves due to the small size of the lesions and the density of infection. It was anticipated that extraction of perchloric acid-soluble components (possibly containing starch) from the infected leaves would reveal whether any localization of these compounds occurred in the pustule after an incubation period extended to 46 hours. A longer incubation period was not employed because the leaves wilted after 48 hours incubation.

Experimental Procedure:

The experiments were performed during May and June, 1974, on samples from the Claremont and Hohenort Estate populations. Shoots (5 - 8 leaves) of healthy and infected poplar were collected early (9 00 - 10 00 hours), the ends of the shoots were cut under water, immersed in water in McCartney bottles and placed in a Conviron growth chamber to equilibrate for 1 - 2 hours. The environmental conditions during the experiment were a light intensity of 18 000 Lux (base of shoot) to 22 000 Lux (shoot apex), relative humidity of 90 - 100% and temperature ranging from 15°C (night) to 18°C (day).

The shoots were exposed to a 12 hour light / 12 hour dark

regime, similar to conditions in the natural environment during May/June, and were covered with bell jars during equilibration and feeding of $^{14}CO_2$. The feeding of $^{14}CO_2$ was performed as described in Section 6 (2). The amount of $^{14}CO_2$ administered was 60 µCi (30 µl) and the duration of the feed was 15 - 20 minutes. Three similar experiments were performed. The time of sampling differed to a certain extent during the three experiments, but samples were taken at specific stages of the photoperiod sequence, namely at the end of the dark phase, after a few hours exposure to light and in the middle or at the end of the light phase in all experiments. The mean sampling intervals were as follows : 1½ hours (light), 7 and 16 hours (dark), 20½ and 24 hours (light), 33 hours (dark) and 46 hours (light).

Leaves were picked randomly at each time period and the laminas were sub-divided for analysis. The infected laminas were divided into pustule region (region containing 20 - 30 pustules per cm^2) and the region adjacent to the pustule (uninfected area surrounding groups of lesions) with a sharp 0,5 cm diameter cork borer. Healthy laminas were sampled with a pair of scissors. Samples (90 - 200 mg fresh weight) were rapidly weighed and tissues were killed by immersion in boiling 80% ethanol. The samples were treated further as shown in Figure 26 to provide four sets of data :

1. Total ^{14}C-assimilate in the ethanol-soluble fraction;

2. Total sugar in the ethanol-soluble fraction;

3. ^{14}C-perchloric acid-soluble components; and

4. Total sugar in perchloric acid-soluble extract.

Perchloric acid (86) was used to dissolve the starch and dextrin according to a modification of the method of Juliano and Varner (54). It was necessary to neutralize the perchloric acid extract with 4 N KOH before preparing each sample for scintillation counting.

^{14}C-levels recovered in ethanol-soluble and perchloric acid-soluble samples were determined as in Section 6 (2) and the results expressed as DPM 100 mg^{-1} fresh weight of sample. Total sugars were assayed by the method of Dubois et al. (28) as described in Appendix 4. The assays were performed on the Pye Unicam SP 1800 spectrophotometer and the results were expressed as µg glucose 100 mg^{-1} fresh weight of sample. The results were shown as mean values for the three experiments with standard errors of the means, or as means of two samples. The data was plotted in Figures 27 - 30 and tabulated in Appendix 7.

SAMPLE

(90 - 100 mg fresh weight)

Homogenised and refluxed in 80% ethanol for 1 hour

Centrifuged at 5 000 x g

<u>Ethanol-soluble fraction</u>

(1) ^{14}C-scintillation spectrometry. Sample prepared as in Section 6 (2).

(2) Total sugar assay (28) after suitable dilution.

Ethanol-insoluble sediment

extracted twice in cold 50% perchloric acid, (a) 3 ml and (b) 1 ml. Centrifuged at 25 000 x g for 20 minutes after each 1 hour extraction period.

Supernatant:

Perchloric acid extract of solubilised starch and dextrin.

Neutralization of sample with 4 N KOH, centrifugation at 20 000 x g.

<u>Neutral perchloric acid soluble extract</u>

(3) ^{14}C-scintillation spectrometry after addition of 10 ml Instagel.

(4) Total sugar assay (28) after suitable dilution.

<u>Figure 26</u>:

Procedure of analysis of the samples from the time-lapse experiment.

Results:

^{14}C-Ethanol-Soluble Components

Levels of ^{14}C-ethanol-soluble components (Figure 27) were higher in the healthy lamina and region adjacent than in the pustule during the first 16 hours of incubation. After 20½ hours, during the light period, levels of radioactivity in all tissues were similar. After 46 hours the region adjacent had higher levels of ^{14}C-components than the pustule and healthy lamina.

^{14}C-Perchloric Acid-Soluble Components

Levels of ^{14}C-perchloric acid-soluble components (Figure 28) appeared to be higher in healthy leaves and the region adjacent than in the pustule during 24 hours of incubation. Levels of ^{14}C-perchloric acid-soluble components in all samples increased slightly during the light phase after 20½ hours, after which the levels declined and were similar in all samples. ^{14}C-levels in the region adjacent showed considerable increases after 46 hours.

Ethanol-Soluble Total Sugar Levels

Total sugar levels in ethanol-soluble components (Figure 29) of healthy and infected leaves showed little variation

during 24 hours of incubation. After 33 hours (during the dark phase of the cycle) the infected leaves appeared to contain higher levels of ethanol-soluble total sugar than healthy leaves and this trend continued to the end of the incubation period.

Perchloric Acid-Soluble Total Sugar Levels

Total sugar levels in the perchloric acid-soluble components in healthy and infected tissues showed no marked differences during the first 16 hours of incubation. However, during the remainder of the incubation period, mean total sugar levels in the region adjacent exceeded levels in the pustule and the healthy lamina. Levels in infected tissues appeared to increase slightly during 33 to 46 hours of incubation.

The zone of starch synthesis as described by several authors (77, 111, 125), would have been located in the pustule samples of poplar leaves due to the difficulty in separating the region adjacent from dense areas of pustules. If accumulation of ^{14}C- and total sugar components had occurred in the zone surrounding the pustules, it should have been reflected in the levels of these components in the pustule samples. While infected tissues showed higher ethanol-soluble total sugar levels than healthy tissues after 33 to 46 hours of incubation, it was concluded that there was not a marked mobilization of ^{14}C-

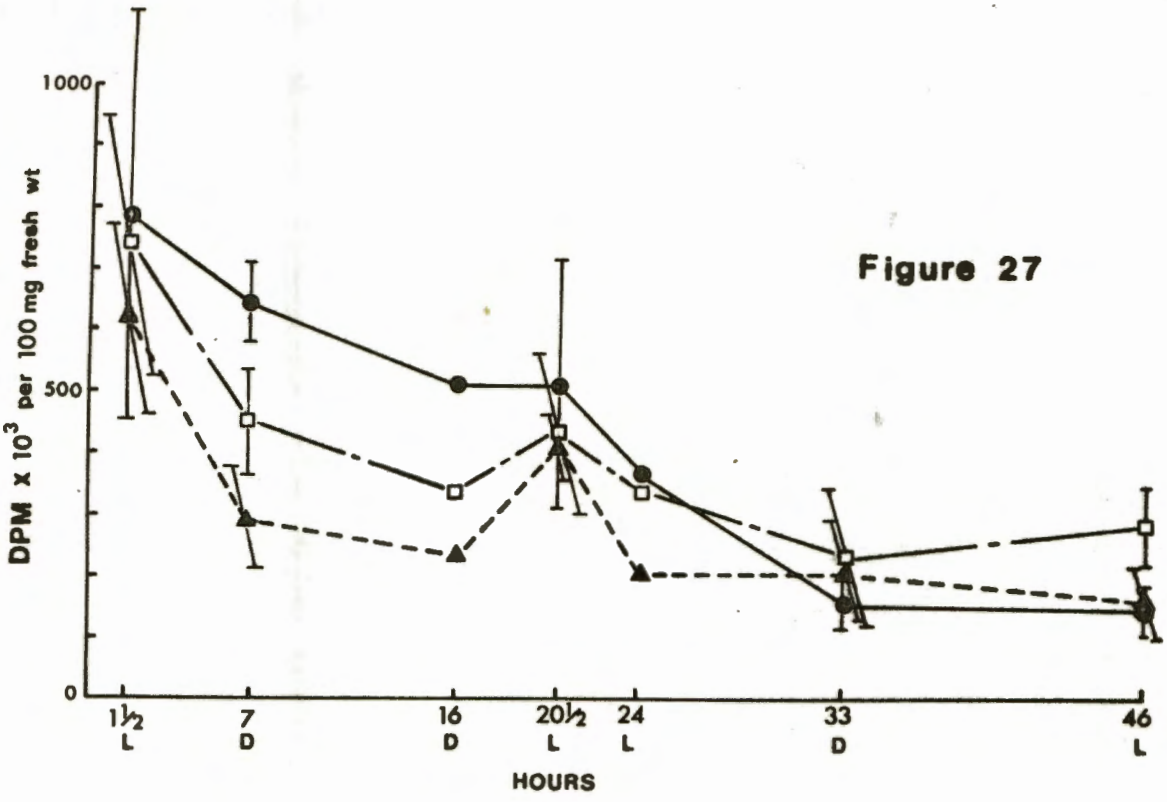


Figure 27

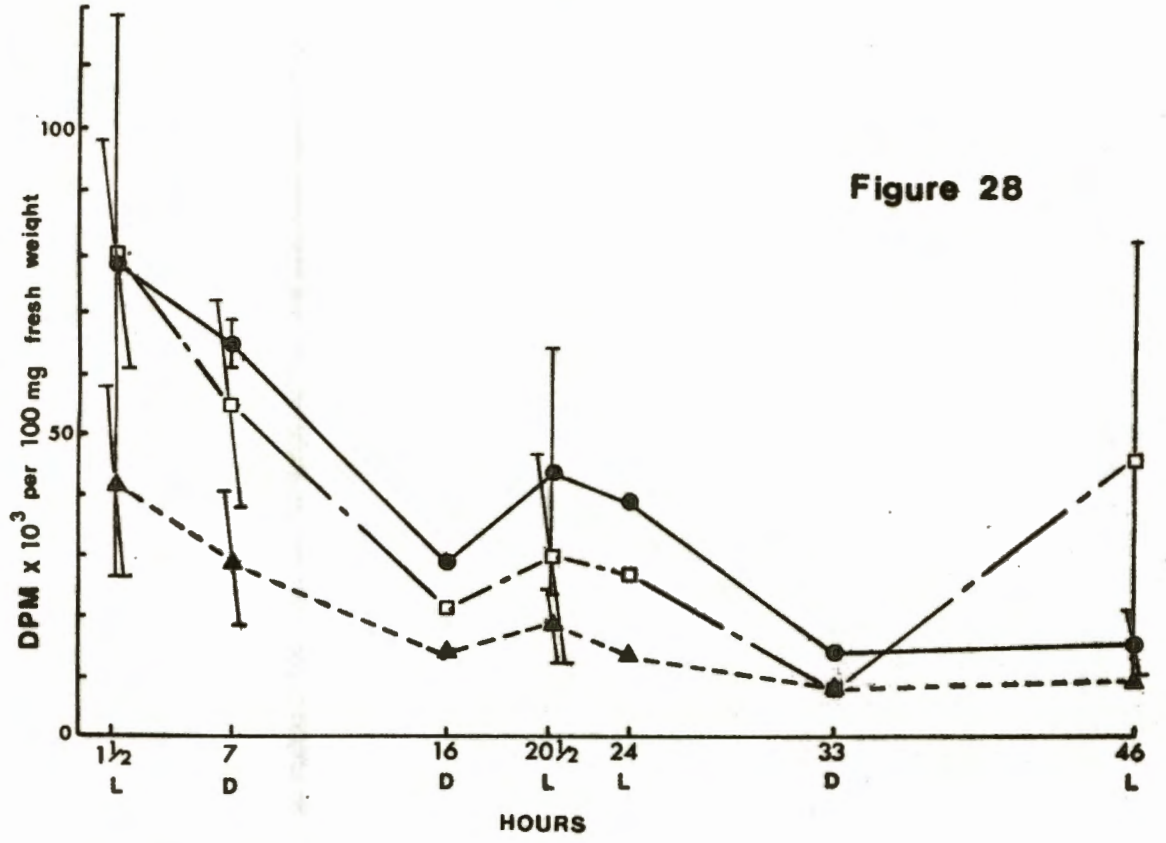


Figure 28

Figures 27 and 28:

Levels of radioactivity (DPM x 10^3 per 100 mg fresh weight) in ethanol-soluble (Fig. 27) and perchloric acid-soluble (Fig. 28) components in healthy and rust-infected poplar leaves at various time intervals after exposure to $^{14}CO_2$ followed by 46 hours incubation in $^{12}CO_2$. Levels shown are means of 3 samples ± standard error of the mean, or means of 2 samples. The light (L) and dark (D) phases of incubation are shown.

Key: ●—● healthy; ▲- - -▲ pustule and □-·-□ region adjacent.

Figure 29

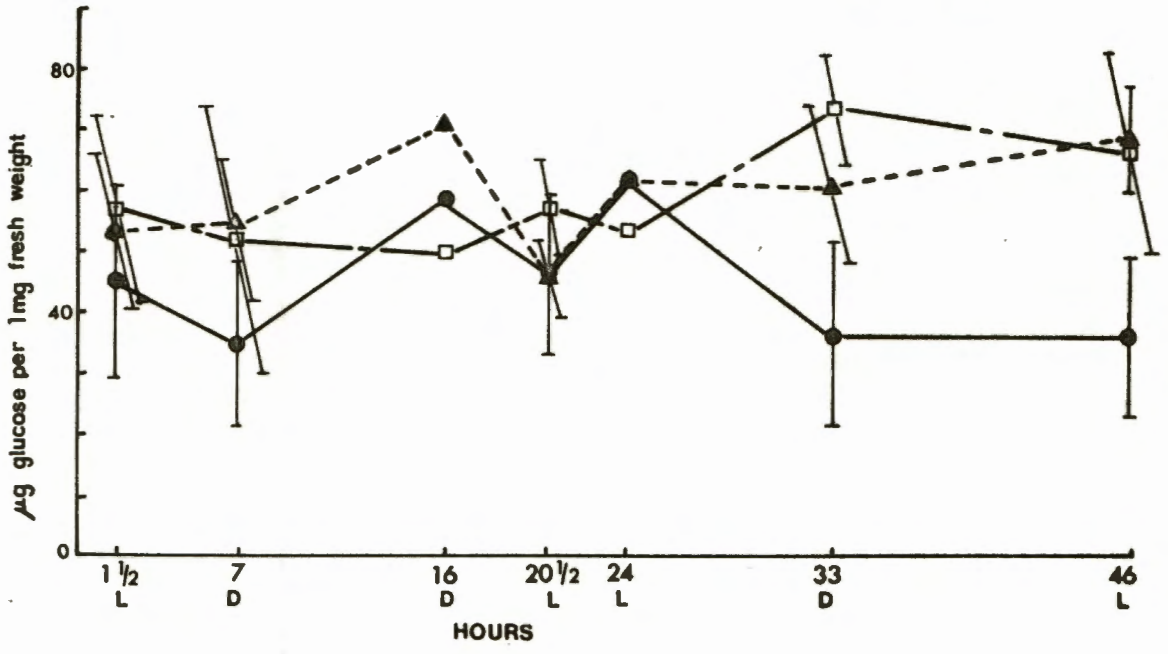
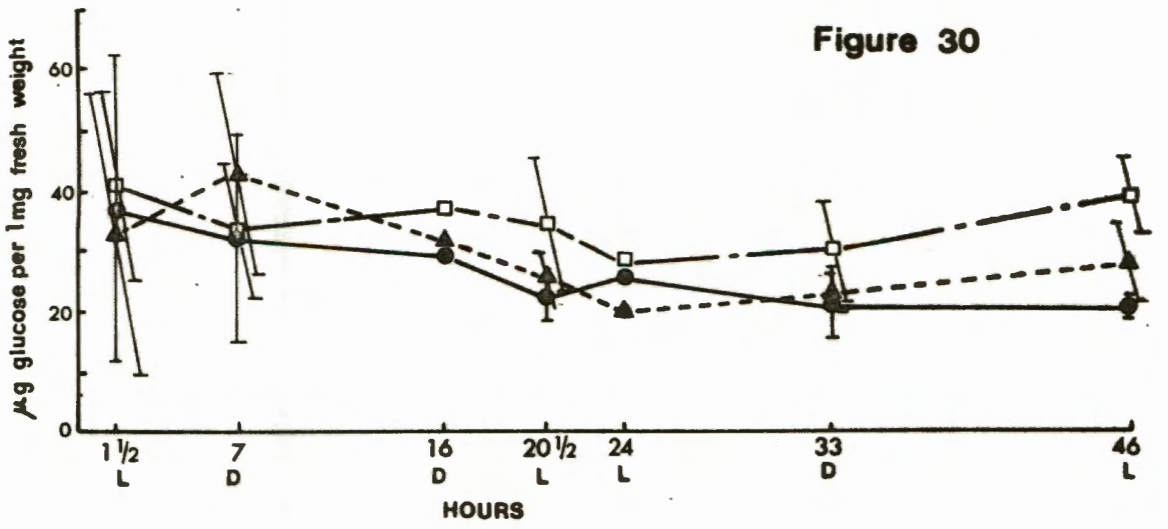


Figure 30



Figures 29 and 30:

Total sugar levels (µg x 10^2 per 100 mg fresh weight) in ethanol-soluble (Fig. 29) and perchloric acid-soluble (Fig. 30) components in healthy and rust-infected poplar leaves at various time intervals after exposure to $^{14}CO_2$ followed by 46 hours incubation in $^{12}CO_2$. Levels shown are means of 3 samples ± standard error of the mean, or means of 2 samples. The light (L) and dark (D) phases of incubation are shown.

Key: ●—● healthy; ▲- - -▲ pustule and □-·-□ region adjacent to pustule.

assimilate to the site of infection nor a deposition of perchloric acid-soluble components around the pustules. The region adjacent (area without pustules) and healthy lamina had similar levels of ^{14}C-components and total sugars. The area of rapid starch synthesis adjacent to rust lesions of Pinto beans described by Wang (132) was not evident in comparable areas (pustule samples) in rusted poplar.

6(4) LEVELS OF ^{14}C-FREE AMINO ACIDS IN HEALTHY AND RUST-INFECTED POPLAR LEAVES.

In order to assess whether infection changed the levels and composition of ^{14}C-free amino acids in poplar leaves, shoots were fed $^{14}CO_2$ as described in Section 6 (2) and the levels and composition of free amino acids were evaluated.

Experimental Procedure:

$^{14}CO_2$ (60 µCi) was fed for 30 minutes to healthy and infected shoots of poplar and the leaves were harvested immediately. The lamina samples (healthy, pustule and region adjacent) of about 1,0 g each were homogenized in 80% ethanol and stored at $0^{o}C$ for 24 hours. Each sample was filtered and then reduced to about 10 ml with a stream of air, followed by removal of pigment and lipid with petroleumbenzine.

Two ml of each sample was placed on the ion-exchange column of a Beckman 120 C automatic amino acid analyser and the eluate from the column was diverted through a flow-cell attached to a Beckman C.P.M. 100 liquid scintillation counter to trace the amino acid radioactivity. The labelling of each amino acid and the total radioactivity in the free amino acids per sample were expressed as DPM 100 mg^{-1} fresh weight of sample (Table 21).

Results:

Levels of ^{14}C-free amino acids constituted a higher proportion of the ^{14}C-ethanol-soluble fraction in the pustule region than in healthy tissue (Table 21) after 30 minutes exposure to $^{14}CO_2$ in the light. Higher levels of free amino acid in the pustule area has been found in other rust infections (111) where pronounced accumulation of tyrosine and phenylalanine was particularly noted. In poplar leaves infected with rust there was greater accumulation of phenylalanine, serine and glutamic acid than in healthy tissues, but the most pronounced accumulation observed was that of glutamine and alanine in the pustule. Asparagine and cystine, found in healthy tissue, were absent from infected leaves. Tyrosine was present in the healthy lamina and pustule, but was not found in the region adjacent. The region adjacent and healthy lamina contained glycine, isoleucine and leucine but these compounds were not present in the pustule.

Table 21:

Levels of radioactivity (DPM 100 mg^{-1} fresh weight) in individual free amino acids of healthy and rust-infected poplar after exposure to $^{14}CO_2$ for 30 minutes.

Amino Acids	Healthy	Pustule	Region Adjacent
	DPM 100 mg^{-1} fresh weight		
Aspartic acid	115	109	293
Threonine	147	493	645
Serine	2761	6349	2174
Asparagine	412	-	-
Glutamic acid	2951	7412	8109
Glutamine	265	5708	113
Proline	-	-	-
Glycine	144	-	153
Alanine	3567	24797	11301
Valine	-	-	-
Cystine	36	-	-
Methionine	-	-	-
Isoleucine	91	-	161
Leucine	91	-	110
Tyrosine	228	86	-
Phenylalanine	1296	1737	1331
Total	12104	46691	24390

6(5) TRANSLOCATION STUDIES IN HEALTHY AND INFECTED POPLAR

Previous evidence (Section 6 : 2) suggested that infection of poplar leaves with rust did not restrict export of ^{14}C-assimilate from these leaves. While it appeared that the rust did not confer a retentive effect on total translocation from the leaf, some mobilization of assimilate did occur in the infected leaf. ^{14}C-ethanol-soluble components formed 34,30% and 28,56% of the recovered radioactivity measured in control pustule and region adjacent respectively in comparison with 11,28% in the healthy lamina after 24 hours incubation. In contrast, levels of ^{14}C-perchloric acid-soluble components were lower (9,70%) in rust-infected tissues than in the healthy lamina (71,75%) after 24 hours.

It appears that host translocation may be affected by a rust infection in two ways. The work of Doodson et al. (26, 27) (cited in Chapter 1, page 3) on translocation patterns in healthy and stripe rust infected wheat showed that the fungus restricted translocation from the infected leaf, but that the infected leaf did not distort translocate from other leaves to the site of infection. Livne and Daly (67) showed that Pinto beans infected with rust on one leaf could influence translocation from other leaves towards the site of infection. Similar effects were observed by Thrower and Thrower (126) in rust-infected broad bean, where a rust-infected leaf influenced translocate to such an extent that translocate was directed

principally to the site of infection at the expense of younger developing leaves. The results of similar work by Pozsár and Király (90) and Gaunt and Manners (32) will be discussed in relation to the results of this investigation.

Extensive studies on translocation of ^{14}C-assimilate in healthy leaves of Eastern Cottonwood plants (Populus deltoides Bartr.) have been published (23, 52, 58, 59) and the conclusions are discussed in relation to the present studies. Translocation studies in poplar were restricted to investigating whether the rust had a retentive effect on export of translocate from the infected leaf and whether the rust infection created a greater "sink" for assimilate than younger, developing leaves. In this study one leaf on a shoot was exposed to $^{14}CO_2$ and the accumulation of radioactivity in various fractions was analysed by liquid scintillation spectrometry. Translocation studies were conducted in two stages because only infected shoots were available when work was commenced in late June, 1974. Healthy material became available during the following season (October/November, 1974). ^{14}C-levels in each leaf were expressed as a percentage of summed levels of radioactivity in the leaves of each shoot.

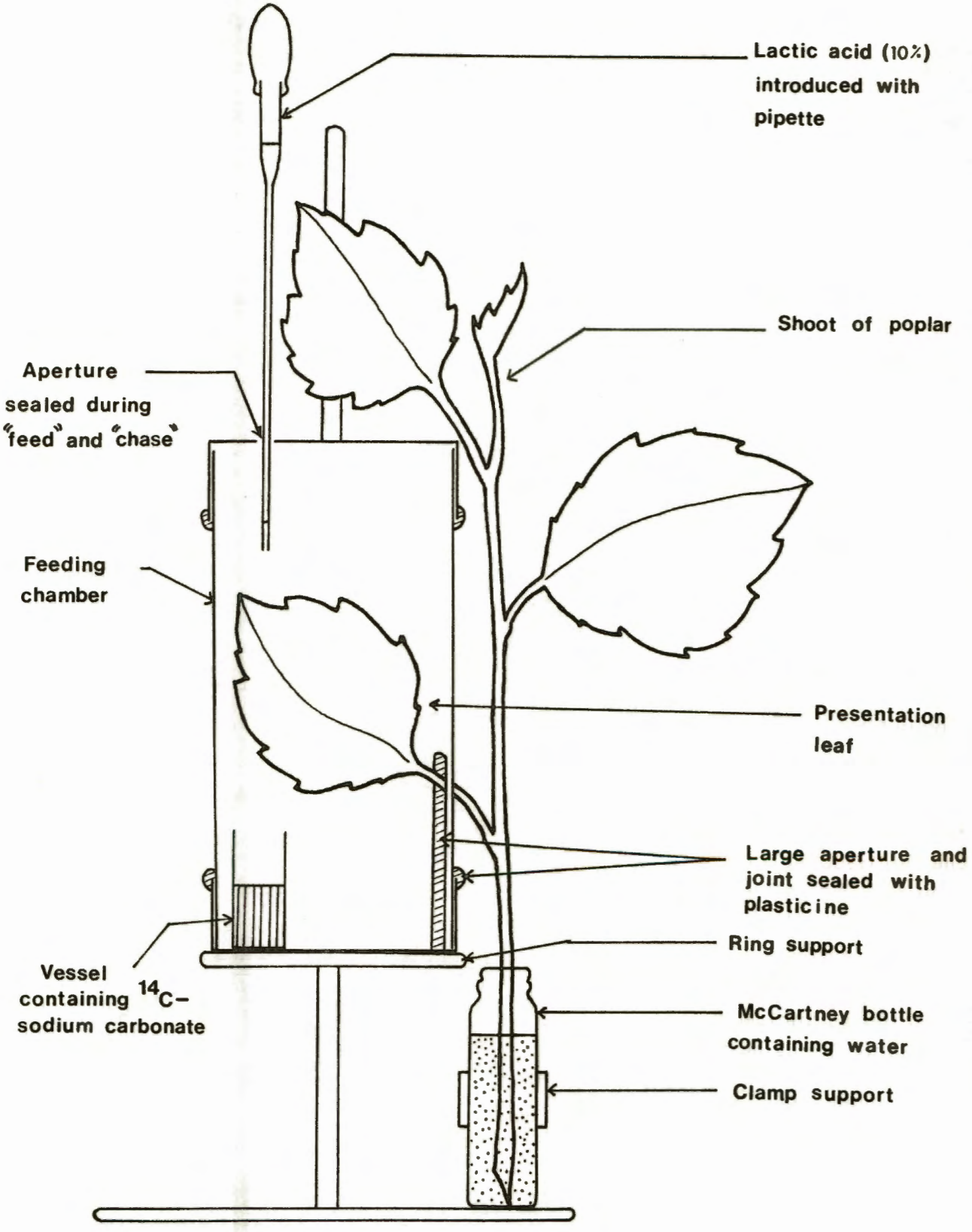


Figure 31:
Method of feeding a single enclosed leaf with $^{14}CO_2$.

Experimental Procedure:

(a) Infected Shoots:

The shoots with freshly cut stems immersed in water in McCartney bottles were equilibrated in a Conviron growth chamber for 1 hour at 23°C, 70% relative humidity and light intensity of 13 000 Lux (base of shoot) to 25 000 Lux (shoot apex). During the equilibration period the lowest leaf on the shoot was enclosed in the feeding chamber, as shown in Figure 31, to isolate it from the rest of the shoot, but the chamber was not sealed during this period. In all shoots the leaf exposed to $^{14}CO_2$ was termed the presentation leaf. Immediately prior to feeding $^{14}CO_2$ the chamber was sealed with plasticine (Figure 31). Using a Pasteur pipette 10% lactic acid (2 - 3 ml) was introduced into the vessel containing 10 µCi sodium carbonate through an aperture in the roof of the chamber. The aperture was sealed with plasticine for the duration of the 30 minute feeding period. The $^{14}CO_2$-liberation was terminated by adding excess 2 N KOH to the reaction vessel, but the chamber was kept sealed throughout the following 2 or 4 hour "chase" period to prevent contamination with $^{14}CO_2$ of the remaining leaves on the shoot. The $^{14}CO_2$ fixation and $^{12}CO_2$ "chase" was conducted in the light.

Shoot 1 was subjected to a 2 hour "chase" and shoot 2

a 4 hour "chase" prior to sampling. Shoot 1 consisted of mature leaves with fewer than 10 lesions per cm^2 and a few patches of high lesion density (> 30 lesions per cm^2), the pustule regions being separable from uninfected regions adjacent. Shoot 2 contained mature leaves so heavily infected that the region adjacent was not separable from the pustules.

The leaves were numbered in ascending order from the lowest to the apex of the shoot. The leaves were divided into pustule and region adjacent when this was possible, as described previously, with the samples ranging in fresh weight from 55 to 300 mg. The samples were immersed in boiling 80% ethanol and only the ^{14}C-ethanol-soluble fraction was extracted for liquid scintillation spectrometry as described in Section 6 (2).

In a subsequent experiment shoots 3 and 4 were treated similarly, but with the additional extraction of ^{14}C-perchloric acid-soluble components (possibly containing starch) as described in Section 6 (3). The only difference in environmental conditions was the temperature of 19°C and light intensity of 17 000 Lux (base of shoot) to 25 000 Lux (shoot apex). Shoot 3 was subjected to a 2 hour "chase" and shoot 4 to a 4 hour "chase" prior to sampling. These shoots differed from shoots 1 and 2 in that there were several young unexpanded healthy leaves at the apex of each

shoots. Infection density of the infected leaves was lower than 10 pustules per cm^2 with a few densely infected areas (> 30 lesions per cm^2).

The radioactivity levels in ^{14}C-ethanol-soluble and ^{14}C-perchloric acid-soluble fractions are expressed as a percentage of the combined radioactivity in all the leaves of the respective shoot, with the fresh weight (mg) and an indication of the age of each leaf given in Table 22.

(b) Healthy Shoots:

Eight healthy, vigorous shoots were investigated in pairs. The environmental conditions were: temperature of 18°C, 70% relative humidity and light intensity of 20 000 Lux (base of shoot) to 32 000 Lux (shoot apex). The position of the presentation leaf (leaf fed $^{14}CO_2$) on each shoot was as follows: the 4th leaf in shoots 5, 6 and 7; the apical leaf in shoots 8 and 9; the second from apical leaves in shoots 10 and 11; the 1st (lowest) leaf in shoot 12. The same procedure for feeding the presentation leaf with $^{14}CO_2$ was employed as described in Section (a) and the feeding time was 20 minutes. All shoots were incubated for two hours prior to sampling and were assayed for ^{14}C-ethanol-soluble components alone as described previously. The levels of radioactivity in each

leaf were expressed as percentages of the combined levels of radioactivity in all the leaves of each respective shoot. The fresh weight (mg) and an indication of the age of each leaf was included in Table 23.

Results:

(a) Rust-Infected Shoots:

The presentation leaf in all shoots retained 96,74 - 99,64% of the ^{14}C-components in each shoot. In the infected shoots 1 and 3 (Table 22), the region adjacent to the pustule in the presentation leaf showed higher ^{14}C-levels than the pustules after 2 hours incubation, which was in agreement with previous results (Section 6 : 2) . In shoot 1, 0,4% of ^{14}C-ethanol-soluble components were found in the 2nd leaf and levels were lower in the remaining leaves of the shoot. In leaves 2 to 5 the pustule region contained a higher % of ^{14}C-ethanol-soluble components than the region adjacent. In the apical leaf (number 5), the pustule contained clearly more ^{14}C-components (0,31%) than the region adjacent (0,02%). After 4 hours the presentation leaf of shoot 2 contained 99,64% of the ^{14}C-ethanol-soluble components and the remaining leaves had a low % of radioactivity. In shoot 2 the %

Table 22: Translocation of ^{14}C-assimilate in rust-infected poplar shoots, showing levels of ^{14}C-ethanol-soluble components in all shoots and ^{14}C-perchloric acid-soluble components in shoots 3 and 4. Levels of radioactivity in each leaf are expressed as a percentage of combined levels of radioactivity in all the leaves of the shoot. Infected leaves were divided into pustule (P) and region adjacent (RA), and healthy leaves were denoted by H. The relative age of each leaf is indicated (see key). The leaves were numbered from the lowest leaf on the shoot to the apical leaf. Fresh weight (mg) of each leaf is shown in parenthesis.

Key: M = mature, fully expanded leaf; Y = young, fully expanded leaf;
VY = very young, not fully expanded leaf.

Leaf Number	Region	Shoot 1 2 hr. "chase"	Shoot 2 4 hr. "chase"	Shoot 3 2 hr. "chase"		Shoot 4 4 hr. "chase"	
		^{14}C-ethanol-soluble		^{14}C-ethanol-soluble	^{14}C-perchloric acid extr.	^{14}C-ethanol-soluble	^{14}C-perchloric acid extr.
1 Presentation leaf.	P	M 12,31 (118)	M 99,64 (152)	M 42,12 (307)	4,60	M 11,93 (604)	9,28
	RA	M 86,61 (78)		M 47,34 (147)	2,68	M 68,59 (169)	7,21
2	P	M 0,22 (58)	M 0,03 (290)	M 0,09 (360)	0,26	M 0,27 (228)	0,08
	RA	M 0,20 (50)		M 0,13 (70)	0,04	M 0,21 (110)	0,08
3	P	M 0,08 (55)	M 0,13 (161)	M 0,10 (240)	0,35	M 0,12 (183)	0,06
	RA	M 0,07 (83)		M 0,14 (187)	0,35	M 0,19 (165)	0,06
4	P	M 0,11 (96)	M 0,04 (295)	M 0,10 (161)	0,05		
	RA	M 0,06 (43)		M 0,07 (135)	0,15		
5	P	M 0,31 (112)	M 0,14 (167)	M 0,02 (50)	0,03		
	RA	M 0,02 (92)		M 0,04 (105)	0,26		
6	P		M 0,02 (230)				
6	H			M 0,04 (520)	0,13	M 0,15 (349)	0,21
7	H			M 0,07 (522)	0,12	M 0,14 (629)	0,14
8	H			Y 0,08 (707)	0,15	M 0,08 (844)	0,15
9	H			Y 0,11 (390)	0,05	Y 0,12 (620)	0,15
10	H			Y 0,16 (527)	0,12	Y 0,20 (471)	0,11
11	H					VY 0,21 (576)	0,13

radioactivity in leaves 2 to 6 was lower per leaf than in shoot 1.

In infected shoots 3 and 4, the younger healthy leaves at the shoot apex contained 1,03% and 1,78% of the ^{14}C-components in each shoot. In both shoots the 2nd leaf, which was nearest to the presentation leaf, had a higher % of radioactivity in the pustule than in the region adjacent when both ^{14}C-components were totalled. The region adjacent in the remaining infected leaves of both shoots had a higher % radioactivity than the pustule and most of the ^{14}C was located in the perchloric acid-soluble extract. The healthy leaves on the shoots had lower ^{14}C levels in both fractions than infected leaves.

The fully expanded healthy leaves had a greater % of radioactivity in the perchloric acid-soluble fraction whereas the very young, not fully expanded leaves had a higher % ^{14}C in the ethanol-soluble fraction. The very young leaves contained levels of radioactivity (0,16 - 0,34%) similar to levels in the more mature leaves (0,17 - 0,36%).

(b) Healthy Shoots:

The presentation leaf of each healthy poplar shoot retained the major proportion of the ^{14}C-ethanol-soluble

Table 23:

Translocation of ^{14}C-ethanol-soluble components in healthy poplar shoots, showing the levels of radioactivity in each leaf as a percentage of the combined radioactivity in all the leaves of each shoot. The presentation leaf (in box) was exposed to $^{14}CO_2$ for 20 minutes followed by a 2 hour "chase". The relative age of each leaf is indicated (see key). The leaves were numbered from the lowest leaf on the shoot to the apical leaf. Fresh weight (mg) of each leaf is shown in parenthesis.

Key: M = mature, fully expanded leaf; Y = young, but fully expanded leaf;
VY = very young, not fully expanded leaf.

Leaf Number.	Shoot 5	Shoot 6	Shoot 7	Shoot 8	Shoot 9	Shoot 10	Shoot 11	Shoot 12
1	M 0,00 (610)	M 0,02 (187)	M 0,02 (272)	M 0,10 (1057)	M 0,14 (945)	M 0,14 (461)	M 0,04 (111)	M 99,73 (538)
2	M 0,00 (238)	M 0,02 (223)	M 0,04 (635)	M 0,02 (249)	M 0,02 (234)	M 0,08 (575)	M 0,01 (241)	M 0,06 (311)
3	M 0,00 (494)	M 0,17 (290)	M 0,06 (703)	M 0,03 (766)	M 0,05 (703)	M 0,03 (496)	M 0,06 (424)	M 0,05 (394)
4	M 98,03 (697)	M 99,65 (444)	M 99,69 (349)	M 0,03 (907)	Y 0,05 (739)	M 0,07 (516)	Y 0,02 (381)	Y 0,06 (369)
5	Y 0,02 (387)	M 0,04 (311)	M 0,07 (535)	Y 0,06 (430)	Y 0,04 (333)	Y 0,02 (467)	Y 99,82 (251)	Y 0,03 (179)
6	VY 1,03 (354)	M 0,02 (488)	M 0,02 (456)	Y 0,06 (480)	Y 0,04 (424)	Y 99,55 (428)	VY 0,06 (87)	VY 0,06 (86)
7	VY 0,93 (166)	M 0,06 (344)	Y 0,07 (390)	VY 99,70 (430)	Y 99,66 (380)	VY 0,10 (143)		
8		M 0,01 (99)	Y 0,02 (283)					

components (98,03 - 99,82%) after 2 hours of incubation and there was limited translocation of ^{14}C-components towards young, unexpanded leaves at the apex of the shoot. This was observed in shoots 5, 7, 10 and 11 (Table 23) where the presentation leaf was the 4th in shoots 5 and 7, the second from apical leaf in shoots 10 and 11. The small, young unexpanded leaves, with a lower fresh weight, localized ^{14}C-levels similar to levels in larger mature leaves.

It appears that the developing tissues at the shoot apex did not influence translocation from the presentation leaf within the 2 hour incubation period to any extent. The mature leaves translocated ^{14}C-assimilate more readily up the shoot than down, although the presence of the stem and roots of the whole plant in the natural state probably modified this trend. The number of young leaves above the presentation leaf did not clearly influence the direction and amount of translocated ^{14}C towards these areas.

The distribution of radioactivity in a shoot is controlled to some extent by the phyllotaxy of the leaves and anatomical features of the conducting tissue. Since data on these aspects was lacking, the apparently random pattern of labelling could not be clarified.

Studies of the related species of poplar, Populus deltoides Bartr., (58) showed that the relative level

of import of ^{14}C-assimilate into a young expanding leaf depended on the stage of development and vascular connections with the exporting leaves of the shoot. Young leaves in the most rapid phase of expansion were usually the main importers of ^{14}C-assimilate, depending on the position of the leaf on the plant.

^{14}C was translocated preferentially to either the right or left half of the lamina, depending on the position of the importing leaf in the phyllotactic sequence and its stage of development. As a young leaf matured, the initial import of ^{14}C-assimilate was directed to the precociously mature lamina tip, then basipetally and laterally with increasing leaf plastochron index (23, 59). Therefore, when a young leaf was half grown, it was exporting from the mature tip and importing to the immature base. This was considered to be a common feature in dicotyledonous species (23).

In P. canescens it was observed that some downward export of ^{14}C-assimilate to mature leaves occurred when the youngest expanding leaves were fed. In Eastern Cottonwood downward export also entered mature leaves, but it was more common for a mature leaf to be supplied with ^{14}C from a source leaf below it. Downward export did not always conform to the phyllotactic sequence.

Translocation in rust-infected poplar contrasted with

the results of Livne and Daly (67) but bore some similarity to the behaviour of stripe rust infected wheat (27) in that infection on one leaf did not distort translocation of assimilate from other leaves to developing regions. However, Pozsár and Király (90) showed that rusted Pinto beans and wheat leaves infected with Puccinia graminis tritici caused abnormally high translocation to the site of infection, and in Pinto beans particularly, competing with the growing point of the host in the regulation of phloem transport.

In both hosts, infection of the first leaves caused a considerable reduction in the development and size of immature leaves. Gaunt and Manners (32) showed that the amount of assimilate exported from the fed leaf in mature wheat plants depended on the requirement for assimilate in the plant, and was greatest during periods of rapid growth by the host or parasite (loose smut). They found that the distribution of assimilate depended upon the position on the plant of the fed leaf, its maturity and the age of the plant. These conclusions were applicable to poplar. However, the presence of the pathogen (32) changed the pattern of assimilate distribution in that totally different sites received labelled assimilate, which had sometimes been translocated over very long distances to the site of infection. While the poplar rust showed slight accumulation of ^{14}C-assimilate at infection sites, it

did not appear to distort translocation of ^{14}C-assimilate from mature leaves to the detriment of young developing tissues. Poplar rust did not appear to conform to the pattern of influence on the host as described for other rust infections.

6(6) CONCLUSIONS

The application of ^{14}C-labelling techniques to the poplar rust association provided greater insight into the nature of the relationship. Infection with rust did not impede translocation of labelled assimilate from the infected leaf, not did the pustules form a "sink" for ^{14}C in competition with uninfected regions for ^{14}C in the shaded portion of the infected lamina.

Translocation studies confirmed this observation, since the presence of rust on the lower leaves of a shoot did not greatly reduce movement of labelled assimilate to younger tissues at the shoot apex. The amount of radioactivity in young developing leaves did not differ greatly whether the mature lower leaves were healthy or infected. The lesions did not appear to compete for assimilate to the detriment of immature leaves. The ultimate effect on the growth and development of young leaves on an infected shoot was not investigated, but from observations in the field the apical leaves of infected shoots differed little

lack of external symptoms of host degradation caused by the presence of rust on poplar leaves, it appears that this host/biotroph relationship is of a benign nature.

Further characteristic symptoms shown by susceptible hosts infected with biotrophic pathogens are increased respiration and more active metabolism of the infected tissue (4). Increased respiration is usually accompanied by a change in the respiratory pathway to the pentose-phosphate sequence (20, 112) with the concomitant production of acyclic polyols such as mannitol and arabitol. However, in a phycomycetous infection which showed a sharp increase in respiration after infection, with values almost double those of controls at initiation of sporulation, there was no apparently significant change to the pentose-phosphate pathway. This was deduced by the absence of acyclic polyols alone (122) since no additional evidence of altered C_6 / C_1 ratios was provided. Although the respiration rate in poplar was not measured, the presence of acyclic polyols in rusted leaves indicates the participation of the pentose-phosphate pathway in these tissues. Levels of polyols are low, however, not exceeding 24% of the total ethanol-soluble sugars, which contrasts with the levels found in some other infections (21, 44). Trehalose is considered to be a significant component of the carbohydrates of rusted tissues (117) and also constitutes a high proportion of ethanol-soluble sugar in phycomycetous associations such as Albugo tragopogonis (Pers.) S.F. Gray infecting leaves of Senecio squalidus L. (68) and Peronospora parasitica (Pers. ex Fr.) Fr. infecting cabbage cotyledons

(122). Trehalose occurs in exceedingly low levels in the poplar rust.

It appears that the poplar rust fungus transforms a small amount of host assimilate into acyclic polyols and trace amounts of trehalose to maintain the flow of carbohydrates to the infection court.

Since polyols constitute such a low proportion of total ethanol-soluble sugars in the pustule it appears that the fungus does not impose an excessive drain on host assimilate. A different method has been proposed for maintaining the "sink" in phycomycetous infections (68) whereby movement of carbon from the autotroph to the heterotroph was associated with invertase activity in infected tissues. High levels of hexoses were observed around the pustules and the considerable amount of trehalose formed in mycelium and spores was supposed to be derived from the hydrolysis of host sucrose and utilization of hexoses.

The pustule regions of rust-infected poplar leaves did not show simultaneous low sucrose and high hexose levels when compared with healthy tissues. Shading of the poplar host was considered to have considerable influence on sucrose levels, since the two populations subjected to shading showed lower sucrose levels than the unshaded population of Season 2. While sucrose levels in the pustule regions during Season 2 were considerably lower than in healthy tissue or the region adjacent, hexose levels in the pustule were similar to those in

comparable healthy tissues. Exceptionally high hexose levels were found in poplar tissue during Season 3, but the pustule regions contained lower hexose levels than healthy tissue.

Infection by obligate biotrophs may result in the accumulation of compounds such as starch at the site of infection (111, 132, 147), retention of these compounds in the tissues immediately adjacent to the biotroph (3) and preferential synthesis of starch in these regions (123). In poplar there was no clear seasonal trend in carbohydrate accumulation in the pustule. Ethanol-insoluble mannose-containing polymers were present in a higher proportion in the pustule than in the region adjacent and healthy lamina and may have been a component of the polysaccharide in the urediniospores, since mannan has been shown to account for up to 15% of the fresh weight of urediniospores of wheat stem rust (114.). There was a higher level of ethanol-insoluble material in the pustule than in uninfected tissue which did not fluctuate during the study period.

Extraction of tissues with hot and cold water revealed that the main component in both fractions was a glucose-containing polymer. Combined levels of hot water-soluble polysaccharide remained fairly constant in infected tissues, but declined in healthy tissues towards the end of the study period, while levels of cold water-soluble polysaccharide showed little variation between healthy and infected tissues.

Considerable increases in dry weight and total nitrogen levels in the lesion area have been observed in some rust-infected hosts (13, 108, 111). In poplar leaves increased dry weight was observed after infection, but total nitrogen levels were lower in diseased than healthy areas, except during the periods when uredosori first appeared on the lamina surface.

On the basis of seasonal studies of host-obligate biotroph interaction with reference to carbohydrate and total nitrogen levels, it appears that the fungus does not constitute a strong "sink" for host assimilate.

The carbon-14 studies confirmed that the poplar rust did not cause a significant accumulation of ^{14}C-assimilate at the site of infection, apart from temporary high levels of ^{14}C-ethanol-soluble components in the region adjacent, after 30 minutes exposure to $^{14}CO_2$. There was no significant accumulation of ^{14}C-ethanol-insoluble or perchloric acid-soluble components at the site of infection, but after incubation for 33 to 46 hours infected tissues maintained higher ^{14}C-and total sugar levels of ethanol-soluble and perchloric acid-soluble components than healthy tissues. After assimilation of $^{14}CO_2$ for 30 minutes the levels of ^{14}C-free amino acids in the pustule exceeded levels in healthy tissues and these results are in agreement with other studies (111). There were qualitative differences in ^{14}C-labelled free amino acids in healthy and diseased tissues and the pustule contained markedly higher levels of radioactivity in glutamine, alanine and serine than healthy tissues.

There was no significant difference in ^{14}C-levels in infected and healthy petioles after 24 hours of incubation, although the infected petioles had significantly lower ^{14}C-levels after 30 minutes exposure to $^{14}CO_2$. It appeared that the rust fungus did not greatly impede translocation from infected leaves. Movement of ^{14}C-assimilate from the presentation leaf was low (1 - 3%) in both healthy and infected leaves and was further reduced when infection density was very high. The presence of young, not fully expanded leaves on the healthy shoots did not increase the flow of ^{14}C to these regions. When the mature lower leaves were infected there was no restriction of ^{14}C translocated to the younger leaves. These translocation studies were of necessity restricted to shoots and therefore, it was not possible to evaluate the effect on translocation to the lower stem and roots. It was evident from these experiments that translocation of ^{14}C-assimilate in a shoot occurred mainly in an upward direction towards young developing leaves.

The capacity of pathogenic biotrophic fungi to induce translocation of substances to the infection court (9, 125), also occurring in fungi of sheathing mycorrhiza (39, 117), appeared to be reduced in poplar rust. The relationship appeared to be balanced in such a manner that the biotroph obtained from the host the assimilate required for growth and sporulation, but did not deplete the reserves of the host to its detriment by depriving young developing tissues or causing premature death.

It is not fully understood how pathogenic biotrophic fungi exert control on the host metabolism in order to divert translocate to the infection court. It is possible that different biotrophs have a variable capacity to alter patterns of translocation in that some are more efficient than others (63). Biotrophs with limited capacity to alter host translocation patterns may be stimulated to retain assimilate or alter translocation patterns by the experimental application of kinetin. In general it appears that biotrophy is favoured by high cytokinin levels (63), although there is little decisive evidence for this statement. The problem of metabolic control has not been investigated in this work but the observed attenuated virulence of the rust on poplar suggests that a delicate balance exists in the metabolic dependence of the rust on the host and this balance may be maintained either by hormones produced by the rust or by rust-induced hormones produced by the host (63).

Since the poplar rust lacks the secondary host in the South Western Cape, it is likely that the compatibility of host and pathogen in this relationship is a necessary condition for survival. It is logical that a parasite which undermines its host's capacity to compete, or destroys its host, will ultimately destroy itself (97). The poplar host is believed to possess a resistance to the rust which is not sufficiently strong to render the association incompatible. The absence of the secondary hosts of this rust in the South Western Cape may be causing the adaptation of the biotroph to survival on one host alone. This

process of adaptation is facilitated by the ready availability of the host during the year due to the comparatively mild climate which enables the retention of leaves for long periods. The poplar rust is therefore proposed as an example of a benign obligate biotroph which exists compatibly with its host.

REFERENCES

1. Akazawa, T. (1956). Nature of protein synthesis in sweet-potato infected with Ceratostomella fimbriata. Science, N.Y., 123, 1075.

2. Allen, P.J. and Goddard, D.R. (1938). A respiratory study of powdery mildew of wheat. Am. J. Bot., 25, 613.

3. Allen P.J. (1942). Changes in the metabolism of wheat leaves induced by infection with powdery mildew. Am. J. Bot., 29, 425.

4. Allen, P.J. (1959). Metabolic considerations of obligate parasitism. Chapter 12. Plant Pathology Problems and Progress, 1908 - 1958. Ed. C.S. Holton et al., The University of Wisconsin Press, Madison.

5. Beevers, H. (1961). Respiratory metabolism of plants. Harper and Row, New York, Evanston & London and John Weatherhill, Inc., Tokyo.

6. Barnell, H.R. (1936). Seasonal changes in carbohydrates of the wheat plant. New Phytol., 35, 229.

7. Bevenue, A. (1949). Determination of sugars in plant materials. Analyt. Chem., 21, 586.

8. Bladon, P. (1964). Physical Methods in Organic Chemistry, Chapter 4, p. 147. Ed. J.C.P. Schwarz, Publishers Oliver and Boyd, Edinburgh and London.

9. Brian, P.W. (1967). Obligate parasitism in fungi. Proc. R. Soc. B., 168, 101.

10. Bushnell, W.R. and Allen, P.J. (1962). Respiratory changes in barley leaves produced by single colonies of powdery mildew. Pl. Physiol., Lancaster, 37, 751.

11. Bushnell, W.R. (1967). Symptom development in mildewed and rusted tissue. In "The dynamic role of molecular constituents in plant-parasite interactions". Ed. C.J. Mirocha and I. Uritani, pp 22 - 39, St. Paul, Minnesota, Bruce Publishing Company.

12. Bushnell, W.R. (1968). In vitro development of an Australian isolate of Puccinia graminis f. sp. tritici. Phytopathology, 58, 526.

13. Calonge, F.D. (1967). Chlorophyll and total nitrogen in barley rust infection. Trans. Br. mycol. Soc., 50 (3), 397.

14. Canny, M.J. and Askham, M.J. (1967). Physiological inferences from the evidence of translocated tracer: a caution. Ann. Bot., N.S, 31, 409.

15. Chester, K.S. (1950), in Yarwood, C.E. (1967). Response to parasites. A. Rev. Pl. Physiol., 18, 419.

16. Coffey, M.D., Bose, A. and Shaw, M. (1969). In vitro growth of gelatin suspensions of uredospores of Puccinia graminis f. sp. tritici. Can. J. Bot., 47, 1291.

17. Coffey, M.D., Bose, A. and Shaw. M. (1970). In vitro culture of the flax rust, Melampsora lini. Can. J. Bot., 48, 773.

18. Coffey, M.D. and Shaw, M. (1972). Nutritional studies with axenic cultures of the flax rust, Melampsora lini. Physiol. Pl. Path., 2, 37.

19. Collins, R.P. and Scheffer, R.P. (1958). Respiratory responses and systemic effects in Fusarium-infected tomato plants. Phytopathology, 48, 349.

20. Daly, J.M., Sayre, R.M. and Pazur, J.H. (1957). The hexosemonophosphate shunt as the major respiratory pathway during sporulation of rust of safflower. Pl. Physiol., Lancaster, 32, 44.

21. Daly, J.M., Inman, R.E. and Livne, A. (1962). Carbohydrate metabolism in higher plant tissues infected with obligate parasites. Pl. Physiol., Lancaster, 37, 531.

22. Daly, J.M., Seevers, P.M., and Ludden, P. (1970). Studies on wheat stem rust resistance controlled at the Sr 6 locus. III Ethylene and disease resistance. Phytopathology, 60, 1648.

23. Dickson, R.E. and Larson, P.R. (1975). Incorporation of ^{14}C-photosynthate into major chemical fractions of source and sink leaves of Cottonwood. Pl. Physiol., Lancaster, 56, 185.

24. Dina, S.J. and Klickoff, L.G. (1974). Carbohydrate cycle of Plantago insularis var. fastigiata, a winter annual from the Sonoran desert. Bot. Gaz., 135 (1), 13.

25. Doidge, E.M. (1926). A preliminary study of the South African rust fungi. Bothalia, 2, 473.

26. Doodson, J.K., Manners, J.G., and Myers, A. (1964a). The distribution patterns of ^{14}C carbon assimilated by the 3rd leaf of wheat. J. exp. Bot., 15, 96.

27. Doodson, J.K., Manners, J.G. and Myers, A. (1964b). Some effects of yellow rust (Puccinia striiformis) on the growth and yield of a spring wheat. Ann. Bot., N.S., 28, 459.

28. Dubois, M. Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analyt. Chem., 28, 350.

29. Dunkle, L.C., Wergin, W.P. and Allen P.J. (1970). Nucleoli in differentiated germ tubes of wheat rust uredospores. Can. J. Bot., 48, 1693.

30. Flor, H.H. (1946). Genetics of pathogenicity in Melampsora lini. J. agric. Res., 73, 335.

31. Gäumann, E. (1950). Principles of plant infection. Crosby Lockwood and Son, London.

32. Gaunt, R.E. and Manners, J.G. (1971). Host-parasite relations in loose smut of wheat. I. The effect of infection on host growth. II. The distribution of ^{14}C-labelled assimilates. III. The utilization of ^{14}C-labelled assimilates. Ann. Bot., 35, 1131.

33. Gerwitz, D.L. and Durbin, R.D. (1965). The influence of rust on the distribution of ^{32}P in the bean plant. Phytopathology, 55, 57.

34. Girolami, G. (1953). Relation between phyllotaxis and primary vascular organization in Linum. Am. J. Bot., 40, 618.

35. Gottlieb, D. and Garner, J.M. (1946). Rust and phosphorus distribution in wheat leaves. Phytopathology, 36, 557.

36. Gussin, A.E.S. (1972). Does trehalose occur in Angiospermae? Phytochemistry, 11, 1827.

37. Hansen, P. and Grauslund, J. (1973). ^{14}C-studies on apple trees. VIII. The seasonal variation and nature of reserves. Physiologia Pl., 28, 24.

38. Hardwick, N.E. (1954). Changes in major chemical constituents of subterranean clover (Trifolium subterraneam L.) during growth. I. The carbohydrates. Aust. J. agr. Res., 5, 372.

39. Harley, J.L. and Lewis, D.H. (1969). The physiology of ectotrophic mycorrhizas. Adv. Microbiol. Physiol., 3, 53.

40. Haverkamp, J., Kamerling, J.P. and Vliegenthart, J.F.G. (1971). Gas-liquid chromatography of trimethylsilyl disaccharides. J. Chromat., 59, 281.

41. Heitefuss, R. and Fuchs, W.H. (1962). Phosphatstoffwechsel und Sauerstoffaufnahme in Weizenkeimpflanzen nach Infektion mit Puccinia graminis tritici. Phytopath. Z., 46, 174.

42. Holligan, P.M. (1971). Routine analysis by gas-liquid chromatography of soluble carbohydrates in extracts of plant tissues. I. A review of techniques used for separation, identification and estimation of carbohydrates by gas-liquid chromatography. New Phytol., 70 (2), 239.

43. Holligan, P.M. and Drew, E.A. (1971). Routine analysis by gas-liquid chromatography of soluble carbohydrates of plant tissues. II. Quantitative analysis of standard carbohydrates, and the separation and estimation of soluble sugars and polyols from a variety of plant tissues. New Phytol., 70 (2), 271.

44. Holligan, P.M., Chen, C. and Lewis, D.H. (1973). Changes in the carbohydrate composition of leaves of Tussilago farfara during infection by Puccinia poarum. New Phytol., 72, 947.

45. Holligan, P.M, McGee, E.E.M. and Lewis, D.H. (1974). Quantitative determination of starch and glycogen and their metabolism in leaves of Tussilago farfara during infection by Puccinia poarum. New Phytol., 73, 873.

46. Hooker, A.L. and Yarwood, C.E. (1966). Culture of Puccinia sorghi on detached leaves of corn and Oxalis corniculata. Phytopathology, 56, 536.

47. Hopkinson, S.M. (1969). The chemistry and biochemistry of phenolic glycosides. Q. Rev. chem. Soc., 23 (1), 98.

48. Hough, L., Jones, J.K.M. and Wadman, W.H. (1950). Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part V. Improved methods for the separation and detection of the sugars and their methylated derivatives on paper chromatograms. J. chem. Soc., II, 1702.

49. Hough, L., Jones, J.S.C. and Wusteman, P. (1972). On the automated analysis of neutral monosaccharides in glycoproteins and polysaccharides. Carbohyd. Res., 21, 9.

50. Howes, N.K. and Scott, K.J. (1972). Sulphur nutrition of Puccinia graminis f. sp. tritici in axenic culture. Can. J. Bot., 50 (6), 1165.

51. Ingram, J.M. and Wood, W.A. (1965). Enzymatic basis for D-arabitol production by Saccharomyces rouxii. J. Bact., 89, 1186.

52. Isebrands, J.G., Dickson, R.E. and Larson, P.R. (1976). Translocation and incorporation of ^{14}C into the petiole from different regions within developing Cottonwood leaves. Planta (Berl.), 128, 185.

53. Jones, H., Martin, R.V. and Porter, H.K. (1959). Translocation of ^{14}C carbon in tobacco following assimilation of $^{14}CO_2$ by a single leaf. Ann. Bot. N.S., 23, 493.

54. Juliano, B.O. and Varner, J.E. (1969). Enzymatic degradation of starch granules in the cotyledons of germinating peas. Pl. Physiol., Lancaster, 44, 886.

55. Keen, N.T. and Williams, P.H. (1969). Translocation of sugars into infected cabbage tissues during clubroot development. Pl. Physiol., Lancaster, 44, 748.

56. Király, Z. and Farkas, G.L. (1962). Relation between phenol metabolism and stem rust resistance in wheat. Phytopathology, 52, 657.

57. Kuprwicz, V.F. (1951) in Scott, K.J. (1972). Obligate parasitism by phytopathogenic fungi. Biol. Rev., 47, 537.

58. Larson, P.R. and Dickson, R.E. (1973). Distribution of imported ^{14}C in developing leaves of Eastern Cottonwood according to phyllotaxy. Planta (Berl.), 111, 95.

59. Larson, P.R., Isebrands, J.G. and Dickson, R.E. (1972). Fixation patterns of ^{14}C within developing leaves of Eastern Cottonwood. Planta (Berl.), 107, 301.

60. Lewis, D.H. and Harley, J.L. (1965a). Carbohydrate physiology of mycorrhizal roots of beech. I. Identity of exogenous sugars and utilization of exogenous sugars. New Phytol., 64, 224.

61. Lewis, D.H. and Harley, J.L. (1965b). Carbohydrate physiology of mycorrhizal roots of beech. II. Utilization of exogenous sugars by uninfected and mycorrhizal roots. New Phytol., 64, 238.

62. Lewis, D.H. and Smith, D.C. (1967a). Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytol., 66, 143.

63. Lewis, D.H. (1973). Concepts in fungal nutrition and the origin of biotrophy. Biol. Rev., 48, 261.

64. Lewis, D.H. (1974). Micro-organisms and Plants : The evolution of parasitism and mutualism. Symposia of the Society for General Microbiology, XXIV. Evolution in the Microbial World, p. 368.

65. Livne, A. and Daly, J.M. (1962). Carbon dioxide fixation in healthy and rust-affected tissues. Phytopathology, 52, 18. (Abstr.)

66. Livne, A. (1964). Photosynthesis in healthy and rust-affected plants. Pl. Physiol., Lancaster, 39, 614.

67. Livne, A. and Daly, J.M. (1966). Translocation in healthy and rust-affected beans. Phytopathology, 56, 170.

68. Long, D.E. and Cooke, R.C. (1974). Carbohydrate composition and metabolism of Senecio squalidus L. leaves infected with Albugo tragopogonis (Pers.) S.F. Grey. New Phytol., 73 (5), 889.

69. Long, D.E., Fung, A.K., McGee, E.E.M., Cooke, R.C. and Lewis, D.H. (1975). The activity of invertase and its relevance to the accumulation of storage polysaccharides in leaves infected by biotrophic fungi. New Phytol., 74, 173.

70. Lunderstädt, J., Heitefuss, R. and Fuchs, W.H. (1962). Aktivität einiger Enzyme des Kohlenhydratstoffwechsels aus Weizenkeimpflanzen nach Infektion mit Puccinia graminis tritici. Naturwissenschaften, 49, 403.

71. Lunderstädt, J. (1966). Effect of rust infection on hexokinase activity and carbohydrate dissimilation in primary leaves of wheat. Can. J. Bot., 44, 1345.

72. Mabry, T.J., Markham, K.R. and Thomas, M.B. (1970). The systematic identification of the flavonoids. Chapter 2. Springer Verlag.

73. Mains, E.B. (1917). The relation of some rusts to the physiology of their hosts. Am. J. Bot., 4, 179.

74. Mason, T.G. and Maskell, E.J. (1928a). Studies on the transport of carbohydrates in the cotton plant. I. A study of the diurnal variation in the carbohydrates of leaf, bark and wood and the effects of ringing. Ann. Bot., 42, 189.

75. Mason, T.G. and Maskell, E.J. (1928b). Studies on the transport of carbohydrates. II. The factors determining the rate and direction of movement of sugars. Ann. Bot., 42, 571.

76. Millerd, A. and Scott, K.J. (1963). Host-parasite relations in powdery mildew of barley. III. Utilization of respiratory energy. Aust. J. biol. Sci., 16, 775.

77. Mirocha, C.J. and Zaki, A.I. (1966). Fluctuation in amount of starch in host plants invaded by rust and mildew fungi. Phytopathology, 56, 1220.

78. Mitchell, D.T. and Roberts, S.M. (1973). Carbohydrate composition of tissues infected by the autoecious rusts Puccinia pelargonii-zonalis and P. malvacearum. Physiol. Pl. Path., 3, 481.

79. Mitchell, D. and Shaw, M. (1968). Metabolism of glucose-^{14}C, pyruvate-^{14}C and mannitol-^{14}C by Melampsora lini. II. Conversion to soluble products. Can. J. Bot., 46, 454.

80. Mitchell, D. and Shaw, M. (1969). The nucleolus of wheat stem rust uredospores. Can. J. Bot., 47, 1887.

81. Mlodzianowski, F. and Siwecki, R. (1975). Ultrastructural changes in chloroplasts of Populus tremula L., leaves affected by the fungus Melampsora pinitorqua Braun. Rostr.. Physiol. Pl. Path., 6, 1.

82. Mooney, H.A. and Billings, W.D. (1960). The annual carbohydrate cycle of alpine plants as related to growth. Am. J. Bot., 47, 594.

83. Morowitz, H.J. (1968). Energy flow in biology. Academic Press, New York and London.

84. Nagai, Y. and Kimura, Y. (1958). Detection of inositol and inositol diphosphate on paper chromatograms. Nature, Lond., 181, 1730.

85. Neales, T.F. and Incoll, L.D. (1968). The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf : a review of the hypothesis. Bot. Rev., 34, 107.

86. Nielsen, J.P. (1943). Rapid determination of starch. Ind. Engng. Chem. analyt. Ed., 15, 176.

87. Northcote, D.H. (1972). Chemistry of the plant cell wall. A. Rev. Pl. Physiol., 23, 113.

88. Oesch, F. and Meier, H. (1967). Trehalose in the cambial sap of Fagus sylvatica L. Phytochemistry, 6, 1147.

89. Pacsu, E., Mora, T.P. and Kent, P.W. (1949). General methods for paper chromatographic analysis of reducing and non-reducing carbohydrates and their derivatives. Science, N.Y., 110, 446.

90. Pozsár, B.I. and Király, Z. (1966). Phloem transport in rust-infected plants and the cytokinin-directed long distance movement of nutrients. Phytopath. Z., 56, 297.

91. Pratt, R. (1938). Respiration of wheat infected with powdery mildew. Science, N.Y., 88, 62.

92. Priestley, C.A. (1963). The carbohydrate resources of young apple-tree under four levels of illumination. Ann. Bot. N.S., 27 (107), 435.

93. Repaś, A. and Nikolin, B. (1968). The separation of phenolic glycosides and carbohydrates from the bark of Populus tremula by gel filtration. J. Chromat., 35, 99.

94. Repaś, A., Nikolin, B. and Dursun, K. (1969). The separation of phenolic glycosides by gel filtration. J. Chromat., 44, 184.

95. Robb, J., Harvey, A.E. and Shaw, M. (1975). Ultrastructure of tissue cultures of Pinus monticola infected by Cronartium ribicola. Physiol. Pl. Path., 5, 1.

96. Roberts, S.M. and Mitchell, D.T. (1974). ^{14}C-assimilation studies on the poplar and pelargonium rust infections. S. Afr. J. Sci., 70 (12), 380.

97. Robinson, R.A. (1973). Horizontal resistance. Rev. Pl. Path., 52 (8), 483.

98. Rohringer, R. and Heitefuss, R. (1961). Incorporation of ^{32}P into ribonucleic acid of rusted wheat leaves. Can. J. Bot., 39, 263.

99. Sakai, A. (1966). Seasonal variations in the amounts of polyhydric alcohol and sugar in fruit trees. J. hort. Sci., 41, 207.

100. Samborski, D.J. and Shaw, M. (1956). The physiology of host-parasite relations. II. The effect of Puccinia graminis tritici Erikss. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. Can. J. Bot., 34, 601.

101. Schipper, A.L. and Mirocha, C.J. (1969a). The histochemistry of starch depletion and accumulation in bean leaves at rust infection sites. Phytopathology, 59, 1416.

102. Schipper, A.L. and Mirocha, C.J. (1969b). The mechanism of starch depletion in leaves of Phaseolus vulgaris infected with Uromyces phaseoli. Phytopathology, 59, 1722.

103. Scott, K.J. and Smillie, R.M. (1966). Metabolic regulation in diseased leaves. I. The respiratory rise in barley leaves infected with powdery mildew. Pl. Physiol., Lancaster, 41, 289.

104. Scott, K.J. (1972). Obligate parasitism by phytopathogenic fungi. Biol. Rev., 47, 537.

105. Seevers, P.M. and Daly, J.M. (1970). Studies on wheat stem rust resistance controlled at the Sr 6 locus. I. The role of phenolic compounds. Phytopathology, 60, 1322.

106. Shaw, M. (1961). The physiology of host-parasite relations. IX. Further observations on the accumulation of radioactive substances at rust infections. Can. J. Bot., 39, 1393.

107. Shaw, M. (1967). Cell biological aspects of host-parasite relations of obligate fungal parasites. Can. J. Bot., 45, 1205.

108. Shaw, M. and Colotelo, N. (1961). The physiology of host-parasite relations. VII. The effect of stem rust on the nitrogen and amino acids in wheat leaves. Can. J. Bot., 39, 1351.

109. Shaw, M. and Hawkins, A.R. (1958). The physiology of host-parasite relations. V. A preliminary examination of the level of free endogenous indole-acetic acid in rusted and mildewed cereal leaves and their ability to decarboxylate exogenously supplied radioactive IAA. Can. J. Bot., 36, 1.

110. Shaw, M. and Manocha, M.S. (1965). The physiology of host-parasite relations. XV. Fine structure in rust-infected wheat leaves. Can. J. Bot., 43, 1285.

111. Shaw, M. and Samborski, D.J. (1956). The physiology of host-parasite relations. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. Can. J. Bot., 34, 389.

112. Shaw, M. and Samborski, D.J. (1957). The physiology of host-parasite relations. III. The pattern of respiration in rusted and mildewed cereal leaves. Can. J. Bot., 35, 389.

113. Shipton, W.A. and Brown, J.F. (1962). A whole-leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology, 52, 1313.

114. Shu, P., Tanner, K.G. and Ledingham, G.A. (1954). Studies on the respiration of resting and germinating uredospores of wheat stem rust. Can. J. Bot., 32, 16.

115. Shu, P. and Ledingham, G.A. (1956). Enzymes related to carbohydrate metabolism in uredospores of wheat stem rust. Can. J. Microbiol., 2, 489.

116. Siddiqui, M.Q. and Manners, J.G. (1971). Some effects of general yellow rust (Puccinia striiformis) infection on ^{14}Carbon assimilation, translocation and growth in a spring wheat. J. exp. Bot., 22, 792,

117. Smith, D., Muscatine, L. and Lewis, D. (1969). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Biol. Rev., 44, 17.

118. Somogyi, M. (1945). Determination of blood sugar. J. biol. Chem., 160, 69.

119. Staples, R.C. and Ledbetter, M.C. (1958). A study by microautoradiography of the distribution of tritium-labelled glycine in rusted Pinto beans. Contr. Boyce Thompson Inst. Pl. Res., 19, 349.

120. Swanson, C.A. (1959). Translocation of organic solutes. In "Plant Physiology". Vol.II. Plants in relation to water and solutes. Ed. F.C. Steward. Chapter 5. p. 481. Academic Press Inc., New York.

121. Taper, C.D. and Liu, P.S. (1969). Sorbitol in Malus leaves and fruits. Can. J. Pl. Sci., 49, 97.

122. Thornton, J.D. and Cooke, R.C. (1974). Changes in respiration, chlorophyll content and soluble carbohydrates of detached cabbage cotyledons following infection with Peronospora parasitica (Pers. ex Fr.) Fr. Physiol. Pl. Path., 4, 117.

123. Thrower, L.B. (1964). Photophosphorylation and starch metabolism in association with infections by obligate parasites. Phytopath. Z., 51, 425.

124. Thrower, L.B. (1965a). On the host-parasite relationship of Trifolium subterraneum and Uromyces trifolii. Phytopath. Z., 52, 269.

125. Thrower, L.B. (1965b). Host physiology and obligate fungal parasites. Phytopath. Z., 52, 319.

126. Thrower, L.B. and Thrower, S.L. (1966). The effect of infection with Uromyces fabae on translocation in broad bean. Phytopath. Z., 57, 267.

127. Trevelyan, W.E., Procter, R.D.P. and Harrison, J.S. (1950). Detection of sugars on paper chromatograms. Nature, Lond., 166, 444.

128. Turel, F.L.M. (1973). Growth of the flax rust Melampsora lini on chemically defined media. Can. J. Bot., 51 (1), 131.

129. von Sydow, B. and Durbin, R.D. (1962). Distribution of ^{14}C-containing metabolites in wheat leaves infected with stem rust. Phytopathology, 52, 169.

130. von Sydow, B. (1966a). Über den Einbau von $^{14}CO_2$ in Weizenblätter nach Infektion mit Puccinia graminis tritici. I. Histologische Untersuchungen. Phytopath. Z., 56, 78.

131. von Sydow, B. (1966b). Über den Einbau von $^{14}CO_2$ in Weizenblätter nach Infektion mit Puccinia graminis tritici. II. Analytische Untersuchungen. Phytopath. Z., 56, 105.

132. Wang, D. (1961). The nature of starch accumulation at the rust infection site in leaves of Pinto beans. Can. J. Bot., 39, 1595.

133. Webb, K.L. and Burley, J.W.A. (1962). Sorbitol translocation in apple. Science, N.Y., 137, 766.

134. Whetter, J.M. and Taper, C.D. (1973). Note on the seasonal occurrence of sorbitol (D-glucitol) in buds and leaves of Malus. Can. J. Bot., 41, 175.

135. Williams, M.W., Martin, G.C. and Stahly, E.A. (1967). The movement and fate of sorbitol-^{14}C in the apple tree and fruit. Proc. Am. Soc. hort. Sci., 90, 20.

136. Williams, P.G., Scott, K.J. and Kuhl, J.L. (1966). Vegetative growth of Puccinia graminis f. sp. tritici in vitro. Phytopathology, 56, 1418.

137. Williams, P.G., Scott, K.J., Kuhl, J.L. and Maclean, D.J. (1967). Sporulation and pathogenicity of Puccinia graminis f. sp. tritici grown on an artificial medium. Phytopathology, 57, 326.

138. Wilson, M. and Henderson, D.M. (1966). British Rust Fungi. Cambridge University Press.

139. Wolff, J.B. and Kaplan, N.O. (1956). D-mannitol-1-phosphate dehydrogenase from Escherichia coli. J. biol. Chem., 218, 849.

140. Wong, A.L. and Willetts, H.J. (1970). Observations on the growth of selected Australian races of wheat stem rust in axenic culture. Trans. Br. mycol. Soc., 55, 231.

141. Yarwood, C.E. (1934). Effect of mildew and rust infection on dry weight and respiration of excised clover leaflets. J. agric. Res., 49, 549.

142. Yarwood, C.E. (1953). Heat of respiration of injured and diseased leaves. Phytopathology, 43, 675.

143. Yarwood, C.E. (1959). Some effects of rust fungi on their hosts. Trans. Br. mycol. Soc., 42, 123.

144. Yarwood, C.E. (1964). Increased host growth associated with rust infections. Phytopathology, 54, 1438. (Abstr.).

145. Yarwood, C.E. (1967). Response to parasites. A. Rev. Pl. Physiol., 18, 419.

146. Yarwood, C.E. and Jacobson, L. (1952). Fungicidal action due to selective absorption of sulphur-35 by fungus-infected leaves. Phytopathology, 42, 478. (Abstr.).

147. Yarwood, C.E. and Jacobson, L. (1955). Accumulation of chemicals in diseased areas of leaves. Phytopathology, 45, 43.

148. Zaki, A.I. and Durbin, R.D. (1962). The effect of rust on the distribution of ^{14}C-containing compounds in bean. Phytopathology, 52, 758.

149. Zaki, A.I. and Mirocha, C.J. (1964). Respiration, photosynthesis and dark fixation of $^{14}CO_2$ by healthy and rusted bean leaves. Phytopathology, 54, 913.

150. Zaki, A.I. and Mirocha, C.J. (1965). Carbon dioxide fixation by rust-infected bean plants in the dark. Phytopathology, 55, 1303.

APPENDIX 1:

Estimate of pustule density during two seasons, 1975 and 1976. Low density of infection was expressed as number of pustules per 1 cm^2 leaf area and high infection density denoted by % leaf area covered with lesions. (See Chapter 4:3)

Date	Infection Level	Number of Lesions ± Standard Error of Mean	% Leaf Area Covered with Lesions ± Standard Error of Mean
21.1.75	Low	1,9 ± 0,7	
30.1.75		4,7 ± 1,3	
10.2.75		11,3 ± 3,1	
24.2.75	High		10,5 ± 6,3
7.3.75			43,4 ± 14,3
24.3.75			27,5 ± 5,7
7.4.75			64,6 ± 15,2
23.4.75			68,8 ± 8,7
3.5.75			61,8 ± 7,8
8.1.76	Low	4,4 ± 1,7	
23.1.76		3,3 ± 0,8	
5.2.76		3,7 ± 1,0	
27.2.76		8,8 ± 1,4	
15.3.76		20,8 ± 2,8	52,9 ± 9,3
29.3.76	High		51,6 ± 7,4

APPENDIX 2:

Dry weight of the seasonal samples from 1971 to 1974. Dry weight expressed as g/1,00 g fresh weight. (See Chapter 4:4).

Month	Healthy Lamina	Pustule	Region adjacent to Pustule
1971:			
October	0,2613	-	-
November	0,2594	-	-
December	0,3161	-	-
1972:			
January	0,4045	-	-
February	0,4008	0,3940	0,4038
March	0,3972	0,4266	0,4049
April	0,4410	0,4201	0,5286
May	-	0,4946	0,4187
1973:			
April	0,4397	0,5177	0,5131
May	0,4568	0,5183	0,4489
June	0,3612	0,5124	0,4862
July	0,2966	0,5316	0,4691
August	0,3419	0,4905	0,4264
October	0,4049	0,5776	0,4965
November	0,4865	0,5282	0,5065
1974:			
January	0,3930	0,4077	0,4866
February	0,3425	0,4356	0,4421
March	0,3989	0,4386	0,4489
April	0,3893	0,4274	0,4587
May	-	0,4274	0,4586

APPENDIX 2. (Continued)

Fresh weight and dry weight of seasonal samples during 1976. Fresh weight expressed in terms of 1 cm^2 leaf area, dry weight expressed in terms of 100 mg fresh weight and 1 cm^2 leaf area (See Chapter 4:4). Values show the mean of 4 replicates and the standard error of the mean. Significant differences to 0,05 level between the means within a fortnightly sample shown by S, no significance by N S.

DATE	FRESH WEIGHT (mg.) PER 1 cm^2 LEAF AREA			SIGNIFI-CANCE.
	Healthy	Pustule	Region Adjacent	
8.1.76	16,6 ± 0,3	16,3 ± 0,4	16,3 ± 0,1	N S
23.1.76	12,1 ± 0,3	14,5 ± 0,4	13,1 ± 0,5	S
5.2.76	13,5 ± 0,2	12,5 ± 0,5	12,9 ± 0,4	N S
27.2.76	11,1 ± 1,2	14,3 ± 0,3	13,1 ± 0,3	S
15.3.76	10,5 ± 0,3	13,6 ± 0,1	13,6 ± 0,3	S
29.3.76	9,6 ± 0,8	10,8 ± 0,8	9,9 ± 0,9	N S
	DRY WEIGHT (mg) PER 100 mg FRESH WEIGHT			
8.1.76	39,1 ± 1,0	36,7 ± 0,6	35,3 ± 0,9	S
23.1.76	38,3 ± 0,8	41,0 ± 1,0	38,2 ± 1,2	N S
5.2.76	40,0 ± 0,5	45,2 ± 1,2	41,9 ± 0,7	S
27.2.76	39,1 ± 1,7	45,2 ± 0,8	42,2 ± 0,4	S
15.3.76	44,5 ± 0,4	48,5 ± 1,1	46,2 ± 0,5	S
29.3.76	51,2 ± 1,9	52,4 ± 1,2	52,4 ± 1,6	N S
	DRY WEIGHT (mg.) PER 1 cm^2 LEAF AREA			
8.1.76	6,1 ± 0,3	5,9 ± 0,1	5,7 ± 0,2	N S
23.1.76	4,6 ± 0,1	5,9 ± 0,2	5,0 ± 0,3	S
5.2.76	5,4 ± 0,0	5,6 ± 0,1	5,4 ± 0,2	N S
27.2.76	4,4 ± 0,6	6,5 ± 0,1	5,6 ± 0,1	S
15.3.76	4,7 ± 0,2	6,6 ± 0,2	6,3 ± 0,1	S
29.3.76	4,9 ± 0,3	5,6 ± 0,3	5,2 ± 0,4	N S

APPENDIX 3.

Total nitrogen levels in healthy and infected poplar leaves during the period April, 1973, to April, 1974. (See Chapter 4:5)

Month	Sample	Dry Weight (mg)	µg N per 100 mg dry weight of sample
April 1973	Healthy	78,5	1660
	Pustule	104,9	1885
	Region adjacent	100,0	1666
May	Healthy	272,5	2093
	Pustule	10,1	1921
	Region adjacent	277,4	1839
June	Healthy	251,2	2491
	Pustule	104,4	2080
	Region adjacent	112,3	2318
July	Healthy	99,9	2238
	Pustule	110,2	2115
	Region adjacent	104,0	1751
August	Healthy	84,8	2852
	Pustule	103,6	1949
	Region adjacent	105,9	2215
October	Healthy	101,5	2793
	Pustule	75,3	2108
	Region adjacent	107,7	2532
November	Healthy	118,6	2240
	Pustule	114,1	2285
	Region adjacent	101,1	2516
January 1974	Healthy	110,1	I524
	Pustule	116,2	2397
	Region adjacent	89,8	1682
February	Healthy	114,9	2534
	Pustule	76,4	2148
	Region adjacent	44,3	2496
March	Healthy	76,8	2173
	Pustule	92,7	1896
	Region adjacent	93,7	2020
April	Healthy	64,0	1842
	Pustule	92,0	1699
	Region adjacent	93,0	2200

APPENDIX 3. (Continued)

Total nitrogen levels in healthy and infected poplar leaves during the period January to March, 1976. Values show the mean of 4 replicates and the standard error of the mean (S.E.M.). Significant differences to 0,05 level between the means within fortnightly samples shown by S, no significance by N S.

Notation: H - healthy; P - pustule; RA - Region adjacent to pustule.

Sample	µg N per 50 mg dry weight.	± S.E.M.	Significance	µg N per 1cm^2 leaf area	± S.E.M.	Significance
8 Jan H	1427,7	186,1	N S	6,6	0,7	N S
P	1396,7	83,1		6,4	0,3	
RA	1224,6	68,0		5,4	0,4	
24 Jan H	1413,1	42,6	S	5,0	0,1	N S
P	1124,7	30,2		5,2	0,3	
RA	1184,9	36,4		4,5	0,5	
5 Feb H	1479,1	38,3	S	6,2	0,2	S
P	987,9	84,3		4,3	0,4	
RA	1109,3	34,7		4,6	0,1	
27 Feb H	1254,3	210,1	S	4,4	0,2	S
P	1050,2	12,5		5,2	0,1	
RA	611,0	111,7		2,7	0,5	
15 Mar H	1385,2	160,6	S	4,9	0,4	N S
P	944,3	42,0		4,8	0,3	
RA	1051,9	23,3		5,1	0,1	
29 Mar H	1171,9	94,8	N S	4,4	0,0	N S
P	1013,9	49,5		4,4	0,2	
RA	1079,9	78,8		4,2	0,1	

Appendix 4:

METHOD OF TOTAL SUGAR ANALYSIS

Total sugar levels in ethanol-soluble and starch fractions were assayed by means of the method of Dubois et al (28). This test enabled the quantitative estimation of mono-, di-, oligo- and polysaccharides but did not include acyclic polyols. The sugars reacted with an orange-yellow colour when treated with phenol and concentrated sulphuric acid.

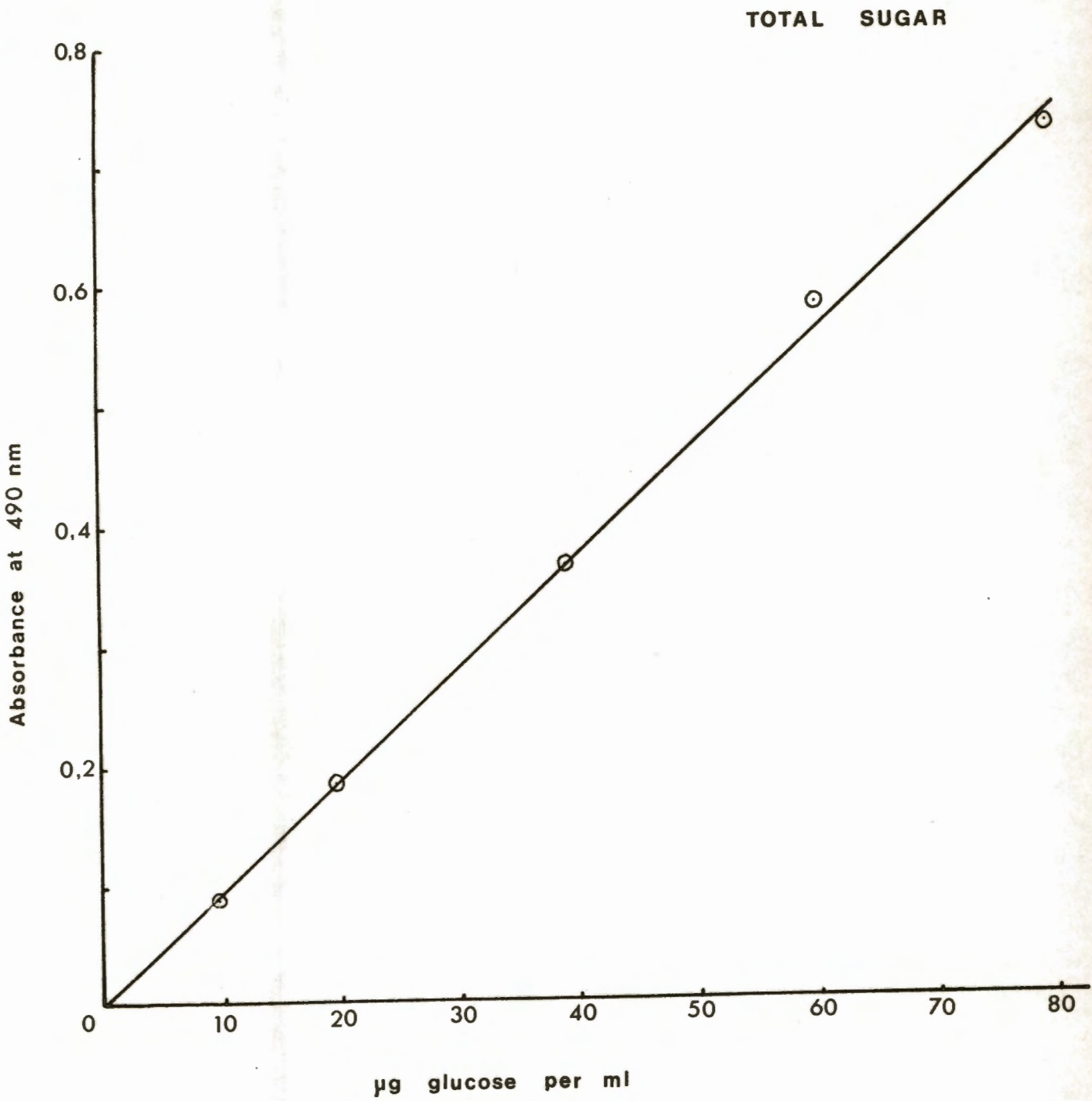
The following components were mixed and allowed to stand for 10 minutes :-

1 ml sugar solution;

1 ml 5% phenol;

5 ml concentrated sulphuric acid.

The solutions were mixed again and incubated for 10 - 20 minutes in a water bath at 25 - 30°C. An orange-yellow colour developed and the absorbance was read at 490nm on a Pye Unicam SP 1800 spectrophotometer. Using the calibration curve (Appendix Figure 1) the total sugar in each sample was calculated and expressed as µg per 100 mg fresh weight.



Appendix Figure 1:
Calibration curve showing total sugar levels in µg per ml of various concentrations of glucose solutions.

Appendix 5 (a):

Levels of ethanol-soluble carbohydrate in healthy and rust-infected poplar leaves during the three seasons studied. Each season was divided into periods of 2 to 4 months and mean levels of carbohydrate were calculated for each tissue region during each period. Standard error of the mean (± S.E.M.) was calculated for means of 3 or more samples. Levels shown as mg g^{-1} dry weight.

Key: t = trace amount

	Carbohydrate levels : mg g^{-1} dry weight (+ S.E.M.)							
SAMPLE	Total Carbohydrate	Arabitol	Fructose	Glucose	Hexitol	Myo-inositol	Sucrose	Tre-halose
SEASON 1 (1971-1972)								
Healthy Lamina								
Oct - Dec	16,11 ± 2,72	-	4,68 ± 1,00	3,44 ± 0,56	-	1,80 ± 0,30	4,21 ± 1,51	-
Jan - Feb	8,09	-	0,93	1,47	-	1,13	2,56	t
Pustule								
Feb - May	11,37 ± 2,98	0,54 ± 0,27	1,15 ± 0,32	2,14 ± 0,70	1,56 ± 0,72	3,51 ± 2,07	2,07 ± 0,97	t
Region Adjacent								
Feb - May	16,29 ± 6,32	t	1,81 ± 0,38	3,99 ± 2,13	t	3,47 ± 3,04	3,89 ± 1,44	t
SEASON 2 (1973-1974)								
Healthy Lamina								
Apr - June	38,56 ± 15,46	-	2,70 ± 0,85	2,53 ± 0,81	t	5,86 ± 1,64	26,89 ± 12,26	t
July - Aug	37,46	-	2,35	1,62	0,32	5,93	27,07	t
Oct - Jan	39,79 ± 6,57	-	10,72 ± 8,13	6,91 ± 3,47	t	5,22 ± 1,45	16,94 ± 3,77	t

Appendix 5 (a): (continued)

SAMPLE	Carbohydrate levels : mg g^{-1} dry weight (± S.E.M.)							
	Total Carbohydrate	Arabitol	Fructose	Glucose	Hexitol	Myo-inositol	Sucrose	Tre-halose.
Pustule								
Apr - June	25,98 ± 11,11	1,20 ± 0,38	2,40 ± 0,47	3,53 ± 0,45	1,41 ± 0,62	2,28 ± 1,21	14,47 ± 10,00	0,49 ± 0,11
July - Aug	11,43	1,30	1,50	2,03	1,39	0,42	4,63	t
Region Ajdacent								
Apr - June	49,27 ± 27,51	-	3,37 ± 0,34	3,13 ± 0,82	t	5,26 ± 4,36	37,04 ± 22,72	0,17 ± 0,09
July - Aug	25,00	t	0,84	1,37	0,90	1,68	19,88	t
Oct - Jan	32,44 ± 4,89	0,35 ± 0,09	1,15 ± 0,30	1,47 ± 0,03	0,32 ± 0,08	7,33 ± 0,55	21,41 ± 5,10	0,41 ± 0,21
SEASON 3 (1974)								
Healthy Lamina								
Mar - Apr	25,33	-	6,02	10,91	-	6,95	1,44	-
Pustule								
Mar - May	23,32 ± 5,92	1,62 ± 0,23	5,23 ± 1,98	8,62 ± 2,90	1,83 ± 0,20	5,76 ± 0,74	t	-
Region Adjacent								
Mar - May	25,58 ± 9,13	t	6,32 ± 3,42	12,13 ± 5,03	t	5,99 ± 0,69	t	-

Appendix 5 (b):

Levels of ethanol-soluble carbohydrate in lamina samples of healthy and rust-infected poplar during Season 1 (1971 - 1972), collected from Cecilia Forest. Levels expressed as mg g^{-1} dry weight.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight							
	Total Carbo-hydrate	Arabi-tol	Fruc-tose	Glu-cose	Hexi-tol	Myo-inosi-tol	Suc-rose	Treha-lose
HEALTHY LAMINA								
Oct. 1971	10,72	-	2,93	2,42	-	1,33	1,89	-
Nov. 1971	19,44	-	6,38	3,55	-	1,72	7,05	-
Dec. 1971	18,16	-	4,72	4,35	-	2,36	3,68	-
Jan. 1972	8,96	-	1,02	1,71	-	1,19	2,14	-
Feb. 1972	7,22	-	0,83	1,24	-	1,06	2,98	t
PUSTULE								
Feb. 1972	12,22	0,18	0,61	3,07	-	7,38	0,98	t
Apr. 1972	5,84	0,36	1,10	0,76	0,84	0,31	1,22	t
May 1972	16,05	1,07	1,73	2,59	2,27	2,84	4,00	0,26
REGION ADJACENT								
Feb. 1972	28,12	-	2,56	8,18	2,31	9,55	3,08	t
Apr. 1972	14,24	t	1,59	2,62	2,76	0,58	6,69	-
May 1972	6,50	0,14	1,29	1,18	0,85	0,28	1,91	0,25

Appendix 5 (b):

Levels of ethanol-soluble carbohydrate in lamina samples of healthy and rust-infected poplar during Season 1 (1971 - 1972), collected from Cecilia Forest. Levels expressed as mg g^{-1} dry weight.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight							
	Total Carbo-hydrate	Arabi-tol	Fruc-tose	Glu-cose	Hexi-tol	Myo-inosi-tol	Suc-rose	Treha-lose
HEALTHY LAMINA								
Oct. 1971	10,72	-	2,93	2,42	-	1,33	1,89	-
Nov. 1971	19,44	-	6,38	3,55	-	1,72	7,05	-
Dec. 1971	18,16	-	4,72	4,35	-	2,36	3,68	-
Jan. 1972	8,96	-	1,02	1,71	-	1,19	2,14	-
Feb. 1972	7,22	-	0,83	1,24	-	1,06	2,98	t
PUSTULE								
Feb. 1972	12,22	0,18	0,61	3,07	-	7,38	0,98	t
Apr. 1972	5,84	0,36	1,10	0,76	0,84	0,31	1,22	t
May 1972	16,05	1,07	1,73	2,59	2,27	2,84	4,00	0,26
REGION ADJACENT								
Feb. 1972	28,12	-	2,56	8,18	2,31	9,55	3,08	t
Apr. 1972	14,24	t	1,59	2,62	2,76	0,58	6,69	-
May 1972	6,50	0,14	1,29	1,18	0,85	0,28	1,91	0,25

Appendix 5 (c):

Levels of ethanol-soluble carbohydrate in lamina samples of healthy and rust-infected poplar during Season 2 (1973 - 1974), collected in Claremont. Levels expressed as mg g^{-1} dry weight.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight							
	Total Carbohydrate	Arabitol	Fructose	Glucose	Hexitol	Myo-inositol	Sucrose	Trehalose
HEALTHY LAMINA								
Apr. 1973	9,06	-	1,25	0,92	0,38	2,62	3,89	-
May 1973	45,26	-	2,65	3,49	-	7,01	31,02	1.09
June 1973	61,35	-	4,19	3,17	0,28	7,96	45,75	-
July 1973	18,70	-	2,32	1,39	0,16	1,88	12,61	0,34
Aug. 1973	56,22	-	2,38	1,85	0,48	9,98	41,53	-
Oct. 1973	52,38	-	26,90	13,37	-	2,64	9,47	t
Nov. 1973	36,78	-	4,03	5,86	t	5,37	21,52	-
Jan. 1974	30,22	-	1,24	1,49	-	7,65	19,84	-
PUSTULE								
Apr. 1973	13,28	0,52	3,11	4,09	0,96	0,73	3,18	0,69
May 1973	16,53	1,26	2,57	3,87	1,27	1,44	5,82	0,30
June 1973	48,12	1,82	1,52	2,64	2,59	4,66	34,40	0,49
July 1973	7,35	0,59	1,35	1,97	0,67	0,23	2,54	t
Aug. 1973	15,50	2,01	1,65	2,09	2,12	0,61	6,73	0,29
Oct. 1973	32,70	1,78	1,04	1,61	2,24	6,48	18,96	0,59
Nov. 1973	39,81	1,36	1,05	1,32	1,46	5,39	26,64	0,59
Jan. 1974	26,83	0,59	2,03	1,89	0,86	9,89	11,18	0,39
REGION ADJACENT								
Apr. 1973	15,59	-	2,69	3,55	0,73	0,34	8,08	0,20
May 1973	28,43	-	3,70	1,55	0,17	1,49	21,21	0,31
June 1973	103,80	-	3,72	4,28	t	13,96	81,84	t
July 1973	31,61	-	1,01	1,13	0,27	2,81	25,93	0,46
Aug. 1973	18,40	0,18	0,67	1,62	1,53	0,56	13,84	t
Oct. 1973	35,93	0,44	0,56	1,52	0,43	7,61	24,76	0,61
Nov. 1973	38,62	0,44	1,41	1,44	0,36	6,27	28.08	0,62
Jan. 1974	22,78	0,17	1,49	1,45	0,17	8,11	11,39	t

Appendix 5 (d):

Levels of ethanol-soluble carbohydrate in lamina samples of healthy and rust-infected poplar during Season 3 (1974), collected from Hohenort Estate. Levels expressed as mg g^{-1} dry weight.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight							
	Total Carbohydrate	Arabitol	Fructose	Glucose	Hexitol	Myo-inositol	Sucrose	Trehalose
HEALTHY LAMINA								
March	21,39	-	4,60	7,36	-	7,85	1,58	-
April	29,28	-	7,45	14,47	-	6,06	1,30	-
PUSTULE								
March	14,96	1,52	2,54	3,73	1,67	5,49	t	-
April	33,71	2,30	9,52	13,05	2,39	6,45	t	-
May	11,34	1,32	1,26	3,51	1,43	3,82	t	-
REGION ADJACENT								
March	15,20	0,83	2,72	4,99	1,14	5,52	t	-
April	43,78	t	13,17	21,84	t	7,35	1,42	t
May	17,76	-	3,08	9,57	t	5,11	t	-

Appendix 5 (e):

Levels of combined ethanol-soluble carbohydrate in healthy and infected petioles of poplar leaves, during the three seasons studied. Levels shown as mg g^{-1} dry weight.

Sample	Combined Carbohydrate mg g^{-1} dry weight	
	Healthy Petiole	Infected Petiole
Nov. 1971	14,60	No infection
Dec. 1971	5,69	"
Jan. 1972	21.72	"
Feb. 1972	14,47	33,75
Mar. 1972	55,52	30,57
Apr. 1972	62,45	30,29
May 1972	All infected	22,50
Apr. 1973	27,67	19,73
May 1973	35,94	27,04
June 1973	69,46	69,17
July 1973	26,91	30,19
Nov. 1973	8,05	17,07
Jan. 1974	All infected	5,07
Feb. 1974	4,55	5,45
Mar. 1974	21,70	18,61
Apr. 1974	35,26	11,46
May 1974	All infected	2,48

Appendix 6 (a):

Levels of acid-hydrolysed hot water-soluble polysaccharide in healthy and rust-infected poplar leaves during April to October, 1973, shown as mg g^{-1} dry weight. The season was divided into two periods, April/May/June and July/October/November. Values shown are means for each compound in each period, showing standard error of the mean.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight		
	Combined Carbohydrate	Glucose	Mannose
HEALTHY LAMINA			
Apr. - June	12,38 ± 1,26	6,73 ± 1,19	0,25 ± 0,02
July - Nov.	7,75 ± 4,22	2,49 ± 1,08	2,87 ± 2,68
PUSTULE			
Apr. - June	5,28 ± 2,39	2,56 ± 1,36	0,74 ± 0,19
July - Nov.	5,93 ± 3,45	2,31 ± 1,95	1,12 ± 0,49
REGION ADJACENT			
Apr. - June	5,33 ± 1,64	2,57 ± 0,87	0,18 ± 0,05
July - Nov.	4,16 ± 2,64	2,47 ± 1,81	0,13 ± 0,08

Appendix 6 (b):

Levels of acid-hydrolysed cold-water soluble polysaccharide in healthy and rust-infected poplar leaves during April to October, 1973, shown as mg g^{-1} dry weight. The season was divided into two periods, April/May and July/August/October. Values shown are mean levels for April/May and means with standard errors of the mean for July/August/October.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight		
	Combined Carbohydrate	Glucose	Mannose
HEALTHY LAMINA			
April/May	0,50	0,39	t
July - Oct.	3,10 ± 0,89	1,29 ± 0,36	0,22 ± 0,03
PUSTULE			
April/May	0,68	0,26	0,33
July - Oct.	0,87 ± 0,31	0,31 ± 0,18	0,23 ± 0,10
REGION ADJACENT			
April/May	2,07	0,66	0,19
July - Oct.	0,76 ± 0,20	0,18 ± 0,07	0,36 ± 0,13

Appendix 6 (c):

Levels of acid-hydrolysed ethanol-insoluble polysaccharide in healthy and rust-infected poplar leaves during November 1973, to May 1974, shown as mg g^{-1} dry weight. The season was divided into two periods, November and January and March/April/May. Values shown are mean levels during November/January and means with standard errors of the mean during March/April/May.

Key: t = trace amount.

SAMPLE	Carbohydrate levels mg g^{-1} dry weight				
	Combined Carbo-hydrate.	Glucose	Mannose	Arabinose	Xylose
HEALTHY LAMINA					
Nov/Jan	5,21	0,66	t	2,99	0,51
Mar - May	4,87 ± 1,86	0,50 ± 0,22	0,18 ± 0,05	2,15 ± 1,23	1,24 ± 0,20
PUSTULE					
Nov/Jan	9,91	0,99	0,35	4,75	2,00
Mar - May	19,40 ± 9,17	2,50 ± 1,22	4,07 ± 2,09	4,55 ± 2,59	4,44 ± 1,92
REGION ADJACENT					
Nov/Jan	12,74	1,33	t	6,82	1,89
Mar - May	10,19 ± 7,25	1,91 ± 1,62	0,31 ± 0,08	2,13 ± 1,45	2,65 ± 1,63

Appendix 7:

Levels of radioactivity (DPM x 10^3 per 100 mg fresh weight) in ethanol-soluble and perchloric acid-soluble components in healthy and rust-infected poplar leaves, at various time intervals after exposure to $^{14}CO_2$ followed by 46 hours incubation. Levels shown are means of 3 samples ± standard error of the mean (S.E.M.), or means of two samples. Leaf samples were: healthy lamina (H); pustule (P); and region adjacent (RA) of infected leaves.

Time (hours)	SAMPLE	DPM x 10^3 per 100 mg fresh weight	
		Ethanol-soluble ± S.E.M.	Perchloric acid soluble ± S.E.M.
1½ light	H	798,9 ± 328,3	79,1 ± 40,1
	P	625,2 ± 169,4	42,2 ± 17,8
	RA	738,4 ± 203,6	80,5 ± 18,3
7 dark	H	650,9 ± 63,8	65,4 ± 38,2
	P	290,3 ± 76,9	27,9 ± 12,5
	RA	454,4 ± 90,9	55,5 ± 17,4
16 dark	H	510,5	28,9
	P	238,1	13,6
	RA	337,5	22,2
20½ light	H	506,7 ± 207,0	44,2 ± 21,5
	P	409,0 ± 50,4	19,2 ± 6,7
	RA	442,3 ± 109,3	29,6 ± 16,9
24 light	H	369,8	39,7
	P	202,0	13,9
	RA	344,5	27,8
33 dark	H	166,4 ± 35,0	14,6 ± 0,4
	P	209,6 ± 78,5	8,8 ± 0,8
	RA	239,4 ± 106,2	8,6 ± 0,6
46 light	H	169,4 ± 41,6	15,2 ± 5,2
	P	162,2 ± 49,1	8,6 ± 0,8
	RA	280,4 ± 60,5	45,3 ± 36,3

Appendix 7: (continued)

Total sugar levels (mg glucose x 10^2 per 100 mg fresh weight) in ethanol-soluble and perchloric acid soluble components of healthy and rust-infected poplar leaves, at various time intervals during the the 46 hour incubation period. Levels shown are means of 3 samples ± standard error of the mean (S.E.M.), or means of two samples.

Leaf samples were: healthy lamina (H); pustule (P); and region adjacent (RA) of infected leaves.

Time (hours)	SAMPLE	Total sugar (µg glucose per 1,0 mg fresh weight)	
		Ethanol-soluble Components ± S.E.M.	Perchloric acid-soluble components ± S.E.M.
1½ light	H	45,0 ± 16,1	38,6 ± 25,9
	P	53,8 ± 13,5	33,6 ± 23,5
	RA	57,2 ± 15,6	41,5 ± 15,4
7 dark	H	36,2 ± 13,3	33,4 ± 17,9
	P	54,3 ± 11,5	42,9 ± 16,9
	RA	52,4 ± 22,4	34,3 ± 11,9
16 dark	H	58,6	31,0
	P	71,7	32,0
	RA	50,6	37,6
20½ light	H	46,4 ± 13,8	22,9 ± 4,4
	P	45,4 ± 6,9	25,8 ± 4,6
	RA	57,1 ± 7,9	34,9 ± 10,5
24 light	H	62,2	25,8
	P	61,4	20,8
	RA	54,5	29,6
33 dark	H	37,8 ± 15,4	22,4 ± 6,3
	P	62,1 ± 12,7	24,2 ± 2,8
	RA	74,1 ± 9,3	31,3 ± 7,1
46 light	H	37,3 ± 13,0	21,8 ± 1,8
	P	69,6 ± 9,5	29,2 ± 6,6
	RA	67,2 ± 16,7	40,0 ± 5,9