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# PHOTOSYNTHESIS, NITROGEN METABOLISM AND SHOOT:ROOT EQUILIBRIA IN LEEKS INFECTED WITH THE RUST **PUCCINIA ALLII** RUD

by

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A thesis submitted for the degree of Doctor of Philosophy at the University of Glasgow

October 1987

**ACKNOWLEDGEMENTS**

I should like to thank my supervisor, Dr. Dale R. Walters, for his advice and guidance throughout the course of this research.

I should also like to thank Dr. Robert K. M. Hay and Dr. Andrew J. Walker for help and advice with certain parts of this work. Thanks are also due to the Engineering Department, West of Scotland Agricultural College, for technical assistance and to my fellow post-graduates for many useful discussions. I should also like to thank Ms. Karen Colquhoun for typing this thesis. Finally, thanks are also due to my parents for their care and encouragement.

I wish to acknowledge financial support from the Agriculture and Food Research Council.

Parts of the work in this thesis appear in the following papers:-

ROBERTS, A.M. and WALTERS, D.R. (1986). Stimulation of photosynthesis in uninfected leaves of rust-infected leeks. Annals of Botany **56**, 893-896.

ROBERTS, A.M. and WALTERS, D.R. (1987). Nitrogen assimilation and metabolism in rusted leek leaves. Physiological and Molecular Plant Pathology (in press).

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**ABSTRACT**

Various aspects of the physiology of rust-infected leeks were studied. Rates of net photosynthesis of healthy leaves of leek were very low in comparison with other $C_3$ species. However, rust infection led to substantial reductions in photosynthetic rates in infected leaves. This was linked to large stimulations in dark respiration and reductions in photorespiration, coupled with reductions in the activity of a variety of photosynthetic enzymes, including RuBPcase, PePcase and 3-phosphoglycerate kinase. Rust infection also led to reductions in total soluble protein and chlorophyll, and chloroplast preparations from infected leaves exhibited much reduced rates of oxygen evolution compared to healthy controls.

Rates of net photosynthesis were markedly increased in uninfected leaves of otherwise rusted leeks. Such leaves also exhibited decreased rates of photorespiration, although dark respiration was not affected. An examination of enzyme activity in uninfected leek leaves revealed that RuBPcase, 3-phosphoglycerate kinase, NADP-glyceraldehyde-3-P-dehydrogenase and NADP malic enzyme activities were all significantly stimulated. Infection of the lower leaves had no effect on the activity of PePcase and NAD malic enzyme in upper, uninfected leaves, nor were protein and chlorophyll content altered.

Construction of a carbon budget revealed that rust infection led to a substantial reduction in assimilate production in all but the two upper, uninfected leaves of infected leeks. Disease also led to reductions in the translocation of assimilates out of all

rusted leaves, although stimulations in assimilate translocation were noted in uninfected fourth leaves.

Although photosynthesis was stimulated in upper, uninfected leaves of rusted leeks, assimilate production by these leaves was slightly lower than controls. Since reductions in photosynthesis by infected leaves appeared to account for reductions in dry weight, the observed stimulations in photosynthesis in uninfected leaves may only have served to alleviate the effects of rust infection.

A detailed examination was made of photosynthesis in localized regions of infected leek leaves. Oxygen evolution, expressed on both a chlorophyll and an area basis, was significantly reduced in pustule regions, coupled with an increase in dark respiration, for the duration of the experiment. The activity of RuBPcase was lower in pustule areas, and losses in total soluble protein and chlorophyll were observed. Areas between pustules exhibited photosynthetic rates near to control values, on a chlorophyll basis, and dark respiration was reduced for at least part of the experiment. No significant alteration in RuBPcase activity, nor in protein or chlorophyll levels, was detected in inter-pustule areas. Rates of photosynthesis were significantly stimulated in tissue from uninfected leaves of rusted plants, coupled with a lowering of dark respiration. RuBPcase activity was significantly stimulated in uninfected leaves towards the end of the experiment, while protein and chlorophyll levels were unaltered.

Phosphate did not appear to limit photosynthesis in any tissue type, since the addition of exogenous phosphate had no appreciable effect on rates of photosynthesis. Stomatal resistance was also

measured, revealing that infection led to reductions in stomatal resistance in pustule regions, while areas between pustules remained unchanged. By the end of the experiment, i.e. 21 days after inoculation, stomatal resistance was considerably higher in uninfected leaves of otherwise rusted leeks, compared to controls.

Cytokinin concentrations were higher in pustule areas at 14 days after inoculation, although trans-zeatin (t-ZR) concentration returned to near control values in pustules by the end of the experiment. Abscisic acid (ABA) concentrations were significantly higher in both infected regions and in uninfected leaves by 14 days after inoculation. However, the concentration of ABA was significantly less in infected regions by 21 days after inoculation, and there was almost eight times more ABA present in uninfected leaves by this time. The concentration of dihydrozeatin (DHZR) was slightly increased in all tissue types compared to healthy controls, by the end of the experiment.

ABA and cytokinin concentrations were always consistently lower in roots of infected plants than in healthy ones at 21 days after inoculation, and with the exception of DHZR, at 14 days after inoculation also. A similar situation existed in sap from healthy and rusted plants, except that no difference in t-ZR concentration could be detected between healthy and rusted sap by 21 days after inoculation. Also, at this time, no ABA was detected in any sample of sap from infected plants.

Pathogen-induced alterations in the growth and nutrient status of the infected leek were found to be complex. Briefly, rust infection led to significant reductions in total plant dry weight,

and to reductions in shoot:root ratio (S:R). Nitrogen content was generally reduced in rusted plants, although concentrations and SAR of nitrogen were usually higher than controls. The amount of phosphate found in rust-infected leeks was usually significantly less than found in healthy controls, although phosphate concentration was again increased compared to controls. SAR's for phosphate were increased in the middle of the experimental period, but were reduced at all other times. The potassium and calcium contents of rusted plants were usually significantly reduced in comparison to healthy controls, and concentrations of these minerals were higher than in non-infected plants, from three weeks after inoculation. Increased SAR's for potassium were observed in rusted leeks, two, three and eight weeks after inoculation, and SAR's for calcium were greater than healthy control values particularly towards the end of the experiment.

The relationship between dry weight accumulation and the uptake of individual and total nutrients was linear, and suggested that a functional equilibrium existed between the shoot and root systems for nutrient uptake. However, closer examination revealed that rust infection significantly altered the equilibirum between shoot and root with respect to the uptake of phosphate, calcium and total nutrients.

Measurements of the inorganic nitrogen content of leaves revealed that infection led to significant increases in nitrate and total nitrogen concentrations, and to reductions in ammonium concentration. Evolution of ammonia gas by rusted leaves was significantly greater than that of healthy plants. Infection also significantly stimulated the degradation of glutamic acid in leaves

of leek and increases were observed in the activities of nitrate reductase and glutamine synthetase. Nitrite reductase activity was unchanged following infection. The reduction in ammonium concentration of infected leaves was explained in terms of increased ammonium utilization, partly via the activity of glutamine synthetase.

SECTION 1

# General Introduction

**The Leek Crop**

The leek (**Allium porrum** L.) is a winter hardy biennial native to the eastern Mediterranean, although wild forms extend into western and southern Russia. Leeks, together with onions (**Allium cepa** L.) are vegetables whose cultivation and use can be traced back for three or four thousand years in the Near East. As a winter vegetable, the leek is a popular choice, since it is available when there are few other fresh vegetables on the market. Market requirements appear to vary in different parts of the country and between the different outlets. Nevertheless, the market is more and more demanding, and in the last decade, leek production in the UK has intensified, mainly due to greater interest in leeks for pre-pack outlets.

Good growth and yields can be obtained on most soil textures, although research has shown that the deep, water retentive loams and peats are most suitable. Leek crops can be grown either by direct drilling in the field, or by sowing in seedbeds and transplanting. The main advantages of the latter are that weed control is easier, greater flexibility and intensity of crop rotations are possible, and the leeks are likely to have a greater length of blanched stem. Considerable labour, however, is involved in transplanting, most of which has to be done in June and July at what is normally the busiest time of the year. Drilling of the crop **in situ** has increased in popularity as more effective herbicides have become available, although full, precisely and uniformly spaced leek crops are not easily produced from drilling because it is difficult to predict the emergence rate of seed under varying soil and climactic conditions, particularly early in spring.

**Major Diseases**

Leeks are prone to attack by several fungal pathogens, including **Sclerotium cepivorum**, a necrotrophic pathogen which causes white rot, and **Phytophthora porri**, the causal agent of white tip disease. A rust, caused by the fungus **Puccinia allii** Rud, has become particularly troublesome over the past few years, due to intensification of production within small land areas with crops present in the ground for nearly the whole year. Data from MAFF reveal that leek rust has been present nearly every year in the United Kingdom since 1919. On a worldwide basis, the rust is predominantly found in Europe, Asia and the West Coast of Africa.

**Puccinia allii** Rud. is an autoecious rust, that is, it has only one host, and was first identified by Sowerby in 1810. Although the rust has several sporing stages, only uredosori are found on leeks. These erupt as bright orange, circular to elongate pustules between the veins of the leek leaf. An additional symptom is chlorotic spotting of the leaf (Dixon, 1981), which appears on plants irrespective of the presence of uredosori, as lesions which are more or less circular up to 5 mm in diameter.

Within the uredosori, uredospores of spherical and elliptical shape are formed, measuring 23-32 $\mu$m x 20-26 $\mu$m, the walls of which are hyaline to yellow and spiny and have a thickness of 1-2 $\mu$m (Saville, 1961). Other sporing stages of the fungus have been identified on different **Allium** hosts. For example, teliosori are found on onion (**A. cepa**), usually scattered among the uredosori. These initiate below the host epidermal surface, leading to the production of chestnut-brown teliospores. These spores are elliptical to obovoid in shape, and measure 28-45 $\mu$m x 20-26 $\mu$m,

their walls being 1-2$\mu$m thick. Other spore forms in the life cycle of **P. allii** have been identified on the hosts **A. shoenoprasium** (chives) and **A. fistulosum** (bunching onions). Spermogonia and aecidia are found together with the aecidiospores, being globoid, of 19-28$\mu$m in diameter, and having a yellow wall 1-2$\mu$m thick.

The major financial losses incurred by rust infection are due to the disfigurement of the leek plant. In eliminating rust lesions, several outer leaves have to be removed. This ultimately leads to a reduction in the weight of the plant for sale, and in the thickness of the stem for competition purposes. Considerable losses are caused on other **Allium** crops. In Chile, for example, losses of up to 83% have been reported in garlic infected with rust. Production of garlic in Israel and of bunching onions in Japan is also limited. Other incidences of major losses due to this pathogen have been reported in Norway and Tanzania, where chives, onions and garlic are affected by rust (Dixon, 1981).

There are several ways in which leeks can be infected by rust from season to season. It seems possible that inoculum could be carried over from one season to the next via contaminated seeds, although Noble and Richardson (1968) stated that this was probably of minor importance. Another possibility is that spread of rust is helped by the presence of infected crop debris. Harvesting of the leek crop involves the stripping of some of the outer leaves to ensure a tidy leek plant. The presence of debris on the soil surface is a possible source of infection for future crops. The structure of many rust fungi means that the uredosorus stage has no mechanism for violent spore discharge. However, spore liberation is by the direct action of wind, or by leaf flutter and stem vibration

caused by wind or heavy rainfall. Viable uredospores of **Puccinia graminis** can be carried hundreds of miles, and Doherty (1981) suggested that, at least in the north of England, the source of infection of **P. allii** was a wind-borne inoculum from a distant source. Finally, research by Simkin and Wheeler (1974) and Parlevliet and Van Ommeren (1976) indicated that barley leaf rust (**Puccinia hordei**) overwintered in Western Europe as uredospores or dormant mycelium on volunteer plants or winter barley plants. Infection from diseased plants in the previous season's crop, or by volunteer leek plants, may be an important vector of disease in the leek crop in the field (Doherty, 1981).

As yet, no varieties of leek have been found to be completely resistant to rust infection, although some cultivars are more susceptible than others. Attempts to control the spread of the disease have involved changes in cultural practices, i.e. the removal and burning of affected debris and volunteer plants. Rust infection can also be controlled by fungicide spraying, but as all sprays used are protectant in action, spraying usually commences at the first sign of rust, or when seen or reported in the vicinity. Sprays of triadimefon or some dithiocarbamates can give reasonably good disease control, but are only effective if applied every two to three weeks. However, an increasingly discriminatory market, coupled with the costs and hazards of chemical control, mean that we should be trying to breed new leek cultivars that demonstrate good resistance to leek rust.

**Aims of the Project**

It seems clear that attempts to breed for resistance to disease will be hampered by our lack of understanding of the host/parasite interaction. Much research has centred on the effects of obligate biotrophic pathogens on physiological processes in host plants such as wheat, barley and beans, and this is reviewed in detail in subsequent chapters. However, to date, no physiological or biochemical investigations have been made to determine the nature of the leek/rust interaction. The research described here attempted to provide some information on the effects of rust infection on the physiology of the leek plant. Particular reference was made to various aspects of photosynthesis, nitrogen metabolism, mineral nutrition and shoot:root interrelationships.

SECTION 2

# General Materials And Methods

**Growth and Maintenance of Plants Before Inoculation**

Seeds of leek, **Allium porrum** L. var. Autumn Mammoth, were sown in seed trays (25 cm x 35 cm, 20 seeds per tray) containing potting compost (Levington). The trays were placed in a ventilated glasshouse under natural daylight supplemented to a 16 h photoperiod with 400W mercury vapour lamps. The maximum daylight temperature was 24°C, falling to a minimum of 9°C at night. When the plants had produced three true leaves, they were placed in individual 4" pots containing potting compost (Levington) and maintained under the conditions described above. Supplementary feeding was supplied twice weekly in the form of Phostrogen liquid feed, with the exception of plants required for mineral experiments (see Section 6).

**Maintenance of Pathogen**

Leek rust, **Puccinia allii** Rud was maintained on stock leek plants, in controlled-environment rooms with a temperature of 18°C during the light period (16 h $d^{-1}$), falling to 10°C during the dark period. The inoculation of stock plants is described below.

**Inoculation and Maintenance of Plants After Inoculation**

When the fourth leaf of experimental plants, and sixth leaf of stock plants had fully emerged, plants were transferred to controlled-environment rooms as described above. Irradiance at the soil surface was 81 $Wm^{-2}$. At this stage, half the plants were inoculated with uredospores of the rust fungus, and the other half were transferred to a separate controlled-environment room and kept under identical conditions as those to be infected. A spore suspension was obtained by lightly brushing the surface of infected leaves with a camel-hair brush and then placing it in a small volume

of distilled water. The resultant spore suspension was painted on the upper and lower surfaces of the leaves. Inoculated plants were kept under a plastic covering for 48 h in order to maintain the high humidity necessary for spore germination. Infected plants were maintained under these conditions until required.

Data were analysed by means of T-tests and analysis of variance.

**Symptom Development in the Leek/Rust System**

| Days After Inoculation | Symptoms | Infection Density |
|---|---|---|
| 11 | Flecking and chlorotic spooting of leaves | ---- |
| 13-14 | Sporulation of rust Orange pustules erupt on leaf surface | 9-10 pustules /cm |
| 24-26 | 'Green islands' formed Chlorophyll retention at infection sites | 9-10 pustules /cm |

SECTION 3

# Effects of Rust on Photosynthesis in Infected and Uninfected Leek Leaves

## INTRODUCTION

During autotrophic growth, plants obtain their substance and energy through the reactions of photosynthesis - the process by which the electromagnetic energy of sunlight is used for the synthesis of carbohydrates ($CH_2O$), and other organic compounds, from carbon dioxide and water. There are many important aspects of carbon fixation which could be affected by disease, including the diffusion of $CO_2$ into the leaf, the production of triose phosphate and the utilization of light.

The diffusion of $CO_2$ from the atmosphere to the sites of carboxylation may be treated as a series of resistances. In laboratory situations, the gas phase resistance is treated as a single resistance, and experimental apparatus is manipulated to ensure that stomatal diffusive resistance is the dominant resistance. The inverse of this composite resistance, i.e. conductance (g), is usually measured using an Ohm's law analogy. The transpiration rate (E) from the leaf is divided by the potential difference (driving force) as shown in Eq. 1:

$$g = \frac{EP}{e_i - e_o} \qquad \text{Eq. 1}$$

where P is atmospheric pressure, ei and eo are the vapour pressures of water inside and outside the leaf respectively. We can then calculate intercellular $CO_2$ (Ci) using the following equation:

$$Ci = Ca - \frac{1.6\ AP}{g} \qquad \text{Eq. 2}$$

where Ca is ambient $CO_2$ partial pressure, A is the $CO_2$ assimilation rate and 1.6 accounts for the fact that $CO_2$ diffusion is slower than

water vapour diffusion. This is sufficient for rough calculations. However, gaseous diffusion in leaves is a very complex process, involving the movement of water vapour, $CO_2$ and air (von Caemmerer and Farquar, 1981).

Thus, when making precise calculations, conductance is calculated as the conductance that would occur in the absence of mass flow, by accounting for the water vapour effect, as shown in Eq. 3.

$$Ci = \frac{(gc - E/2)\ Ca - AP}{(gc + E/2)} \qquad \text{Eq. 3}$$

where gc is conductance to $CO_2$.

Once inside the chloroplast, $CO_2$ is enzymatically combined with ribulose-1,5-bisphosphate (RuBP) to form an unstable six carbon intermediate which dissociates into two molecules of 3-phosphoglycerate (PGA). The enzyme involved is ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPcase). This enzyme's properties have a large influence on the gas exchange behaviour of leaves.

RuBPcase is a very large enzyme, consisting of 16 subunits, eight large, catalytic subunits and eight small subunits, and it comprises approximately 20% of the total protein in leaves. RuBPcase catalyzes the oxygenation, as well as the carboxylation of RuBP (see Lorimer, 1981). The interaction between $CO_2$ and $O_2$ is competitive (Ogren and Bowes, 1971) and so the presence of $O_2$ will lower the affinity of the enzyme for $CO_2$.

It is now known that the enzyme requires $Mg^{2+}$, $CO_2$ and the correct pH to be fully active (Laing, Ogren and Hageman, 1975; Lorimar, Badger and Andrews, 1976). In darkness, the enzyme is inactive because the pH and magnesium concentrations in the chloroplast stroma are low. In the light, hydrogen ions in the stroma are pumped inside the thylakoids and exchanged for $Mg^{2+}$, thereby raising both the pH and $Mg^{2+}$ concentration in the stroma. Thus, RuBPcase is activated.

The regeneration of the $CO_2$ acceptor, RuBP, requires light-derived chemical energy and a series of reactions termed the reductive pentose phosphate (RPP) pathway. The conversion of sunlight into chemical energy involves both cyclic and non-cyclic photophosphorylation. These are light-dependant processes that jointly provide ATP and reduced ferredoxin (or its product, NADPH) which are essential for $CO_2$ assimilation (Figure 1). Both processes occur within the chloroplast.

In cyclic photophosphorylation, energy from light is converted into the pyrophosphate bonds of ATP via electron transport, with no need for added chemical substrates. Cyclic photophosphorylation in isolated chloroplasts requires an added catalyst, ferredoxin (Fd), an iron-sulphur protein.

$$ADP + Pi \xrightarrow[\text{ferredoxin}]{\text{light}} ATP \qquad \text{Eq. 4}$$

Non-cyclic photophosphorylation involves the formation of ATP, coupled to an oxidation-reduction reaction in which electrons from water are transferred in the light to Fd with a concommitant evolution of molecular oxygen.

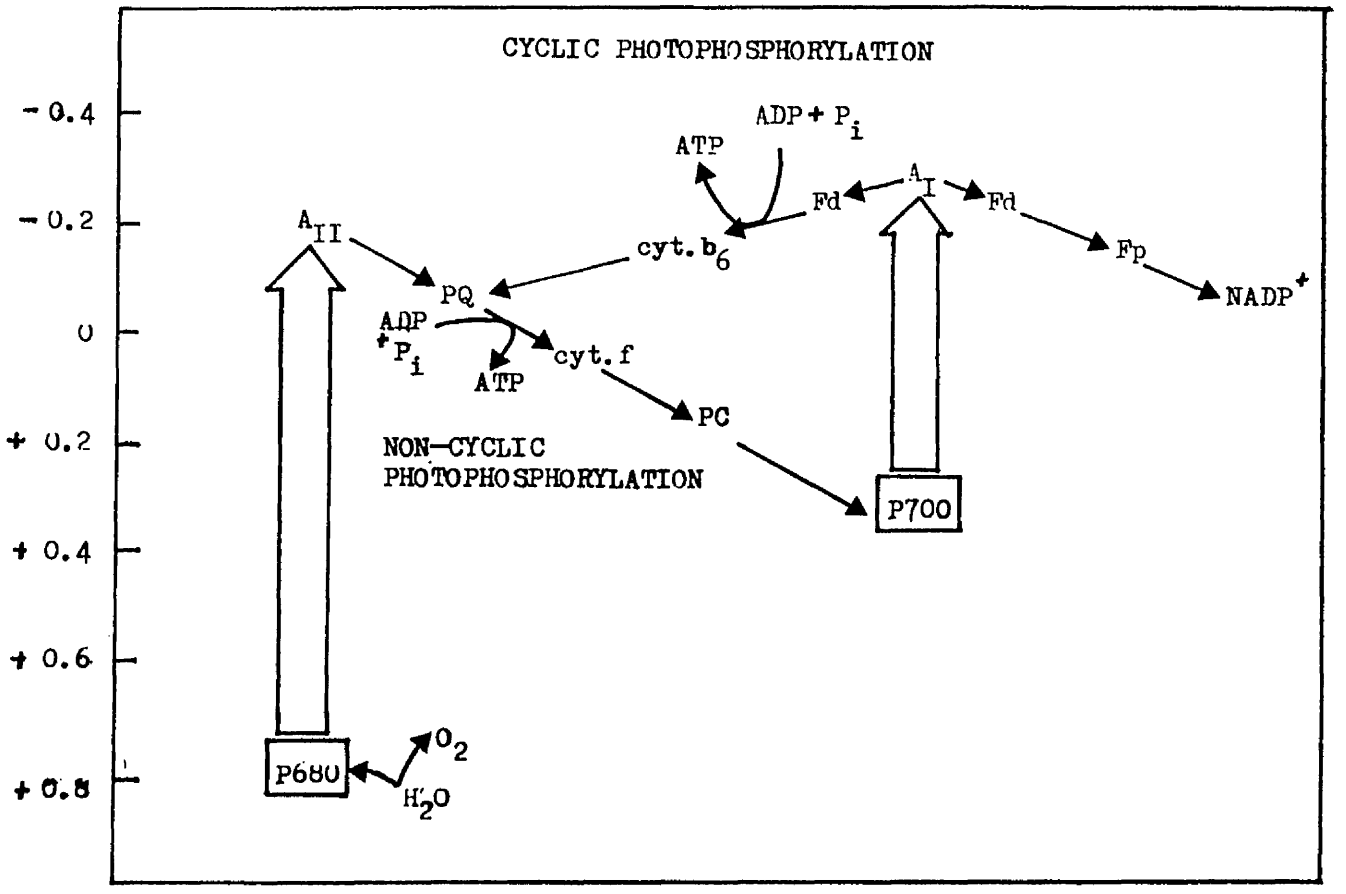



Figure I. Mechanisms of non-cyclic and cyclic photophosphorylation in chloroplasts. Non-standard abbreviations: P680, reaction centre chlorophyll of photosystem II; $A_{II}$, primary electron acceptor of photosystem II; PQ, plastoquinone; cyt.f, cytochrome f; PC, plastocyanin; P700, reaction centre chlorophyll of photosystem I; $A_I$, primary electron acceptor of photosystem I; Fd, ferredoxin; Fp, flavoprotein (ferredoxin-NADP reductase); cyt.$b_6$, cytochrome $b_6$.

$$2H_2O + 4Fd_{ox} + 2ADP + 2Pi \xrightarrow{light} 4Fd_{red} + 2ATP + O_2 + 4H^+$$

Eq. 5

The reduced Fd may then, in turn, reduce NADP in a reaction catalysed by a flavoprotein enzyme, ferredoxin-NADP reductase. This occurs independently of light.

The heart of the system effecting cyclic and non-cyclic photophosphorylation is a light-driven transport of electrons from two different active chlorophylls (P680, P700), located on the thylakoid membranes of chloroplasts, to each of two acceptors (A-II, A-I). Simply, in non-cyclic reactions, electrons from water flow through two photochemical reactions, photosystems II and I, to Fd and then to NADP. Cyclic transport however, involves electron flow through only one photoreaction, photosystem I, which cycle in a closed system without net oxidation or reduction (Figure 1).

The ATP and NADPH generated by photosynthetic electron transport are used in the RPP pathway. The conversion of PGA to triose phosphate requires ATP and NADPH (1 mole each per mole PGA) and the phosphorylation of RuBP requires another ATP. The carbon in PGA is derived from RuBP and $CO_2$. For the cycle to be autocatalytic, one molecule of triose phosphate must re-enter the regenerative phase of the pathway, leading to a build-up of intermediates. The RPP pathway is illustrated in Figure 2.

Infection by obligately biotrophic pathogens usually leads to a reduction in the rate of net photosynthesis (Livne, 1964; Raggi, 1978; Mitchell, 1979; Gordon and Duniway, 1982a; Ahmad, Farrar and Whitbread, 1983; Walters and Ayres, 1983), and a stimulation in dark

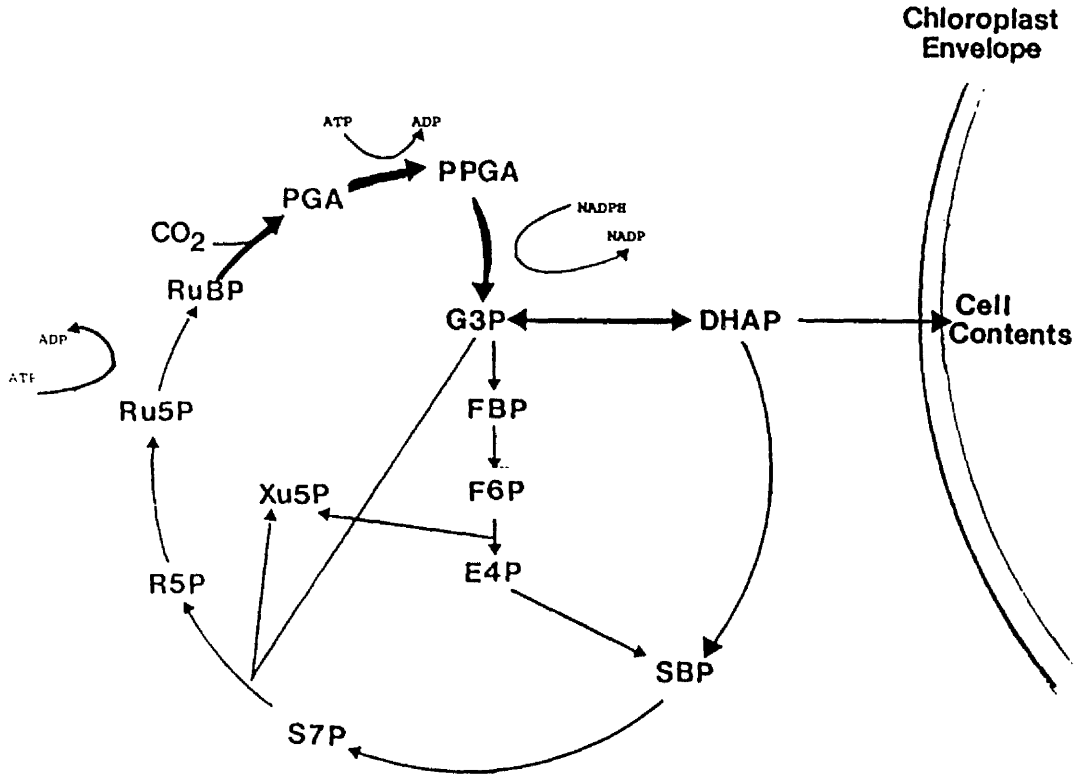



Figure 2. The photosynthetic carbon reduction cycle. The route from atmospheric $CO_2$ to dihydroxyacetone phosphate (DHAP), which is exported from the chloroplast, is indicated by the heavy line. Reduction of the initial product, phosphoglyceric acid (PGA)is initiated by phosphorylation to di-phosphoglyceric acid (PPGA), followed by reduction to glyceraldehyde-3-phosphate (G3P). Starch may be formed in the chloroplast by further metabolism of fructose bisphosphate and fructose-6-phosphate (FBP, F6P). The $CO_2$ acceptor molecule, ribulose bisphosphate (RuBP), is regenerated from the 3-carbon sugars (G3P and DHAP) in the complex series of reactions indicated, which involve erythrose-4-phosphate (E4P), sedoheptulose bisphosphate and sedoheptulose-7-phosphate (SBP, S7P), and the 5-carbon sugar phosphates of xylulose, ribose and ribulose (Xu5P, R5P and Ru5P). The cycle is driven in the direction of net synthesis by ATP and NADPH generated in the light-dependent reaction of photosynthesis.

respiration (see Walters, 1985). Photorespiration was reduced in certain plant/pathogen interactions, e.g. oak/powdery mildew (**Microsphaera alphitoides**) (Hewitt and Ayres, 1977); barley/powdery mildew (**Erysiphe graminis** f.sp. **hordei** (Marchal)) (Ayres, 1979); wheat/stem rust (**Puccinia graminis tritici**) (Kiraly and Farkas, 1957). However, an increase in photorespiration was reported in barley infected with brown rust (Owera, Farrar and Whitbread, 1981).

Various mechanisms have been proposed to explain the effect of phytopathogens on host $CO_2$ exchanges. It should be noted that these effects are not necessarily mutually exclusive, and it is possible that a variety of mechanisms could account for the observed effects. Increased rates of dark respiration in infected plants lead to an increasing proportion of newly fixed assimilate being lost via the respiratory process. There is some uncertainty regarding the contribution of the pathogen itself to increased respiration, and while Ayres (1979) suggested that it was very small, Owera **et al** (1981) argued that the pathogen was solely responsible for the increased dark respiration observed in barley infected with brown rust.

It has been suggested that the most likely cause of increased respiratory activity in diseased plants is the enhanced operation of the oxidative pentose phosphate pathway (Daly, 1976). This pathway is apparently located in the cytosol, and its activity is limited by $NADP^+$ availability. A variety of mechanisms have been suggested to explain in what way this particular pathway is affected by disease. Thus, the increased activity may be due to the release of $NADP^+$ into the cytosol (Ryrie and Scott, 1968) following chloroplast breakdown (Dyer and Scott, 1972). Other research has reported the

presence of pentose phosphate pathway dehydrogenases in chloroplasts (Anderson, Toh-Chin and Kyung-Eun, 1974), and it has been suggested that reductions in photosynthesis in diseased plants lead to the breakdown of control mechanisms on the two dehydrogenases (Chakravorty and Scott, 1982), resulting in increased activity of the pathway. Williams and Pound (1964) suggested that the stimulation of existing pathways, and not the pentose phosphate pathway, is responsible for enhanced rates of respiration in infected plants. Infections by obligate biotrophs generally lead to reductions in photorespiration, which have been linked to reductions in the activities of enzymes involved in the photorespiratory cycle. Glycolate oxidase activity was reduced in several pathosystems, namely flax/rust (Kakkar, 1966); wheat/stem rust (Kiraly and Farkas, 1957), oak/powdery mildew (Hewitt and Ayres, 1977) and barley/powdery mildew (Walters and Ayres, 1984).

Although net photosynthesis will be indirectly affected by changes in dark respiration and photorespiration, several studies have been undertaken to determine the direct effect of disease on photosynthetic rates. Since photosynthesis is a complicated, intricate process, with several control mechanisms, it seems feasible that disease may affect the process in a number of different ways.

Changes in stomatal resistance appear to play a small part in the observed alterations in photosynthesis in rusted and mildewed plants. Thus, Sempio, Majernik and Raggi (1966) found a decrease in stomatal resistance (rs) in early bean rust infection, with increases being reported in the later stages. A small decrease in rs was also found in brown rusted barley (Owera **et al**, 1981).

Powdery mildew infections led to small increases in rs in pea (Ayres, 1976), barley (Ayres, 1979) and oak (Hewitt and Ayres, 1975).

Much work has centred on the effects of disease on photosynthetic pigments. Much of the research however, has yielded contradictory results. Thus, photosynthesis per unit chlorophyll has been shown to decrease (Allen, 1942; Scott and Smillie, 1963). However, when Scott and Smillie's original data were recalculated, it was shown that net photosynthesis per mg chlorophyll was actually 50% higher in diseased leaves than in healthy controls (Waygood, Pao and Godavari, 1974). Hewitt (1976) has demonstrated that total chlorophyll levels were reduced by infection, but reductions in photosynthesis occurred earlier than reductions in chlorophyll. The chlorophyll a/chlorophyll b ratio also decreased in oak following infection by powdery mildew (Hewitt, 1976). So and Thrower (1976) reported a reduction in chlorophyll levels in the **Vigna sesquipedalis**/rust interaction, which was found to be significantly correlated with the reduction in photosynthesis in infected hosts. However, no correlation was detected in the wheat/**Puccinia striiformis** interaction (Doodson, Manners and Myers, 1964).

A great deal of research has concentrated on the effect of disease on the enzymes of photosynthesis. Powdery mildew infection led to a substantial decrease in the activity and amount of RuBPcase in leaves of sugar beet (Gordon and Duniway, 1982b), and barley (Walters and Ayres, 1984). Two possible mechanisms exist to explain the reduction in amount of RuBPcase in mildewed and rusted plants. First, the large subunit of the RuBPcase molecule is synthesized on 70S ribosomes in the chloroplast (Wildner, 1981), and since mildew

infection induces a reduction of ribosomes and rRNA in chloroplasts (Plumb, Manners and Myers, 1968; Bennett and Scott, 1971; Dyer and Scott, 1972; Callow, 1973) it seems likely that any interference with ribosome production may also affect the assembly of the RuBPcase molecule. Second, RuBPcase is the largest source of reutilizable reduced nitrogen in the leaf (Huffaker and Miller, 1978) and therefore any alterations in nitrogen metabolism in diseased hosts will also affect the amount of RuBPcase. This is discussed in more detail later.

RuBPcase activity is affected by several factors, including the concentration of cytoplasmic inorganic phosphate (Pi) (Wildner, 1981; Edwards and Walker, 1982). Reductions in Pi will result in a decline in ATP/ADP, and in the activity of RuBPcase (Herold, 1980). It is interesting to note that polyphosphate accumulation has been shown to occur in rusted wheat (Bennett and Scott, 1971) which may indicate a depletion of Pi from adjacent cells (Scott, 1972). Fungal sequestration of Pi may also have been responsible for the observed reductions in the activity of PePcase in sugar beet (Magyarosy, Schurmann and Buchanan, 1976) and barley (Walters and Ayres, 1984) infected with powdery mildew. The activities of other RPP enzymes were also reduced following infection. Thus, 3-phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase ($NAD^+$ and $NADP^+$ activated) activities were reduced in powdery mildewed barley (Walters and Ayres, 1984). It is possible, therefore, that RuBPcase in mildewed leaves may not be solely responsible for limiting the flux of carbon through the RPP pathway (Gordon and Duniway, 1982b).

Recent research has indicated that uninfected tissues within an infected host may compensate for lowered photosynthetic activity in diseased parts, thus ensuring survival of both host and pathogen. Stimulation in photosynthetic rates in upper, uninfected leaves has been demonstrated in mildewed pea (Ayres, 1981), barley (Williams and Ayres, 1981; Walters and Ayres, 1983) and rusted leek (Roberts and Walters, 1986). Walters and Ayres (1983) have suggested that the stimulation in net photosynthesis in uninfected leaves of mildewed barley seems to be due, in part, to an increase in the amount and activity of RuBPcase, and increases in the activities of PePcase and NADP malic enzyme.

Very few studies have examined more than one aspect of photosynthesis in any one pathosystem. It was decided, therefore, to gather as much information as possible about photosynthesis in the leek/rust system, since such a study should provide a valuable insight into the growth and development of the infected host. Furthermore, leeks infected with rust are a good system for any physiological study, since disease symptoms do not appear until 12-14 days after inoculation, allowing detailed measurements to be made for some time prior to sporulation.

## MATERIALS AND METHODS

The growth and inoculation of leeks (**Allium porrum** L. var. Autumn Mammoth) are described in Section 2 - General Materials and Methods.

**Gas Exchange Measurements**

Gas-exchange measurements were carried out on four infected plants, four healthy plants and four infected plants with upper fourth leaves left uninfected. Net photosynthesis was measured after intact, attached leaves were enclosed in a glass cylindrical leaf chamber of internal dimensions 17 x 3 cm. Air, with a relative humidity of 70 per cent entered the chamber at 0.2 l $min^{-1}$. Leaf-surface temperature was measured with a chromel-constantan thermocouple and was maintained at 21°C by means of a thermostatically regulated water jacket surrounding the leaf chamber. Leaves were illuminated from above by metal halide lamps (Thorn 400W, Kolorarc). A muslin filter was suspended between the leaf chamber and the light source, to ensure an irradiance of 100W $m^{-2}$ at the leaf surface.

The leaf in the chamber was allowed to equilibrate for 20 min, then concentrations of $CO_2$ were measured with an infra-red gas analyser (Analytical Development Company, Hoddesdon, Herts). Photorespiration was measured by the "oxygen inhibition" method (Zelitch, 1971), and was considered equal to the increase in $CO_2$ uptake after air containing 2 per cent oxygen, 300 v.p.m. $CO_2$, balance nitrogen (British Oxygen Company, Special Gases, London) was passed through the chamber in place of normal air. Dark respiration was measured after the leaf chamber was enclosed in a black jacket and the light was switched off (Roberts and Walters, 1986).

**Protoplast Isolation (Edwards & Walker, 1982)**

Leaves from healthy and rusted plants were harvested at various stages after inoculation. The surface of each leaf was brushed gently with a toothbrush in order to break through the epidermal

tissue, and then submerged in a solution of 0.5 M sorbitol to prevent air entering the leaf during the cutting process. Leaf segments, approximately 1 mm in width were prepared by cutting with a scalpel. Six grammes of tissue were sectioned in this manner. The sorbitol solution was then removed with a pipette, and replaced with 25 $cm^3$ of an enzyme medium, which contained 0.5 M sorbitol, 1 mM $CaCl_2$, 0.05% BSA, 2.5% cellulase and 0.5% pectinase (all from Sigma Chemical Co. Ltd., Poole, Dorset) adjusted to pH 5.5. The enzyme medium and leaf segments were placed in a glass petri-dish, which was then incubated at 25°C under constant illumination ($100W\,m^{-2}$) using a 400W metal halide lamp (Thorn, Kolorarc).

After three hours, the enzyme medium was removed and discarded because it contained little chlorophyllous material. The remaining segments were washed three times with 20 $cm^3$ aliquots of 0.5 M sorbitol and 1 mM $CaCl_2$. After each washing, the released protoplasts and chloroplasts were filtered through a coarse-mesh filter (1 mm aperture) followed by a nylon gauze (80 $\mu$m aperture, Henry Simon). The first filter retained leaf segments while the second retained released vascular strands. Subsequently, the filtrate preparation was maintained on ice.

The filtrate was then centrifuged at 250 g for 2 min, and the supernatant discarded. Approximately 100 $\mu$l of a solution containing 0.5 M sucrose, 1 mM $CaCl_2$ was added to each centrifuge tube, and the pellet resuspended by gentle shaking. Five $cm^3$ of the same solution was then added to each tube and the contents thoroughly mixed. Two $cm^3$ of a solution containing 0.4 M sucrose, 0.1 M sorbitol, 1 mM $CaCl_2$, 5 mM MES-KOH (pH 6.0) was then layered onto each tube, followed by 1 $cm^2$ of 0.5 M sorbitol, 1 mM $CaCl_2$,

5 mM MES-KOH (pH 6.0). After centrifugation at 250 g for a further 4 min, the protoplasts collected in a band between the layers of sugar solution, and were removed with a pasteur pipette. The purified protoplasts were stored on ice.

**Preparation of Chloroplasts from Protoplasts**

One $cm^3$ aliquots were taken from the stock of protoplasts, and to this was added 5 $cm^3$ of a solution of 0.5 M sorbitol, 1 mM $CaCl_2$. After centrifugation at 250 g for 2 min, the protoplast pellet was suspended in 1 $cm^3$ 0.4 M sorbitol, 50 mM HEPES-KOH (pH 7.6), 10 mM $NaHCO_3$, 1 mM EDTA. For protoplast extracts, a 1 $cm^3$ syringe was used, the tip being removed leaving an opening of approximately 2 mm diameter. A 20 $\mu$m nylon mesh (Henry Simon) was placed over the opening. The protoplasts were broken by sucking into the syringe and ejecting four times. The protoplast extracts obtained from this procedure were centrifuged at 250 g for 90 secs and the subsequent chloroplast pellet was resuspended in 100 $\mu$l of the same medium (0.4 M sorbitol, 50 mM HEPES-KOH) (pH 7.6). The volume was brought to 1 $cm^3$ and the chloroplast preparations were stored on ice, generally being used within one hour.

**Chloroplast Intactness Assay**

Chloroplast intactness was determined by ferricyanide-dependant oxygen evolution before and after osmotic shock (Lilley, Fitzgerald, Rieitis and Walker, 1975). Chloroplasts were assayed in a medium containing 0.4 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA and 50 mM HEPES-KOH (pH 7.6). For osmotic shock of chloroplasts, 0.1$cm^3$ of the chloroplast suspension was added to 0.9 $cm^3$ of water and stirred for one minute. One $cm^3$ of double strength assay medium (0.8 M sorbitol, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 4 mM EDTA, 100 mM HEPES-

KOH, pH 7.6) was then added to the chloroplast suspension. For assay of the intact preparation, 0.1 $cm^3$ of chloroplasts was added to 1.9 $cm^3$ normal strength assay medium.

Ten mM D,L-glyceraldehyde was then added to the assay, which inhibited the reductive pentose phosphate (RPP) pathway and its associated $O_2$ evolution. Potassium ferricyanide (3 mM) was also added. Oxygen evolution was followed in an oxygen electrode (Rank Brothers), and three minutes after illumination, 5 mM $NH_4Cl$ was added. The rate of light-dependent $O_2$ evolution was measured after the addition of $NH_4Cl$ with shocked (A) and unshocked (B) chloroplasts. The percentage intactness of the original preparation was calculated as follows:

$$\% \text{ intactness} = \frac{A - B}{A} (100) = 100 - (100\ B/A) \qquad \text{Eq. 6}$$

Chloroplasts isolated from protoplasts were on average 90% intact using this criterion for intactness.

**Photosynthesis by Isolated Chloroplasts**

Oxygen evolution was followed in an oxygen electrode (Rank Brothers) at 22°C. The assay medium consisted of 0.4 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 10 mM $NaHCO_3$, 0.3 mM Pi and 50 mM HEPES-KOH (pH 7.6). Catalase (100 units) was also included as a protective agent against any peroxide which might be formed during photosynthesis. Red light was provided by a lamp equipped with suitable filters, giving a quantum flux of about 800 $\mu$mol $m^{-2}s^{-1}$. Chlorophyll was determined in acetone from A = 645 nm and A = 663 nm.

**RuBPcase Assay**

Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) activity was assayed as described by Callow (1974), assays being conducted in the range where there was a linear relationship between the amount of enzyme added and activity detected. Leaves from healthy and rusted leek plants, including uninfected leaves from infected plants, were harvested and frozen. Samples (500 mg) were ground in a pre-chilled pestle and mortar with 5 $cm^3$ of 0.05 M tris-HCl, pH 8.0, containing 2 mM $EDTANa_2$ and 3 mM Dithiothreitol (DDT), at 2 $^oC$. Homogenates were centrifuged at 18,000 rpm for 20 min at 4$^oC$. Assays were performed on crude homogenates using a technique based on that of Paulsen and Lane (1966). Assay media contained the following: 50 $\mu$l 0.35 M Tris-HCl, pH 8.0, 20 $\mu$l 0.125 M magnesium acetate, 10 $\mu$l $NaH^{14}CO_3$ (50 $\mu$Ci/$\mu$mol (S.A.), Amersham), 100 $\mu$l serially diluted enzyme (1 in 100) and 20 $\mu$l 0.05 M DDT. Media were preincubated at 25$^oC$ for 10 min for optimal activation of the enzyme (Lorimer **et al**, 1976). After preincubation, 10 $\mu$l of a solution of 0.5 mg ribulose 1,5-bisphosphate (bisodium salt) in 100 $\mu$l distilled water, were added to assay media. The reaction was allowed to proceed for 10 min, after which the reaction was stopped by the addition of 100 $\mu$l 2N HCL. After drying aliquots (0.5 $cm^3$), water (0.5 $cm^3$) followed by 10 $cm^3$ of an LSC cocktail (Emulsifier Safe, Packard) scintillant was added.

**Phosphoenolpyruvate carboxylase (PePcase) Activity**

The activity of PePcase (EC 1.1.1.37) was determined using the methods described by Donkin and Martin (1980). Leaf samples (300 mg) were homogenized in a pre-chilled pestle and mortar with acid washed sand with 3 $cm^3$ of the following buffer: 50 mM Tris,

pH 8.3; 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DDT. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. Assays were performed directly on the supernatant. Enzyme activity was assayed spectrophotometrically in a coupled enzyme assay measuring NADH oxidation by decrease in extinction at 340 nm. Each cuvette contained in a total volume of 1 $cm^3$ the following reagents: 0.1$\mu$ mol NADH; 8 units malate dehydrogenase; 60 $\mu$mol Tris buffer (pH 8.0); 6$\mu$mol $MgCl_2$; 0.12$\mu$mol EDTA; 3$\mu$mol DTT; 6$\mu$mol $NaHCO_3$; 106$\mu$l (approx.) enzyme extract. The reaction was initiated by the addition of 50$\mu$l of a solution of 2$\mu$mol PEP (tricyclohexylamine).

**Activity of Other Photosynthetic Enzymes**

Leaves (500 mg) were ground in a 100 mM $K_2PO_4$ buffer (pH 7.2) containing 50 mM DDT at 2°C, the homogenates being centrifuged at 10,000 rpm for 20 min. The supernatant was used as the source of all enzymes to be studied. All assays were performed according to the methods of Winter, Foster, Edwards and Holtum (1982). Assays were performed at 25°C by following the change in absorbance of a pyridine nucleotide at 340 nm in a 1.2 $cm^3$ reaction mixture. Reactions were initiated by the compound stated last, as described below.

P-glycerate kinase (EC 2.7.2.3); 50 mM MES/KOH, pH 7.0; 2 mM $MgCl_2$; 0.08 mM NADH; 3 IU glyceraldehyde-3-P-dehydrogenase; 20 $\mu$l enzyme extract; 3 mM 3-P-glyceric acid; 1 mM ATP.

NADP-glyceraldehyde-3-P-dehydrogenase (EC 1.2.1.13): 50 mM HEPES-KOH, pH 8.0; 10 mM $MgCl_2$; 5 mM GSH; 5 mM ATP; 0.2 mM NADPH; 0.6 IU P-glycerate kinase; 40$\mu$l enzyme extract; 2 mM 3-P-glyceric acid.

NAD-glyceraldehyde-3-P-dehydrogenase (EC 1.2.1.12): as for NADP linked activity, but 0.08 mM NADH was used instead of NADPH, and 20$\mu$l enzyme extract instead of 40 $\mu$l.

NADP malic enzyme (EC 1.1.1.40): 50 mM MES-KOH, pH 7.5; 3 mM $MgCl_2$; 0.25 mM NADP; 100$\mu$l enzyme extract; 3 mM malate.

NAD malic enzyme (EC 1.1.1.38): 50 mM HEPES-KOH, pH 7.2; 0.2 mM $EDTANa_2$; 5 mM DDT; 2 mM NAD; 40$\mu$l enzyme extract; 5 mM malate; 5 mM $MnCl_2$; 75$\mu$M coenzyme A.

Protein was determined by the method of Lowry, Rosebrough, Farr and Randall (1951), and a standard curve was prepared from stock Bovine Serum Albumin (BSA) solutions (see Appendix I).

## RESULTS

### Carbon Dioxide Exchanges

Rates of net photosynthesis of healthy fourth leaves of leek were very low in comparison with other $C_3$ species (typical rates of net photosynthesis for leeks and barley being 3 mg $CO_2$ $dm^{-2}$ $h^{-1}$ and 30 mg $CO_2$ $dm^{-1}$ $h^{-1}$, respectively). Although sporulation occurs at between 12-14 days after inoculation in the leek rust system, net photosynthesis was substantially reduced in rusted fourth leaves by eight days after inoculation and, by 20 days after inoculation, net photosynthesis was only 30% of rates in healthy control leaves (Figure 3). This was accompanied by a very large stimulation of dark respiration, eight days after inoculation in infected leaves, coupled with a reduction of photorespiration (Figure 4), twelve days after inoculation.

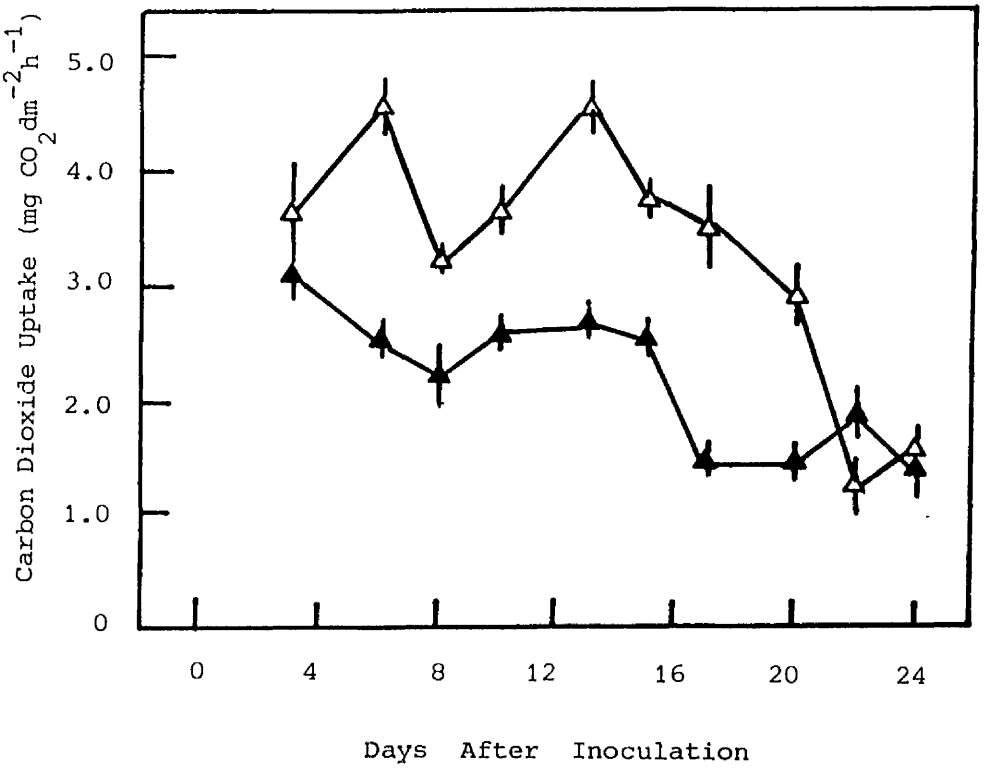



Figure 3. The effect of rust infection on photosynthesis by fourth leaves of leek (▲). Healthy controls (△). Bars indicate standard error of four replicate values for each point.

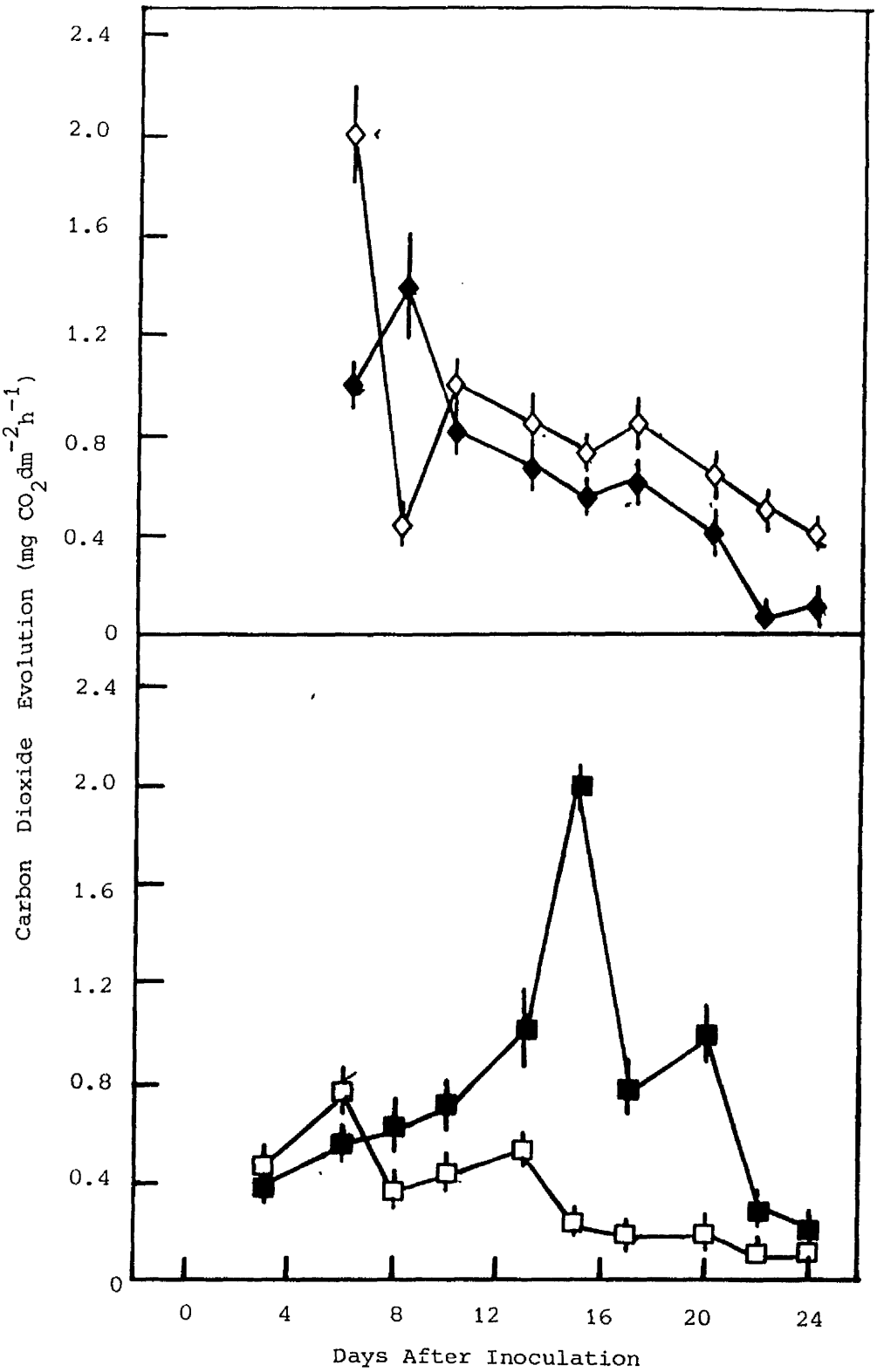



Figure 4. The effect of rust infection on (a) photorespiration (◆) and (b) dark respiration (■) by fourth leaves of leek. Healthy controls (◇, □). Bars indicate standard error of four replicate values for each point.

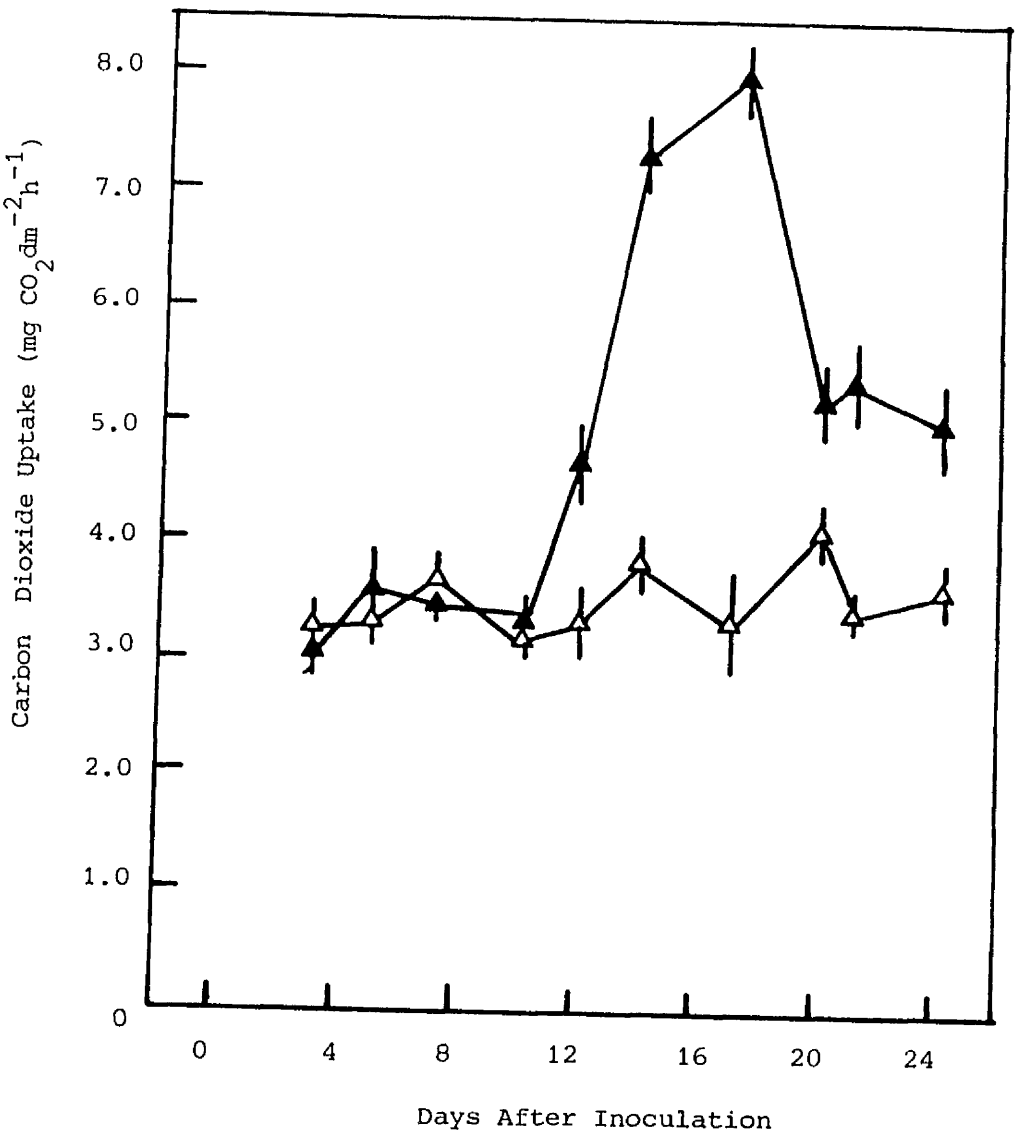



Figure 5. The Effect of rust infection on the lower three leaves of leek on photosynthesis in uninfected fourth leaves (▲). Healthy controls (△). Bars indicate standard error of four replicate values for each point.

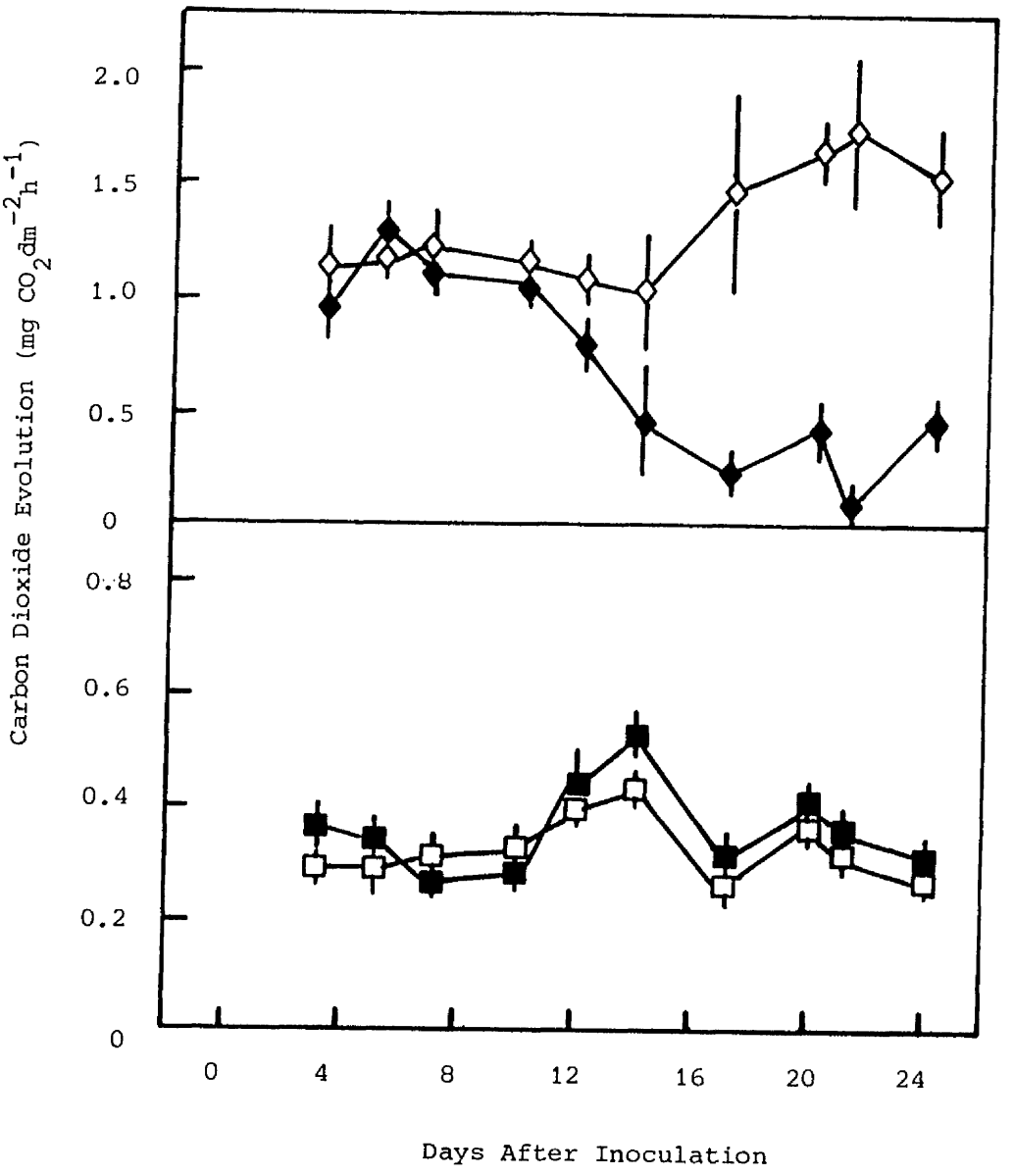



Figure 6. The effect of rust infection on the lower three leaves of leek on (a) photorespiration of uninfected fourth leaves (◆) and (b) dark respiration of uninfected fourth leaves (■). Healthy controls (◇, □). Bars indicate standard error of four replicate values for each point.

Carbon dioxide exchange was also examined in uninfected fourth leaves of rusted leek plants. These leaves showed a very marked increase in the rate of net photosynthesis in comparison with controls (Figure 5). This stimulation of net photosynthesis started at 10-12 days after inoculation and was still evident at 24 days after inoculation. Uninfected leaves of rusted leeks also exhibited a decreased rate of photorespiration by 14 days after inoculation but no change in rates of dark respiration (Figure 6) (Roberts and Walters, 1986).

**Photosynthesis by Isolated Chloroplasts**

Much of the work involved the determination of a satisfactory method for the isolation of intact or class A (Hall, 1972) chloroplasts from leek leaves. Chloroplasts isolated from protoplasts as described in the Materials and Methods showed an average of 90% intactness. The chloroplast preparations obtained from healthy leaves of leek exhibited very low rates of oxygen evolution. For example, rates obtained from leek chloroplasts were between 12-26 moles $O_2$ $mg^{-1}$ chlorophyll $h^{-1}$ as compared to values from barley of 150-200 moles $O_2$ $mg^{-1}$ chlorophyll $h^{-1}$. Nevertheless, chloroplast preparations from rusted leeks exhibited much reduced rates of oxygen evolution when compared to rates from healthy leek preparations (Figure 7) for the duration of the experiment.

**Enzymes of Carbon Assimilation**

Rust infection significantly reduced the activities of RuBPcase (Figure 8) and PePcase (Figure 9) in diseased leaves. This reduction in RuBPcase activity was apparent by only 7 days after inoculation, and by 10 days after inoculation the activity of

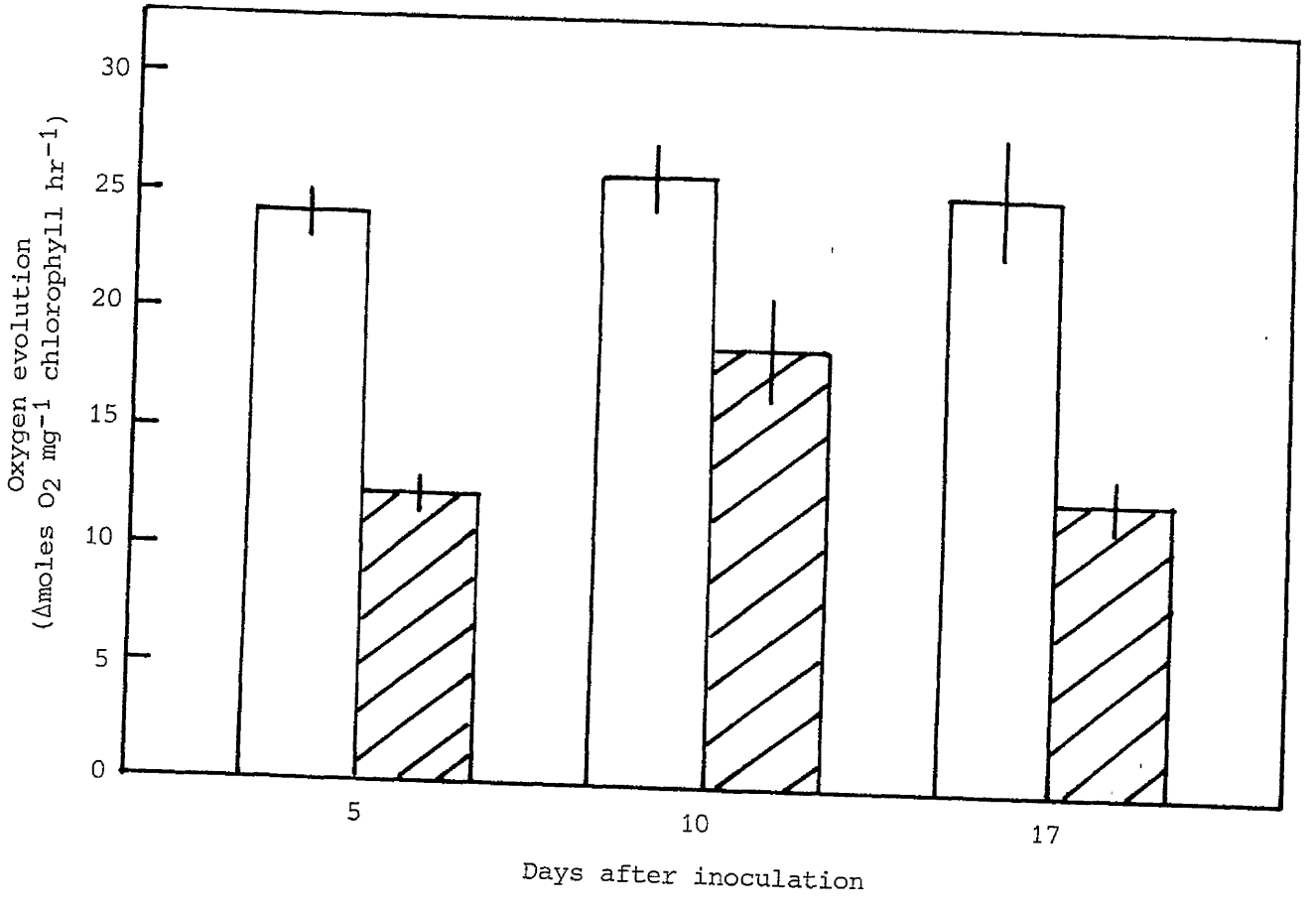



Figure 7. Effect of rust infection on oxygen evolution by isolated chloroplasts of leek. (▨). Healthy controls (□). Bars indicate standard error of four replicate values for each histogram bar. Differences significant at $P<0.01$ (**) and $P<0.001$ (***).

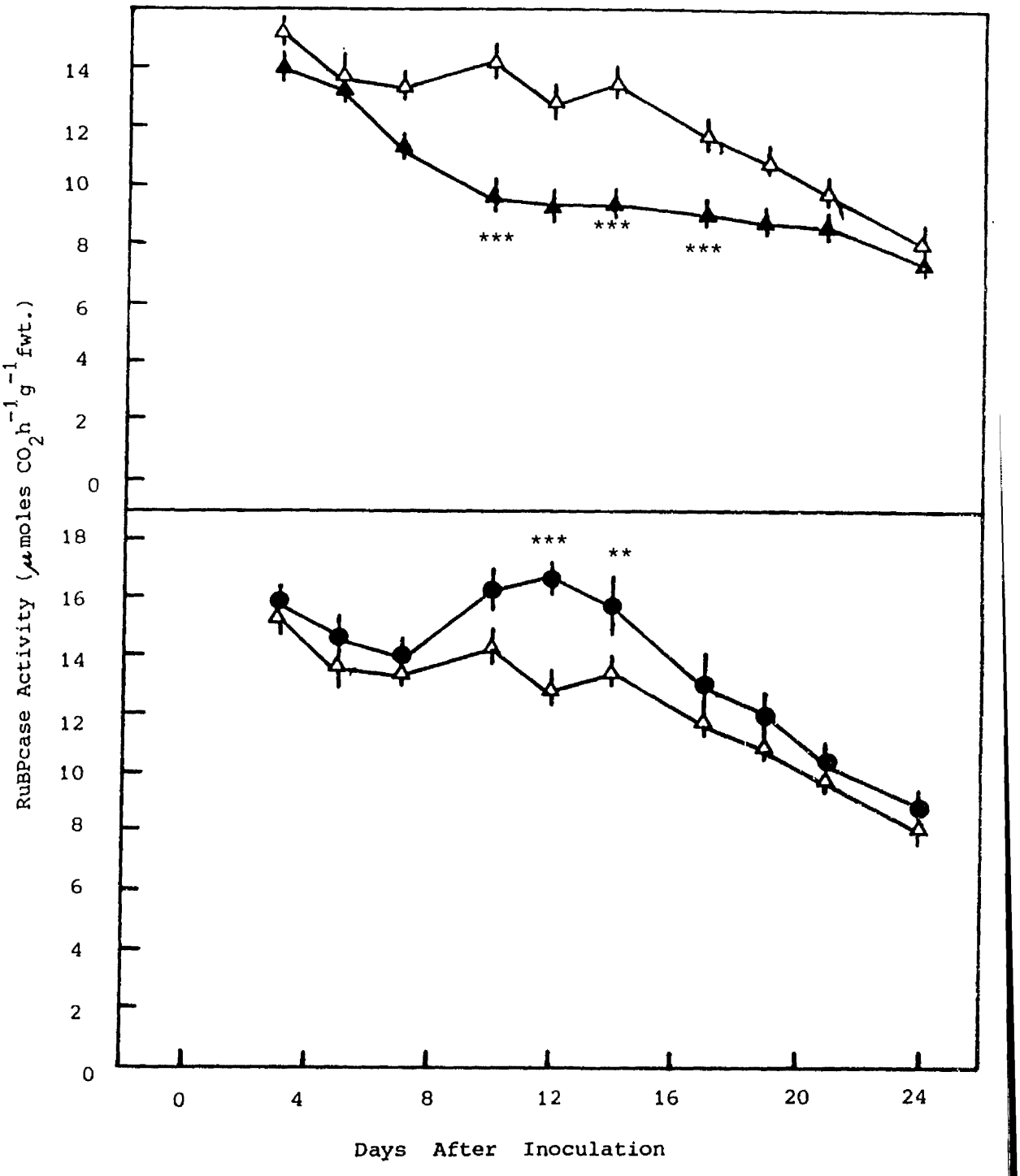



Figure 8. The effect of rust infection on the activity of RuBPcase in (a) rusted leek leaves (▲) and (b) uninfected leaves of otherwise rusted leeks (●). Healthy controls (Δ). Each value is the mean of four replicates with standard error. Differences significant at $P < 0.01$ (**), $P < 0.001$ (***).

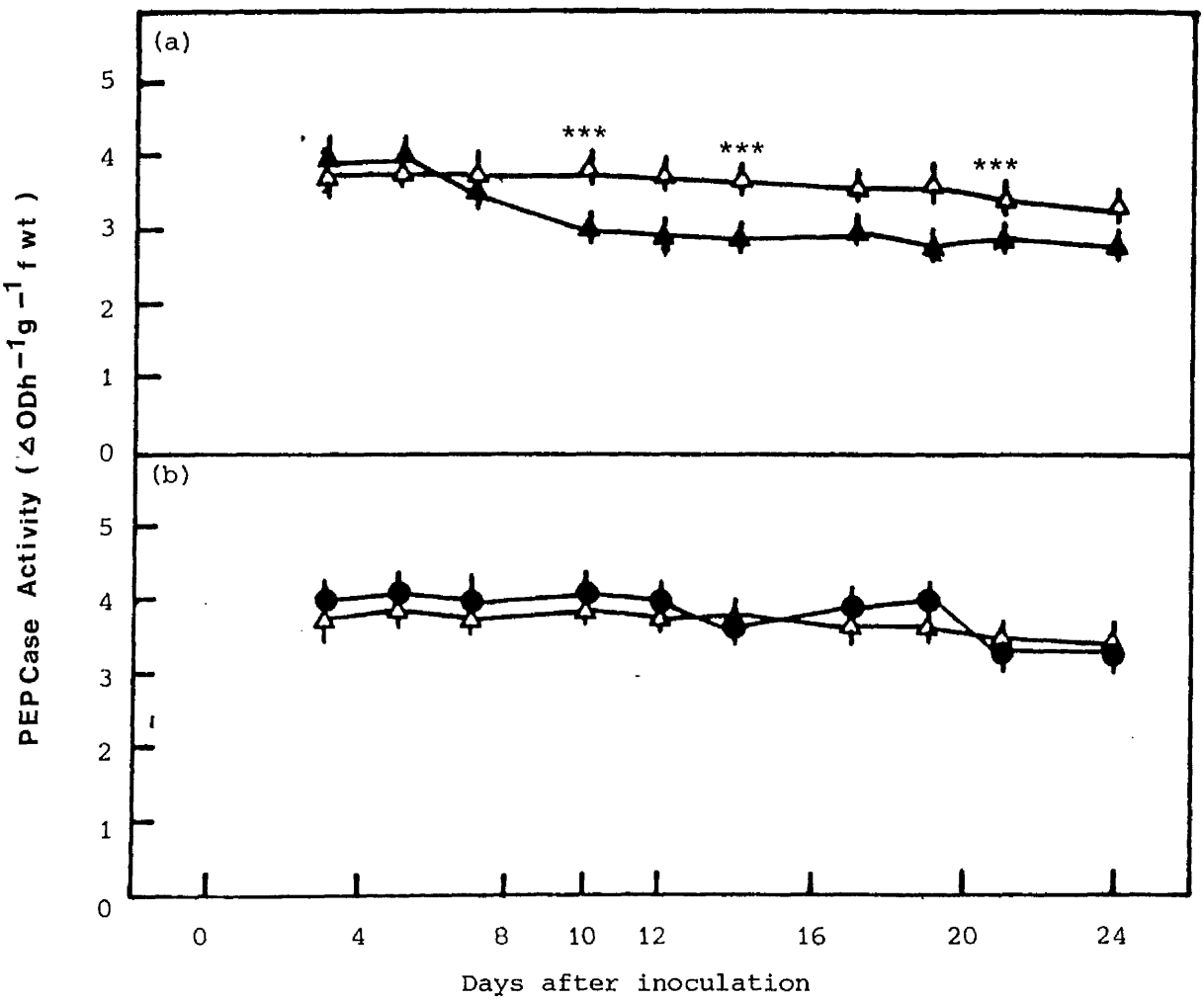



Figure 9. Effect of rust infection on the activity of PEPcase in leeks; (a) rusted leaves (▲), (b) uninfected leaves of otherwise rusted plants (●). Healthy controls (△). Each value is the mean of four replicates with standard error. Differences significant at $P<0.001$ (***).

PePcase was also reduced. There was also a substantial reduction in the activities of other photosynthetic enzymes in infected leaves. Thus, activities of 3-phosphoglycerate kinase, glyceraldehyde-3-P-dehydrogenase ($NAD^+$ and $NADP^+$) were all significantly reduced in infected leaves ($P<0.001$) by 12 days after inoculation. With the exception of NAD-glyceraldehyde-3-P-dehydrogenase, these reductions in activities were still present at 21 days after inoculation (Table 1).

Enzyme activity was also assayed in uninfected fourth leaves of leeks, where all the lower leaves were diseased. RuBPcase activity was significantly stimulated in these leaves, by 10 days after inoculation (Figure 8). The stimulation lasted until 17 days after inoculation, by which time no difference was found when compared to healthy control plants. The activity of PePcase remained unchanged in uninfected leaves for the duration of the experiment. However, the activities of 3-phosphoglycerate kinase, NADP-glyceraldehyde-3-P-dehydrogenase and NADP malic enzyme were all significantly stimulated ($P<0.001$) at 12, 14 and 21 days after inoculation. The activity of NAD malic enzyme remained unchanged until 21 days after inoculation, when a stimulation was noted. There was a transient stimulation in NAD-glyceraldehyde-3-P-dehydrogenase activity at 12 days after inoculation ($P<0.05$), but values returned to near control levels for the remainder of the experiment (Table 1).

**Total Soluble Protein and Chlorophyll Content**

Infection on the lower leaves had no effect on the protein and chlorophyll content of the upper uninfected fourth leaves of leek plants (Table 2). Total soluble protein and chlorophyll were substantially decreased in infected leaves by 7 days after

| Enzyme | | Enzyme activity ($\Delta$OD $h^{-1}$ $g^{-1}$ f wt) Days after inoculation | | |
|---|---|---|---|---|
| | | 12 | 14 | 21 |
| 3-phosphoglycerate kinase (EC 2.7.2.3) | Healthy | 0.94 ± 0.031 | 0.97 ± 0.016 | 0.86 ± 0.014 |
| | Infected | 0.74 ± 0.022[a] | 0.64 ± 0.019 | 0.58 ± 0.014[a] |
| | Uninfected | 1.14 ± 0.021[a] | 1.16 ± 0.008[a] | 1.09 ± 0.023 |
| NADP-glyceraldehyde-3-P-dehydrogenase (EC 1.2.1.13) | Healthy | 0.85 ± 0.017 | 0.82 ± 0.031 | 0.76 ± 0.023 |
| | Infected | 0.53 ± 0.014 | 0.41 ± 0.039[a] | 0.44 ± 0.020[a] |
| | Uninfected | 1.14 ± 0.030[a] | 1.25 ± 0.021[a] | 1.18 ± 0.011[a] |
| NAD-glyceraldehyde-3-P-dehydrogenase (EC 1.2.1.12) | Healthy | 0.64 ± 0.036 | 0.55 ± 0.057 | 0.51 ± 0.036 |
| | Infected | 0.54 ± 0.019[a] | 0.44 ± 0.019[a] | 0.49 ± 0.022 |
| | Uninfected | 0.60 ± 0.039[b] | 0.58 ± 0.037 | 0.53 ± 0.024 |
| NADP malic enzyme (EC 1.1.1.40) | Healthy | 0.94 ± 0.112 | 1.05 ± 0.041 | 0.66 ± 0.023 |
| | Infected | 0.39 ± 0.039[a] | 0.45 ± 0.037[a] | 0.39 ± 0.038[a] |
| | Uninfected | 1.44 ± 0.127 | 1.17 ± 0.039[a] | 1.27 ± 0.137[a] |
| NAD malic enzyme (EC 1.1.1.38) | Healthy | 1.28 ± 0.048 | 1.39 ± 0.024 | 1.07 ± 0.049 |
| | Infected | 1.01 ± 0.047[a] | 0.83 ± 0.033[a] | 0.77 ± 0.025[a] |
| | Uninfected | 1.29 ± 0.049 | 1.39 ± 0.018 | 1.40 ± 0.008[a] |

Table 1. Activities of enzymes involved in photosynthesis in rusted leek leaves and in uninfected leaves of otherwise rusted leek plants. Values are mean of four replicates with standard error. Differences significant at $P<0.001^a$ and $P<0.05^b$.

| | Days after inoculation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 5 | 7 | 10 | 12 | 14 | 17 | 19 | 21 | 24 |
| (a) Total soluble protein (mg protein $g^{-1}$ f wt) | | | | | | | | | | |
| Healthy | 24.04 ± 0.042 | 23.77 ± 0.222 | 23.35 ± 0.215 | 22.93 ± 0.246 | 22.19 ± 0.168 | 22.07 ± 0.143 | 21.84 ± 0.092 | 21.58 ± 0.215 | 20.79 ± 0.184 | 20.80 ± 0.069 |
| Infected | 23.11 ± 0.307 | 23.17 ± 0.125 | 21.58[a] ± 0.276 | 21.88[a] ± 0.107 | 19.53[a] ± 0.205 | 18.14[a] ± 0.097 | 15.85[a] ± 0.241 | 15.12[a] ± 0.095 | 14.38[a] ± 0.172 | 14.28[a] ± 0.068 |
| Uninfected | 24.08 ± 0.076 | 23.99 ± 0.098 | 23.56 ± 0.238 | 23.46 ± 0.224 | 22.17 ± 0.108 | 22.59 ± 0.257 | 21.83 ± 0.091 | 21.78 ± 0.083 | 21.28 ± 0.348 | 20.51 ± 0.210 |
| (b) Chlorophyll (mg chlorophyll $g^{-1}$ f wt) | | | | | | | | | | |
| Healthy | 3.64 ± 0.621 | 3.61 ± 0.450 | 3.58 ± 0.351 | 3.65 ± 0.202 | 3.64 ± 0.214 | 3.41 ± 0.305 | 3.44 ± 0.219 | 3.21 ± 0.391 | 3.14 ± 0.101 | 3.13 ± 0.117 |
| Infected | 3.71 ± 0.404 | 3.60 ± 0.209 | 3.45[b] ± 0.249 | 3.47[b] ± 0.267 | 3.19[a] ± 0.204 | 2.98[a] ± 0.265 | 2.71[a] ± 0.169 | 2.73[a] ± 0.145 | 2.53[a] ± 0.201 | 2.50[a] ± 0.214 |
| Uninfected | 3.69 ± 0.349 | 3.70 ± 0.410 | 3.64 ± 0.314 | 3.69 ± 0.214 | 3.65 ± 0.314 | 3.66 ± 0.219 | 3.49 ± 0.213 | 3.31 ± 0.214 | 3.20 ± 0.313 | 3.21 ± 0.196 |

Table 2. Total soluble protein and chlorophyll content in rusted leek leaves and in uninfected leaves of otherwise rusted plants. Values are the mean of four replicates with standard error. Differences significant at $P<0.001^a$ and $P<0.01^b$.

inoculation (Table 2). Levels remained much lower than control values for the remainder of the experiment.

## DISCUSSION

The leeks used in this study were 3-4 months old, and even under glasshouse and growth cabinet conditions, growth was very slow. Therefore, it is not surprising that rates of net photosynthesis of healthy fourth leaves of leek at this stage of growth were very low in comparison with other $C_3$ species. Leeks exhibited photosynthetic rates of about 3 mg $CO_2$ $dm^{-2}$ $h^{-1}$ while typical rates of photosynthesis for healthy barley plants are in the order of 30 mg $CO_2$ $dm^{-2}$ $h^{-1}$. However, infection led to reductions in the rates of net photosynthesis in intact leaves of leeks. Gas exchange measurements revealed that photosynthetic rates began to decline at about seven days after inoculation, although sporulation did not occur until 12-14 days after inoculation. Similar reductions in photosynthetic rates have been reported by a variety of other authors (see Walters, 1985 for references). Rusted leek leaves also exhibited a very large stimulation in dark respiration coupled with a reduction in photorespiration.

Large stimulations in rates of dark respiration will obviously account, in part, for some of the decrease in photosynthesis in infected leaves. Several authors have reported similar increases in dark respiration following infection (Scott and Smillie, 1966; Daly, 1976). It has been suggested that increased respiratory activity may not be deleterious to the plant cell, but may be associated with the creation of a metabolic sink (Livne and Daly, 1966), the

accompanying mobilization of energy sources and nutrients, production of energy and enhanced biosynthetic activity, all of which may enable the plant to respond more effectively to infection and permit expression of resistance. However, increased respiration, creation of a metabolic sink, and the accompanying increased flow of nutrients to infection sites may be advantageous to the pathogen which relies on the host for nutrients necessary for it s multiplication and reproduction.

What is certain is that increases in rates of dark respiration lead to an increasing proportion of newly fixed photosynthate being lost from the host (Ayres, 1979). A number of factors are known to influence dark respiration rates of plant tissues. These include the capacity of the phosphorylative system, substrate availability, concentration of enzymes catalyzing respiratory reactions, supply of inorganic ions (e.g. phosphate) hormonal concentrations, oxygen tensions, shifts in respiratory pathways, wounding and temperature (Millerd and Scott, 1962). The respiratory responses of host tissues following infection could be invoked by one or more of these factors. Uncoupling of oxidative phosphorylation as a possible cause of increased respiration following infection was initially proposed by Allen (1953). Since that time, Kiraly and Farkas (1955) demonstrated that exogenous application of DNP (dinitrophenol), an uncoupler of oxidative phosphorylation, had no effect on oxygen consumption in wheat leaves infected with stem rust. The use of synthetic uncouplers, however, is not an ideal comparison with the effects of disease, and results obtained from such studies are often open to misinterpretation. Healthy wheat leaves demonstrated marked increases in the rate of oxygen consumption following DNP

application. Observations of this type can be explained by increased synthesis following infection or by uncoupling of oxidative phosphorylation. Daly (1976) concluded that the most likely cause of increased respiration was increased activity of the oxidative pentose pathway which supplies carbon skeletons for synthesis of materials, e.g. phenolics, involved in plant defence reactions. Both possibilities, i.e. uncoupling of oxidative phosphorylation and increased operation of the pentose pathway mean that assimilate which would otherwise be available for host growth is utilized in a non-productive manner (Ayres, 1979).

Leek leaves infected with rust also exhibited reductions in photorespiration. The mechanism underlying this reduction is at present unknown. However, other research has suggested that reductions in the activities of photorespiratory enzymes may be responsible for this reduction. In rusted flax (Kakkar, 1966) and stem rusted wheat (Kiraly and Farkas, 1957) glycolate oxidase activity was reduced by infection. In addition to reduced glycolate oxidase activity, Walters and Ayres (1984) reported a decrease in the activity of RuBPoxygenase in mildew infection of barley. Our results indicate that RuBPcarboxylase activity is substantially reduced in rusted leek leaves. Since photorespiration is also reduced, we suggest that RuBPoxygenase activity may also be reduced. Although there are no measurements of total RuBPcase protein in rusted leaves, there is between a 20-30% reduction in total soluble protein in infected leaves, and it is tempting to suggest that much of the lost protein may be RuBPcase. Indeed, Gordon and Duniway (1982b) suggested that powdery mildew infection of sugar beet engendered a preferential degradation of RuBPcase protein. We

speculate that a similar situation may exist in rusted leek leaves, since a 12% reduction in total soluble protein in our system is associated with a 37% reduction in RuBPcase. This would undoubtedly contribute to a reduction in the oxygenase function of the enzyme. It is also possible that peroxisome ultrastructure and the association of peroxisomes with chloroplasts may be affected following infection, leading to alterations in glycolate metabolism.

In an attempt to understand the underlying mechanisms of the reduction in photosynthetic carbon assimilation in rusted leaves, we examined chloroplast functioning and the activity of a variety of photosynthetic enzymes in such leaves. The chloroplast preparations obtained from healthy leeks did not demonstrate exceptionally high rates of oxygen evolution. Perhaps this should not be surprising, especially in view of the low rates of net photosynthesis shown by healthy leeks. It is also important to note that rates of transport of photosynthetic products between the cell and the medium are probably reduced compared with rates of transport between cells in the intact leaf (Leegood and Walker, 1979). However, intact chloroplasts do permit the study of photosynthesis in a system which avoids the limitations of the intact leaf, for example stomatal limitation of gas exchange.

Chloroplasts isolated from rusted leaves displayed much slower rates of oxygen evolution than preparations from healthy controls. Similar results were reported by Magyarosy **et al** (1976) for chloroplasts isolated from sugar beet leaves infected with powdery mildew. They suggested that mildew effected a preferential inhibition of non-cyclic photophosphorylation by isolated chloroplasts of host leaves. Evidence was provided to suggest that

the inhibition stemmed from a diminution in electron flow from water to NADP and led to a inhibition of photosynthetic $CO_2$ assimilation and a shift in products. Chloroplast ultrastructure was also affected by disease. Although the inner and outer chloroplast membranes were unaffected, infection by the parasite caused a marked change in the morphological organization of the stroma and grana regions of the chloroplast. Thylakoid stacking was disturbed and large lipid globules, but not starch granules, were present in the stroma (Magyarosy **et al**, 1976). Similar thylakoid disturbances were reported in rusted flax and sunflower (Coffey, Palevitz and Allen, 1972) and rusted cowpea leaves (Heath, 1974). It is possible that similar changes occurred in chloroplasts from rusted leek leaves which could perhaps account, in part, for the reduction in oxygen evolution rates reported here.

It is interesting to note that other research suggests that chloroplasts in rusted leaves can operate as well as control chloroplasts. Ahmad **et al** (1983) demonstrated that chloroplasts freshly isolated from rusted barley leaves did not show impaired rates of oxygen evolution. Similarly, Wynn (1963) showed no reduction in photophosphorylation by chloroplasts isolated from rust-infected oats. It is difficult to reconcile these results with those obtained for rusted leek chloroplasts. However, experimental procedures vary between different researchers, and in the case of rusted barley chloroplasts, mechanical isolation techniques were used (Ahmad **et al**, 1983). Also, the generalization of results between different pathosystems should be avoided, and hence the situation in rusted leeks will probably not be mirrored in rusted barley or oats.

Undoubtedly, the large reduction in the activity of RuBPcase in diseased plants must account in part for the observed reduction in $CO_2$-fixation in intact rusted leaves. Similarly, powdery mildew infection led to a substantial decrease in the activity and amount of RuBPcase in sugar beet leaves (Gordon and Duniway, 1982b) and barley (Walters and Ayres, 1984). Gordon and Duniway (1982b) suggested that the reduction in activity of RuBPcase could be correlated with reductions in mesophyll conductance. They also report that the reduction in total soluble protein is somewhat less than the reduction in RuBPcase. Allowing for small discrepancies in calculations and experimental techniques, they suggest that infection with powdery mildew seems to engender a somewhat preferential degradation of RuBPcase protein. This is similar to the situation in senescing leaves, where loss of RuBPcase accounts for most of the decrease in total soluble protein (Friedrich and Huffaker, 1980; Peterson and Huffaker, 1975; Wittenbach, 1978).

Walters and Ayres (1984) suggested that the reduction in activity and amount of RuBPcase in mildewed barley was linked to the nitrogen economy of the plant. RuBPcase represents the largest source of re-utilizable reduced nitrogen in the leaf (Huffaker and Miller, 1978). Powdery mildew infection of barley resulted in a reduction in the nitrate content of all tissues (Walters and Ayres, 1980; Murray and Ayres, 1986a) and large amounts of nitrogen are transferred to the fungus (Bushnell and Gay, 1978). Thus, a reduction in the nitrogen content of leaf tissues may have been the stimulus for the net loss of RuBPcase in mildewed barley (Walters and Ayres, 1984). It is doubtful that a similar situation exists in rusted leek leaves, since subsequent experiments will show that

total plant nitrogen is actually higher in rusted leeks.

As previously mentioned in the Introduction, RuBPcase activity is influenced by several factors, including $CO_2$, $Mg^{2+}$ and pH (Laing **et al**, 1975; Lorimer **et al**, 1976). Activation of the enzyme is a readily reversible equilibrium process, the final position of equilibrium being dependant on $[CO_2]$, $[Mg^{2+}]$ and $[H^+]$ (Lorimer **et al**, 1976). It involves the ordered addition of $\updownarrow {}^{A}CO_2$ to the enzyme in a rate-determining step followed by the rapid addition of $Mg^{2+}$ to form the activated ternary complex, as shown in Eq. 7 (Lorimer **et al**, 1976).

$$\underset{\substack{\text{(inactive}\\\text{enzyme)}}}{E + {}^{A}CO_2} \overset{\text{slow}}{\rightleftharpoons} E\,{}^{A}CO_2 + M \rightleftharpoons \underset{\substack{\text{(active}\\\text{enzyme)}}}{E\,{}^{A}CO_2\,M} \qquad \text{Eq. 7}$$

It is important to note that a number of chloroplast metabolites, notably 6-phosphogluconate and NADPH, also influence the final position of equilibrium. Although their effect appears to be secondary (Badger and Lorimer, 1981; McCurry, Pierce, Tolbert and Orme-Johson, 1981), any disruption in chloroplast metabolism caused by disease may influence the generation of 6-phosphogluconate and NADPH and in turn influence the activation/stabilisation of RuBPcase. This could play an important part in the reduction of RuBPcase activity in rusted leek leaves, although this must remain speculation in the absence of suitable kinetic data.

Gordon and Duniway (1982b) have suggested that RuBPcase in mildewed leaves may not be responsible for limiting the flux of carbon through the reductive pentose phosphate pathway, since the activities of other enzymes in that pathway may be reduced. This

$\updownarrow {}^{A}CO_2$ - ATMOSPHERIC $CO_2$

appears to be the case in rusted leek leaves, since the activities of PePcase and the other enzymes studied were all substantially reduced following infection. Previous research has indicated that organic acid production, including malate and glycerate was reduced in sugar beet leaves following infection with powdery mildew (Magyarosy **et al**, 1976). These workers suggested that such a reduction in organic acid production may have been due to the reduced activity of PePcarboxylase and malate dehydrogenase in cell-free extracts from powdery mildew-infected sugar beet leaves. Walters and Ayres (1984) also reported reductions in the activity of 3-phosphoglycerate kinase and glyceraldehyde-3-P-dehydrogenase ($NAD^+$ and $NADP^+$ activated) in mildewed barley leaves and suggested that this could lead to a reduction in the regeneration of RuBP and possibly also a reduction in the storage of various carbohydrates. The reduction in RuBPcase activity in rusted leek leaves could be influenced by several of the factors discussed here, and previously (see Walters, 1985). It is also possible that the activity of RuBPcase is reduced because total soluble protein was reduced. Due to difficulties in obtaining rusted plant material, it has been impossible to determine RuBPcase protein itself, but it seems reasonable to suggest that much of the loss of total soluable protein in infected leaves will constitute loss of RuBPcase protein.

Total chlorophyll levels were reduced in leaves infected with rust by seven days after inoculation, that is, at least two days after photosynthesis began to decline in rusted leaves. Several authors have examined the possibility that failure of photosynthesis derives from the loss of chlorophyll which sooner or later follows infection. However, the evidence presented is very contradictory.

Thus, Allen (1942) found photosynthesis per mole of chlorophyll in mildewed barley increased in the early stages of infection, then declined. The evidence for such a decline was supported by Scott and Smillie (1963), although upon recalculation of their original data, Waygood, Pao and Godavari (1974) concluded that photosynthesis per mg chlorophyll was 50% higher in mildewed leaves than in healthy ones.

In mildewed oak, as in rusted leeks, photosynthesis declined before total chlorophyll levels were reduced by infection (Hewitt, 1976). It was noted that infection reduced the ratio of chlorophyll a/chlorophyll b, from 3.30 in healthy leaves to 1.90 in infected leaves. No such alteration in chlorophyll ratios was found in rusted leek leaves (results not shown). So and Thrower (1976) found a significant correlation between reductions in photosynthesis and chlorophyll levels after rust infection of **Vigna sesquipedalis,** while Doodson **et al** (1965) found that photosynthesis declined more rapidly than chlorophyll levels in wheat infected with **Puccinia striiformis.** The reduction in chlorophyll levels reported for leeks infected with rust may not play a role in the initial decline of photosynthesis in the very early stages of disease, but may in fact be involved as disease progresses.

This discussion so far has solely been concerned with the effect of rust infection on infected leaves. However, infection appears to have very important effects on any uninfected plant parts. Thus, rates of net photosynthesis were stimulated in uninfected leaves of otherwise rusted leeks. Experiments involving rusted bean leaves revealed that heavy infection of primary unifoliate bean leaves stimulated rates of $^{14}CO_2$ fixation in

uninfected trifoliate leaves on the same plant by more than 50% (Livne, 1964). A similar, but less striking situation was observed in sheaths of rusted wheat leaves and in cotyledons of rust-infected safflower (Livne, 1964). Later research by Livne and Daly (1966) on rusted bean, related the stimulated rates of $^{14}CO_2$ fixation in uninfected leaves to alterations in the pattern of assimilate translocation. They found that there was increased export of assimilate out of healthy leaves and assimilate import into rusted leaves. Recent work by other groups has shown that powdery mildew of pea and barley stimulated photosynthesis in upper, uninfected leaves of these plants (Ayres, 1981; Williams and Ayres, 1981; Walters and Ayres, 1983). This stimulation appeared to be due to increased RuBPcase amount and activity in uninfected leaves of mildewed barley leaves, coupled with increased PePcase and NADP malic enzyme activities (Walters and Ayres, 1983). However, the stimulation of photosynthesis and enzyme activity reported by these authors was transient, lasting no more than five days.

In rust-infected leeks the increases in net photosynthesis of uninfected leaves were very large and long-lived, unlike the responses reported for the barley/mildew system (Walters and Ayres, 1983). The very large stimulation in $CO_2$-fixation was obviously linked to increased RuBPcase activity in uninfected leaves, although RuBPcase activity returned to near control levels while photosynthetic rates remained stimulated. The activity of PePcase in uninfected leaves was not altered following infection of the lower leaves, yet the activities of 3-phosphoglycerate kinase, NADP malic enzyme, NADP-glyceraldehyde-3-P-dehydrogenase and NAD-glyceraldehyde-3-P-dehydrogenase were all stimulated in uninfected

leaves, the latter, however, showing only a transient stimulation. All these increased rates of enzyme activity may help account for part of the very large stimulation in photosynthesis of uninfected leaves of rust-infected leeks (Roberts and Walters, 1986).

It appears that photosynthetic rate may be affected by the end product of the process (Neales and Incoll, 1968; Geiger, 1976) and it is also known that sites of fungal infections are vigorous sinks for the attraction of host assimilates (Scott, 1972). On the basis of these hypotheses, it is tempting to suggest that the stimulation in photosynthetic rate in uninfected leaves could be due to the export of assimilates from these young developing leaves to heavily infected leaves. However, some authors argue that "end product inhibition" cannot be reconciled with many experiments reported in the literature (Geiger and Giaquinta, 1982), and indeed, there is little evidence that suggests assimilate import into mature monocot leaves (Farrar, 1984). Furthermore, work on brown rust of barley has indicated that there is very little export of assimilate from uninfected to infected leaves (Farrar, 1984).

In view of the stimulations of net photosynthesis in uninfected leaves of rusted leeks, it seems pertinent to examine the effects of partial defoliation on the growth and physiology of the remaining tissue. It has been suggested that the stimulation of net photosynthesis observed in the remaining leaves on partially defoliated plants may be the result of a diversion of increasing amounts of some promotive factors, e.g. cytokinins, from the roots to the remaining leaves, thus increasing their photosynthetic capacity over a period of several days (Wareing, Khalifa and

Treharne, 1968; Carmi and Koller, 1979). This is very interesting in view of the results presented by Vizarova and Minarcic (1974), who found a transient increase in cytokinin levels of shoots of mildewed barley, and of Poszar and Kiraly (1966), who demonstrated increased cytokinin activity in extracts of rust-infected leaves when compared to healthy controls. It is important to note, however, that in partially infected plants, the diseased leaves act as vigorous "sinks", presumably not just for assimilates but perhaps for other substances, including cytokinins. In partial defoliation experiments on the other hand, some sink activity is lost and, unless regrowth occurs, is not replaced (Walters, 1985). However, the possibility of a direct effect of the pathogen on the hormonal balance of the plant should not be ruled out (Walters, 1985) and this is examined in detail in Section 5.

Stimulation of photosynthesis in uninfected leaves of infected plants is potentially important, since it may enable such leaves to compensate, in part, for the reduction in photosynthetic activity that occurs in infected tissues (Roberts and Walters, 1986). Furthermore, such a response may enable the plant to maintain a functional equilibrium with respect to root and shoot activities (Walters, 1985).

SECTION 4

# Growth and Whole Plant Photosynthesis: The Construction of a Carbon Budget for Healthy and Rust-Infected Leeks

## INTRODUCTION

Reductions in plant dry weight as a result of rust infection (e.g. Lancashire and Latch, 1966; Manners and Myres, 1973) are often attributed to reductions in rates of net photosynthesis observed in infected leaves of these plants (e.g. Livne, 1964; Mitchell, 1979). However, photosynthesis has been shown to be stimulated in uninfected leaves of rusted and mildewed plants (Roberts and Walters, 1986; Walters and Ayres, 1983), although the exact contribution of such changes, to the growth and survival of the host plants are not known.

Why rates of net photosynthesis should be increased in uninfected leaves of rusted and mildewed plants is not known. It has been suggested that photosynthetic rate may be affected by the end product of the process (Neales and Incoll, 1968; Geiger, 1976) and further, it is also known that sites of fungal infections are vigorous sinks for the attraction of host assimilates (Scott, 1972). Based on these hypotheses, it is tempting to suggest that the stimulation in photosynthesis in uninfected leaves of infected plants could be due to the export of assimilates from these younger developing leaves to heavily infected leaves. However, there is little evidence that suggests assimilate import into mature monocot leaves (Farrar, 1984) and furthermore, work on brown rust of barley has shown that there is little export of photosynthate from uninfected to infected leaves (Farrar, 1984).

In order to examine assimilate production and translocation by uninfected and infected leaves of rust-infected leeks, simple carbon budgets were constructed for individual leaves. These budgets were

based on measurements of photosynthesis and dry weights of single leaves and were compared to changes occurring in the whole plant.

## MATERIALS AND METHODS

The growth and inoculation of leeks (**Allium porrum** L. var. Autumn Mammoth) are described in Section 2 - General Materials and Methods. Measurements were made of the rates of photosynthesis, photorespiration and dark respiration by both healthy and rusted whole leek plants. Net photosynthesis was measured after plants were enclosed in a large perspex plant chamber of internal dimensions 29 x 29 x 57 cm. The soil surface in the pots was covered tightly with plastic film, in order to prevent any carbon dioxide being released due to respiration in the soil. Air with a relative humidity of 70 per cent entered the chamber at 0.5 l $min^{-1}$, and was stirred within the chamber by means of a small battery-operated fan. The temperature within the chamber was measured with a chromel-constantan thermocouple and was maintained at 21-22$^{o}$C by means of a thermostatically regulated water tank placed directly above the plant chamber. Plants were illuminated from above as described in Section 3, and were allowed to equilibrate for approximately 45 minutes before concentrations of $CO_2$ were measured with an infra-red gas analyser (Analytical Development Co., Hoddesdon, Herts). Dark respiration was measured after the lights had been switched off and the chamber was darkened by means of a black cloth. Photorespiration was measured by the "oxygen inhibition" method (Zelitch, 1971), as described in Section 3.

Following gas exchange measurements for the whole plant, rates of photosynthesis, dark respiration and photorespiration were

determined for each individual leaf on each plant, by placing each leaf in a cylindrical glass leaf chamber as described in Section 3. After gas exchange measurements had been completed plants were harvested, leaf areas were determined using a leaf area meter (△T Devices), and plants were divided into shoot and root material. Oxygen uptake by excised root segments from healthy and rusted plants was followed in an oxygen electrode (Rank Brothers), after root segments were placed in a solution containing 1 mM $KH_2PO_4$ and 2.5 mM $CaSO_4$ and the chamber was darkened. Fresh weights of leaves, stem and roots were measured, and plants were then dried to constant weight at 80°C in a forced draught oven (Gallenkamp) before dry weights were determined.

## RESULTS

Rust infection led to a 21% reduction in the rate of photosynthesis, on a whole plant basis, compared to healthy controls ($P<0.1$; Table 3). However, the combined rates of photosynthesis by each individual leaf suggested that photosynthesis was reduced by 31% in rusted plants (Table 4). It is difficult to explain this discrepancy, although it is possible that the large plant chamber used in whole plant measurements afforded a less efficient transfer of $CO_2$ from air to the leaves. Rust infection also led to a reduction in the rate of photorespiration and a stimulation in dark respiration in whole plants ($P<0.02$; Table 3).

Photosynthesis was reduced in the three lowest leaves of rusted leek plants, i.e. in those leaves where rust infection was present. In the upper, uninfected leaves of otherwise rusted plants, photosynthesis was stimulated compared to healthy control leaves

| Plant | Photosynthesis (mg $CO_2$ $dm^{-2}$ $h^{-1}$) | Photorespiration (mg $CO_2$ $dm^{-2}$ $h^{1}$) | Dark respiration (mg $CO_2$ $dm^{-2}$ $h^{-1}$) | | Root respiration (µmoles $CO_2$ $g^{-1}$ fw $h^{-1}$) |
|---|---|---|---|---|---|
| | | | Day | Night | |
| Healthy | 1.66 ± 0.156 | 0.55 ± 0.054 | 0.40 ± 0.023 | 0.41 ± 0.011 | 5.23 ± 0.056 |
| Rusted | 1.31 ± 0.055 | 0.29 ± 0.008 | 0.62 ± 0.052 | 0.62 ± 0.031 | 4.82 ± 0.038 |

Table 3. The effect of rust infection on photosynthesis, photorespiration and dark respiration (shoot and root) by leeks on a whole plant basis. All values are the mean of four replicates with standard error.

| Leaf number | Photosynthesis (mg $CO_2$ $dm^{-2}$ $h^{-1}$) | | Photorespiration (mg $CO_2$ $dm^{-2}$ $h^{-1}$) | | Dark respiration (mg $CO_2$ $dm^{-2}$ $h^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Day | | Night | |
| | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted |
| 1 | 2.65 ± 0.290 | 1.43 ± 0.030 | 0.63 ± 0.011 | 0.25 ± 0.021 | 0.43 ± 0.014 | 0.97 ± 0.038 | 0.51 ± 0.015 | 0.94 ± 0.018 |
| 2 | 2.82 ± 0.242 | 1.34 ± 0.044 | 0.62 ± 0.010 | 0.45 ± 0.035 | 0.44 ± 0.032 | 0.97 ± 0.061 | 0.45 ± 0.031 | 0.91 ± 0.023 |
| 3 | 4.85 ± 0.331 | 2.23 ± 0.130 | 0.63 ± 0.013 | 0.45 ± 0.023 | 0.34 ± 0.022 | 0.93 ± 0.015 | 0.38 ± 0.007 | 0.92 ± 0.031 |
| 4 | 4.81 ± 0.305 | 6.93 ± 0.124 | 0.62 ± 0.017 | 0.27 ± 0.021 | 0.41 ± 0.015 | 0.54 ± 0.038 | 0.42 ± 0.045 | 0.51 ± 0.022 |
| 5 | 5.75 ± 0.098 | 6.24 ± 0.314 | 0.55 ± 0.029 | 0.38 ± 0.013 | 0.41 ± 0.014 | 0.43 ± 0.031 | 0.42 ± 0.031 | 0.49 ± 0.015 |

Table 4. Photosynthesis, photorespiration and dark respiration in individual leaves of healthy leeks and in leeks infected with rust. All values are the mean of four replicates with standard error.

(Table 4). Rates of photorespiration were substantially reduced in each leaf of rusted plants, while dark respiration was increased in all but the youngest, uninfected leaf, compared to healthy control plants (Table 4).

Total leaf area, shoot dry weight and root dry weight were reduced following infection, although the reductions in shoot and root dry weights were not significantly different compared to controls (Table 5). It seems difficult to reconcile a 21% reduction in whole plant net photosynthesis with only a 10% reduction in the dry weight of infected plants. However, when measurements were made, the two lowest leaves on infected plants were rapidly senescing and further, the leeks were a substantial size prior to inoculation. Thus, although Owera **et al**, (1981), using infected first leaves of barley, suggested that all of the reduction in dry weight in these leaves could be accounted for by reductions in photosynthesis and transport from these leaves, in our system, reductions in photosynthesis may not necessarily lead to equivalent reductions in dry weight. An examination of individual leaves revealed that in rusted plants, both the leaf area and the dry weight of each leaf was reduced compared to controls (Table 6). The information obtained from these experiments allowed the estimation of assimilate production and translocation by individual leaves of healthy plants, and the construction of a carbon budget for healthy and rusted leeks.

Rust infection led to a substantial reduction in assimilate production, expressed as mg $CH_2O$ $day^{-1}$, in all but the upper uninfected leaves. Total assimilate production, per plant, per day, was reduced in rusted leeks compared to uninfected controls.

| Plant | Total leaf area ($cm^2$) | Total FW shoot (g) | Total DW shoot (g) | Total FW root (g) | Total DW root (g) | Root:shoot ratio |
|---|---|---|---|---|---|---|
| Healthy | 283.67 ± 28.787 | 38.59 ± 4.456 | 3.72 ± 0.192 | 35.68 ± 0.990 | 1.00 ± 0.021 | 0.267 ± 0.003 |
| Rusted | 187.33 ± 19.643 | 27.91 ± 2.184 | 3.36 ± 0.219 | 29.09 ± 3.131 | 0.91 ± 0.076 | 0.272 ± 0.014 |

Table 5. The effect of rust infection on total leaf area, shoot and root dry weight and root:shoot ratio in leeks. All values are the mean of four replicates with standard error. FW = fresh weight, DW = dry weight.

| Leaf number | Leaf area ($cm^2$) | | Fresh weight (g) | | Dry weight (g) | |
|---|---|---|---|---|---|---|
| | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted |
| 1 | 27.66 ± 2.986 | 22.00 ± 2.369 | 1.16 ± 0.103 | 1.18 ± 0.126 | 0.17 ± 0.020 | 0.15 ± 0.015 |
| 2 | 33.33 ± 3.962 | 25.67 ± 2.966 | 1.85 ± 0.293 | 1.27 ± 0.076 | 0.20 ± 0.024 | 0.15 ± 0.033 |
| 3 | 47.00 ± 5.866 | 32.33 ± 4.113 | 2.47 ± 0.323 | 1.51 ± 0.302 | 0.23 ± 0.030 | 0.17 ± 0.052 |
| 4 | 54.67 ± 6.126 | 35.66 ± 4.813 | 2.99 ± 0.310 | 1.30 ± 0.231 | 0.26 ± 0.030 | 0.20 ± 0.023 |
| 5 | 36.33 ± 4.182 | 33.33 ± 1.203 | 1.72 ± 0.294 | 1.20 ± 0.141 | 0.15 ± 0.017 | 0.18 ± 0.088 |

Table 6. Leaf area, fresh weight and dry weight of individual leaves of healthy and rusted leeks. All values are the mean of four replicates with standard error.

Disease led to a reduction in assimilate translocation out of the lowest three rust-infected leaves, and a stimulation in the translocation out of the uninfected fourth leaves. The translocation rate for the uninfected fifth leaf was similar to control values (Table 7). Total plant growth in one 16 hour photoperiod was reduced by approximately 34% in infected plants. On the basis of this information, a carbon budget was constructed for leaves of healthy and rusted plants, and this is shown in Table 8.

## DISCUSSION

Measurements of whole plant photosynthesis were made in an attempt to further explain the disease-induced alterations in photosynthetic rates by single intact leaves of leeks. Rust infection led to a reduction in photosynthesis by whole rusted leeks, although it is difficult to reconcile a 21% reduction in photosynthetic rates with only a 10% reduction in the dry weight of infected plants. However, it is important to remember that when measurements were made, the two lowest leaves on infected plants were rapidly senescing. This will not always have been the case, and, it is also important to note that these plants were of a substantial size prior to inoculation. Owera **et al** (1981), using infected first leaves of barley, suggested that all of the reductions in dry weight in these leaves could be accounted for by reductions in photosynthesis and transport from these leaves. However, the plants used in their experiments were extremely small, and in such a situation, rapid reductions in photosynthesis will probably cause dramatic growth reductions. On the other hand, the leeks used in these experiments were very large, and, in such a

| Leaf number | Healthy | Rusted |
|---|---|---|
| 1 | 11.40 | 5.03 |
| 2 | 14.80 | 5.36 |
| 3 | 36.40 | 11.40 |
| 4 | 41.50 | 38.80 |
| 5 | 33.10 | 32.90 |
| Total production plant$^{-1}$ day$^{-1}$ | 137.2 | 93.49 |
| Plant growth in 1 16 h photoperiod (mg) | 472 | 315 |

Table 7a. Assimilate production by individual leaves of control and rusted leeks over a 16 h period (mg $CH_2O$ day$^{-1}$).

| Leaf number | Healthy | Rusted |
|---|---|---|
| 1 | 19.8 | 9.2 |
| 2 | 21.1 | 9.0 |
| 3 | 36.0 | 14.2 |
| 4 | 36.0 | 48.9 |
| 5 | 43.0 | 44.1 |
| Total translocation plant$^{-1}$ day$^{-1}$ | 155.9 | 125.4 |

Table 7b. Assimilate translocated out of individual leaves (mg $CH_2O$ day$^{-1}$).

**See Appendix III**

| | First leaf | | Second leaf | | Third leaf | | Fourth leaf | | Fifth leaf | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted | Healthy | Rusted |
| Net photosynthesis | 19.8 | 10.7 | 21.1 | 10.05 | 36 | 16 | 36 | 51.3 | 43 | 46 |
| Leaf respiration | 3.2 | 3.2 | 3.3 | 3.2 | 2 | 2 | 3.1 | 4.1 | 3.1 | 3.2 |
| C storage in leaf | 0 | 1.5 | 0 | 1.5 | 0 | 1.77 | 0 | 0* | 0 | 1.88** |
| Fungal respiration | 0 | 4.05 | 0 | 3.97 | 0 | 4.3 | 0 | 0 | 0 | 0 |
| Gross fungal C uptake | 0 | 5.55 | 0 | 5.47 | 0 | 6.07 | 0 | 0 | 0 | 0 |
| Translocation | 19.8 | 9.2 | 21.1 | 9.0 | 36 | 14.23 | 36 | 51.3 | 43 | 44.12 |

* No estimate was made for carbon storage in fourth leaves of infected plants since, at the time of measurements, these leaves were already fully expanded and were probably net exporters of carbon. Furthermore, because these leaves were uninfected, there was no reduction in assimilate translocation out of them.

** A value for carbon storage in uninfected fifth leaves of infected plants was estimated because these leaves were still rapidly expanding when measurements were made.

Table 8. Carbon budget for individual leaves of rusted leeks. ($\mu g\ C\ m^{-2}\ s^{-1}$)

system, reductions in photosynthesis may not necessarily lead to equivalent reductions in dry weight.

In constructing the carbon budget, no estimate was made for carbon storage in fourth leaves of infected plants since, at the time of measurements these leaves were already fully expanded and were probably net exporters of carbon. Furthermore, because these leaves were uninfected, there was no reduction in assimilate translocation out of them. A value for carbon storage in fifth leaves of infected plants was estimated because these leaves were still rapidly expanding when measurements were made.

The leaf carbon budget can be used to examine, amongst other things, the uptake of carbon by the pathogen. Gross fungal carbon uptake in rusted third leaves was calculated to be 6.07$\mu$g C, which represents a loss of 37.5% of host photosynthate to the fungus. This appears to be in general agreement with the work of Owera **et al**, (1981) who, using rusted first leaves of barley, reported a loss of approximately 33% of host photosynthate to the brown rust fungus. However, it is interesting to note that in the brown rust/barley system, despite a loss of carbon to the fungus, the dry weight of the infected first leaf was actually increased compared to control values (Owera **et al**, 1981). Similar increases in weight of infected leaves have been reported elsewhere (Manners and Myres, 1973; Van der Waal, Smeitink and Mann, 1976). In the results presented here for rusted leeks, the dry weight of the infected third leaf was reduced. It may seem difficult to reconcile these results with those obtained for infected barley leaves, but, whereas there was only a 27% reduction in photosynthesis in brown-rusted barley leaves, photosynthesis was reduced by 56% in rusted leek leaves.

This large reduction in photosynthesis, coupled with an increasing loss of assimilate to the fungus, must surely lead to a reduction in the dry weight of the infected leek leaves.

Changes in the movement of assimilates in plants induced by fungal infection have been known for many years (e.g. Daly, 1976; Bushnell and Gay, 1978), although the precise mechanism whereby these changes are elicited is still not known. When first-formed unifoliate leaves of French bean were infected by the rust **Uromyces appendiculatus**, the amount of recently fixed carbon exported dropped from 50% to 2%, whereas import to the next (trifoliate) leaf increased from <1% to 32% (Livne and Daly, 1966). When single leaves of wheat were infected by the rust **Puccinia striiformis**, they could not attract assimilates from the other leaves (Doodson **et al**, 1965). However, later experiments by Siddiqui and Manners (1971) showed that in completely infected plants, the proportion of $^{14}C$-labelled assimilate moving to the leaves was increased and that to the roots was decreased. Thus, the pattern of translocation in the whole plant was altered.

An examination of the pattern of translocation of assimilates in rusted leeks revealed that there was an increase of approximately 50% in translocation out of uninfected fourth leaves of rusted leek plants. A small increase in translocation out of uninfected fifth leaves was also observed, although these leaves were not fully expanded at the time of the experiment. It seems important to determine the destination of the assimilate from these uninfected leaves. From the carbon budget constructed for rusted leeks, we can tell that this assimilate was not being translocated to the first and second rusted leaves, since these leaves were senescing at the

time measurements were made. In addition, the weight of these leaves was considerably lower than would be expected if assimilate were being imported from the upper leaves. Infected third leaves did not appear to be importing this assimilate either, since their weights were reduced following infection. Also, there was not enough fungal material present to allow for the import of almost 50 $\mu$g C into these leaves, because levels of infection were consistently light on rusted third leaves. Thus, there appears to be no evidence to support the import of assimilate into infected lower leek leaves from uninfected fourth leaves.

However, the question concerning the fate of this exported assimilate still remains. It is possible that assimilate was imported into the uninfected fifth leaf of otherwise rusted leek plants, since the increase of 16% in dry weight was accompanied by only an 8.5% increase in the rate of photosynthesis. However, even if this was the case, it could not completely account for the 50 $\mu$g C, and so it seems reasonable to assume that a proportion of assimilate was being transported to the roots. This is a good possibility, since root weight and root respiration were hardly affected by infection. However, the rate of carbon accumulation in roots of infected plants has been estimated as approximately 10 $\mu$g C $m^{-2}$ $s^{-1}$, and in the uninfected leaf as approximately 11.5 $\mu$g C $m^{-2}$ $s^{-1}$, leaving approximately 38 $\mu$g C $m^{-2}$ $s^{-1}$ (from the original 50 $\mu$g C) unaccounted for. However, the stem‡ forms a very large part of the mature leek, and although we have no supporting evidence, it is possible that the stem could act as a large "sink" for assimilate in rusted leeks.

‡ Correctly, the sheaths of mature leaves

Assimilate production by rusted leek plants as a whole, was reduced by 32% over one day, compared to healthy plants. This is interesting, since the daily dry weight production of rusted plants was reduced by approximately 34%, compared to healthy controls. If this loss in dry weight (34%) is matched by a reduction in assimilate production (32%), we must ask the following question: What is the significance of the stimulation in photosynthesis by uninfected leaves of rusted leeks? Although photosynthesis was increased in these leaves, assimilate production by these leaves on a daily basis was slightly lower than controls. We can assume that the reductions in photosynthesis, and hence the reductions in assimilate production by the first, second and third leaves of rusted plants were responsible for reductions in plant growth on a daily basis. Therefore, the stimulation in photosynthesis by upper, uninfected leaves may help to alleviate the effects of rust infection, which could, in fact, be worse in the absence of such stimulations in photosynthetic rates.

An examination of the carbon budget reveals that the carbon uptake by the fungus on the first, second and third leaves was 16.5$\mu$g C $m^{-2}$ $s^{-1}$. Obviously, this carbon was not all lost in fungal spores since the reduction in plant dry weight was not great enough to account for this extra loss of carbon. In fact, although the dry weight reduction in rusted plants was not quite matched by reductions in the % assimilate produced, this discrepancy was only 2-4%. This would mean a mere loss of 3-6$\mu$g C $m^{-2}$ $s^{-1}$ in fungal spores. However, this range of values is in agreement with rates of spore production in brown-rusted barley (Owera **et al**, 1981). Thus, at the time measurements were made in these experiments, most of the

carbon "locked" within the infected leaf actually stayed in the host/parasite system and was not lost via fungal spores.

SECTION 5

# Studies involving localized regions of diseased leek leaves: photosynthesis and growth regulators

## INTRODUCTION

Like many foliar diseases, rust infection leads to a reduction in rates of net photosynthesis, and several causes for this reduction have been proposed (see Section 3 for references). However, it has recently been suggested that gross photosynthesis per unit chlorophyll, and thus per chloroplast, is enhanced in rusted tissue (Owera **et al**, 1981), which seems incompatible with partial inhibition of photophosphorylation and reductant generation suggested by several authors (Montalbini and Buchanan, 1974; Magyarosy **et al**, 1976). Further, a suggestion that carbon fixation by chloroplasts is reduced as a result of lowered host cytoplasmic inorganic phosphate levels (Whipps and Lewis, 1981) is not consistent with the idea that photosynthesis is increased in diseased chloroplasts (Owera **et al**, 1981).

### Photosynthesis in Discrete Regions of Diseased Leaves

In the light of some of the apparent inconsistencies in results obtained by several workers, it is surprising that little research has been conducted on localized regions of the diseased leaf. Some of the conflicting results may be attributed to differences between host/parasite combinations, and to differences in the experimental conditions used by different researchers. Environmental factors will be important in determining photosynthetic rates, and include leaf temperature, relative humidity, resistance to $CO_2$ diffusion and the concentration of $CO_2$ within the experimental system. Also, photosynthesis in a rust-infected leaf may be shown to increase or decrease depending on the basis upon which results are expressed, for example, per unit chlorophyll or per unit leaf area (Owera **et al**, 1981).

Much of the research involving studies of discrete regions of diseased leaves has concentrated on two pathosystems, namely bluebell infected with the rust **Uromyces muscari** (Scholes and Farrar, 1985) and barley infected with the rust **Puccinia hordei** (Scholes and Farrar, 1986). Although the experimental conditions used were identical in both systems, the results obtained were very different for each system. Briefly, oxygen evolution, both per unit area and per unit chlorophyll was reduced in rust pustules on bluebell in comparison to green areas of an infected leaf and to healthy leaves (Scholes and Farrar, 1985). However, autoradiographic and oxygen evolution studies on brown-rusted barley leaves showed that the decline in net photosynthesis could be attributed largely to a reduction in the rate of net photosynthesis in the regions between pustules. Within areas of the leaf invaded by the fungus, gross photosynthesis was increased in comparison to control tissue (Scholes and Farrar, 1986).

Concentration of inorganic phosphate in the medium of isolated chloroplasts, or in the cytoplasm of intact tissue, markedly affects both photosynthetic rate and the apportioning of newly fixed carbon between starch and cytoplasmic metabolites (Walker and Herold, 1977; Herold and Walker, 1979). Fungal sequestration of phosphate by pathogens may contribute to observed reductions in photosynthetic rates in infected tissue (Whipps and Lewis, 1981). However, Scholes and Farrar (1986) demonstrated that the addition of Pi to brown-rusted barley tissue (pustules or regions between pustules) had very little effect on rates of oxygen evolution, which suggested that Pi was not previously limiting the rate of photosynthesis in rusted tissue. Similar results were reported by Ahmad **et al** (1984), who

reported that chloroplasts from brown-rusted barley leaves contained an increased concentration of phosphorous, that the flux through the phosphate translocator was greater in diseased than in healthy leaves and that fluxes of Pi into the host cytoplasm were sufficiently large compared with the flux to fungal polyphosphate to enable host cytoplasmic Pi to be maintained at concentrations similar to those of healthy plants.

Since previous gas exchange measurements for rusted leeks were carried out on whole infected leaves (see Section 3), the results obtained were a mean of both uninvaded and fungally invaded tissue. Although these results give a general overview of photosynthetic rates in rusted leeks, they cannot accurately predict any changes which may occur in some parts of the leaf but not others. It was decided, therefore, to make a detailed examination of photosynthesis in localized regions of infected leek leaves, including the effect of exogenously applied Pi on photosynthesis. There has been much speculation concerning the effects of fungal infection on growth regulators and the influence of such alterations on the host/pathogen interaction (see Goodman, Kiraly and Wood, 1986). While examining photosynthesis in discrete regions of rusted leek leaves, it seemed prudent to determine levels of certain growth regulators in these areas. Alterations in levels of cytokinins and abscisic acid, for example, could influence the physiological behaviour of the infected leaf.

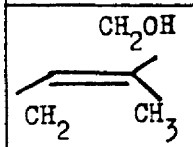
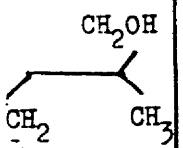
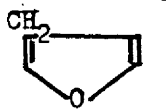
**Cytokinins and Abscisic Acid (ABA) in Healthy Systems**

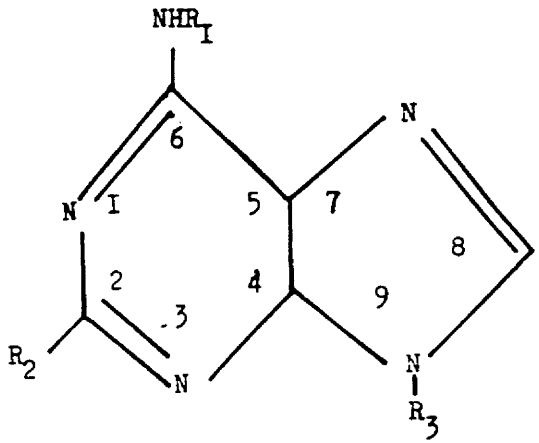
Growth in a complex multicellular organism is usually the result of the interrelated processes of cell division and cell expansion (Horgen, 1984). The existence of specific substances

which can control cell division in plants was postulated many years before such substances were finally discovered. Many materials were investigated as potential sources of cell division promoting substances. Particularly rich sources, as evidenced by activity in tobacco pith culture, included autoclaved DNA (Miller, Skoog, Saltza and Strong, 1955). Miller **et al**, (1956) went on to isolate a pure, highly-active, cell-division inducing factor from autoclaved herring sperm DNA, which they identified as 6-(furfurylamino) purine and called kinetin. Kinetin was not a component of DNA, but was formed during its breakdown and rearrangement during autoclaving. In 1963, Letham (1963) isolated a second cytokinin from immature kernels of **Zea mays** and named it zeatin. Further studies resulted in the isolation of zeatin riboside, zeatin ribotide (the 5$^{-}$-monophosphate) and other cytokinins (Letham, 1963). The chemical structures of several cytokinins are shown in Figure 10.

In addition to their effects on cell division, cytokinins exhibit a wide range of physiological effects when applied externally to whole plants, tissues and organs. One of the most striking responses to cytokinins is the redifferentiation of certain plant tissues to form organs. Research by Skoog and Miller (1957) showed that the ratio of auxin and cytokinin in the medium could control organ formation in tobacco pith culture. Some evidence for the control of growth and differentiation in plant tissue cultures by endogenous cytokinins comes from studies of crown gall tissues, and Weiler and Spanier (1981) demonstrated that crown gall tissues from a variety of plants produce large quantities of cytokinins.

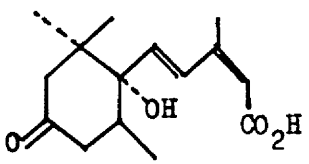
Cytokinins can cause an increase in size of leaf and cotyledon tissue by a process involving only cell enlargement (Miller, 1956;

| SUBSTITUENTS | | | TRIVIAL NAME | ABBREVIATION |
|---|---|---|---|---|
| RI | R2 | R3 | | |
|  | H | H | Zeatin | $io^6Ade$ |
| | H | Ribofuranosyl | Zeatin Riboside | $io^6A$ |
|  | H | H | Dihyrozeatin | $H_2io^6Ade$ |
|  | H | H | Kinetin | |




General Chemical Structure
Of Cytokinins

Figure IO. Chemical structures, trivial names and IUPAC-IUB abbreviations for some cytokinins. The structure of (+)Abscisic acid is shown below.




(+) - (S) - ABA

Letham, 1971), and it has been suggested that normal leaf development could be controlled by the gibberellin/cytokinin ratio (Horgen, 1984). A remarkable effect of externally applied cytokinins is their ability to delay the rate of chlorophyll disappearance and protein degradation which usually accompanies senescence, first reported by Richmond and Lang (1957) in detached leaves of **Xanthium.** More recent studies have revealed that several processes involved in senescence are influenced by cytokinins, including stabilization of polysome aggregates (Berridge and Ralph, 1969) and a delay of the reduction in several membrane-associated activities in the chloroplast (Thomas, 1975; Woolhouse and Batt, 1976). Cytokinins also suppress senescence-linked changes in respiration rate and mitochondrial coupling (Tetley and Thimann, 1974).

In relation to the "anti-senescence" effects of cytokinins described above, these compounds also have the ability to direct the movement of numerous substances to areas of the plant treated with exogenous cytokinins. Tobacco leaves treated with a single localized spot of kinetin and supplied with $^{14}C$-labelled amino acids showed accumulation of radioactivity totally coincident with the localized area of cytokinin application (Mothes, 1960). Localized application of cytokinins to yellowing leaves produces green areas where senescence is much reduced (Horgen, 1984), which mimic the green islands which are observed in some diseased leaves. Cytokinins also exhibit a complex relationship with light in influencing chloroplast development, and appear to stimulate the synthesis of certain chloroplast constituents during the light-dependent etioplast to chloroplast transformation in greening

seedlings and tissue culture (Horgen, 1984). The major effect of cytokinins on chloroplast structure appears to be an increase in the internal membrane system (Khokhlova, 1977), and the effects described here appear to be independent of cell division inducing activity (Seyer, Marty, Lescure and Peaud-Lenoel, 1975; Naito, Tsuji and Hatakeyama, 1978).

Just as cytokinins are involved in the promotion of growth, abscisic acid (ABA) is an important inhibitor of growth. ABA is present in all vascular plants in which it has been sought, and in most fungi, bacteria and algae. However, apart from the clearly-demonstratable inhibitory action of ABA when applied to growing organs, there is some evidence to suggest that it does act as a slight brake on normal growth (Milborrow, 1974; Walton, 1980).

ABA was originally isolated, as its name suggests, as an abscission-accelerating susbtance (Ohkuma, Addicott, Smith and Thiessen, 1965), but subsequent research has shown that it has only a slight effect when applied to intact plants. Separate research has established that there is usually a correlation of ABA contents with dormancy (El Antably, Wareing and Hillman, 1967), and although the case for a role for ABA in seed dormancy is better established than for buds, the correlation is not exact (Wareing and Saunders, 1971). Karssen (1982) suggested that ABA had an indirect effect on the germination of lettuce, because applications of synthetic ABA abolished their red-light-induced ability to germinate; however, Braun and Khan (1975), measuring the endogenous ABA levels in lettuce seeds suggested that germination was not always correlated with a decrease in ABA content.

Wright (1969) made the important observation that the amount of a growth-inhibiting material in wheat shoots increased dramatically when they lost water, and this growth inhibitor was later identified as ABA (Wright and Hiron, 1969). Chilling and salinity were also found to cause increased ABA levels, and it might be supposed that these treatments act by causing incipient loss of turgor and so operate indirectly via the wilting response (Milborrow, 1984). Beardsell and Cohan (1975) and Wright (1977) found a sudden increase in ABA content when leaf water potentials fell below a critical value, and suggested that the slight differences in the position of the threshold between plants and species may be related to the inherent drought tolerance of the leaf.

Knowledge of how ABA is transported within the plant is essential to an understanding of how and where it produces its effects, and yet the topic has received little attention. Hocking, Hillman and Wilkins (1972) found that (±)-[$^{14}$-C]-ABA applied to the leaf of a pea seedling was widely distributed within the plant within 24 h and 18% was present in the root nodules. Two mechanisms have been identified for the movement of ABA into cells of bean root segments (Astle and Rubery, 1980). First, there was a passive entry of the uncharged molecule (ABAH), presumably by partitioning into the lipid phase of the membranes followed by diffusion into the cytosol. The second method of entry was brought about by a saturable carrier which was specific for ABA, and was restricted to the elongating zone. The most probable mechanism for the ABA carrier is an $ABA^-/H^+$ symport (Rubery and Astle, 1982; Goldsmith, 1977).

Movement of solutes is influenced by ABA concentration, e.g. ABA inhibition of $K^+$-absorption into castor oil (**Ricinus communis**) petioles (Malek and Baker, 1978). On the other hand, ABA has been found to stimulate the accumulation of ions by roots (Collins and Kerrigan, 1974; Karmoker and Van Steveninck, 1978) and also to inhibit this process (Shaner, Mertz and Arntzen, 1975). It appears that the promotion or inhibition of these processes by ABA depended on the conditions under which the plants had been grown and the temperature during experiments (Pitman, Luttge, Lauchli and Ball, 1974). The production, but not the activity, of certain enzymes appears to be affected by ABA. The most studied example is the formation of $\alpha$-amylase by barley aleurone cells (Chispeels and Varner, 1966) which is powerfully stimulated by gibberellic acid and inhibited by ABA. Ho and Varner (1974) proposed that the inhibition of $\alpha$-amylase synthetase was caused, in part, by an effect on translation by ABA. The structure of ABA is shown in Figure 10.

**The Involvement of Cytokinins and ABA in the Diseased Plant**

Under conditions of stress, changes occur in the concentration of some or all plant growth regulators which can profoundly affect the physiology and often the morphology of the plant. In this sense, pathogenesis may be regarded as a chronic stress effect (Pegg, 1981). Therefore, it is not surprising that plant disease may show greatly exaggerated symptoms of growth substance activity and enhanced tissue levels of some growth regulators. However, very little information is available concerning the effects of pathogens on the concentration of cytokinins and ABA. Furthermore, many of the results obtained are based on bioassay techniques and therefore they may not be a reliable representation of the effects of disease

on growth regulators **in vivo.**

Cabbage tissue infected by the club-root fungus **Plasmodiophora brassicae** has been shown to contain very high amounts of cytokinins (Dekhuijzen and Overeem, 1971; Butcher, El-Tigani and Ingram, 1974). Pegg (1981) suggested that the possible involvement of cytokinins in the stimulated cell division seen in infected club root tissue may be closely related to the host-parasite interaction. Furthermore, Vizarova and Minarcic (1974), using bioassay techniques, reported an increase in cytokinin levels in roots of mildewed barley up to and including the fourth day after inoculation. Following this period, cytokinin levels in roots returned to normal values, but increases in cytokinin concentration were detected in the leaves.

It has long been known that infections caused by rusts and powdery mildews often result in the development of green islands around infection sites (e.g. Bushnell and Allen, 1962). These authors, using extracts from conidia, suggested that a primary effect of this extract was possibly a redirection of the transport system of the leaves. Later research (Thrower, 1965; Pozsar and Kiraly, 1966) indicated that rust infection and kinetin had similar effects on the long-distance transport of metabolites within the host plant. In addition, a complex of active mobilization inducers (including cytokinins) was detected in uredospores and mycelium of the bean rust fungus. It is interesting to note that these components had different Rf values to the cytokinins extracted from rust-infected host plants (Sequeira, 1973), suggesting that the observed increases in cytokinin concentration in infected leaves were of host origin (Poszar and Kiraly, 1966). Recently, Scholes and Farrar (1986) found that rates of photosynthesis were increased

in pustule regions on brown rusted barley leaves. They speculated that perhaps increased concentrations of cytokinins, thought to be associated with green-island formation, may be responsible for the changes in photosynthesis they observed. This is possible, since it is known that cytokinins stimulate chlorophyll synthesis and promote grana and thylakoid production in chloroplasts (Parthier, 1979). Further, it has been suggested that cytokinins stimulate photosynthesis by increasing protein synthesis in areas to which they are supplied (Meidner, 1967).

Although synthesis of ABA by plant pathogenic fungi has not been reported, it would seem that this hormone produced in the diseased plant has an important role in expression of such symptoms as leaf abscission, inhibition of extension growth, and inhibition of flowering (Goodman, Kiraly and Wood, 1986). To date, the only fungal disease in which an increased tissue level of ABA has been demonstrated is **Verticillium albo-atrum**, which showed a fivefold increase of this hormone (Pegg and Selman, 1959).

The precise role of plant growth regulators in many plant-pathogen interactions is still unclear. The possibility exists that in many cases the physiological effects of individual growth regulators interact, and therefore the contributions made by any one compound could be difficult to determine. However, an accurate quantification of these compounds would probably be a useful indicator of their individual roles within the host plant. It was decided, therefore, to examine two cytokinins and ABA in rusted leek plants using a monoclonal antibody immunoassay.

## MATERIALS & METHODS

The growth and inoculation of leeks (**Allium porrum** L. var. Autumn Mammoth) are described in Section 2 - General Materials and Methods.

### Photosynthetic Oxygen Evolution

Oxygen evolution was followed in an oxygen electrode (Rank Brothers) at 22°C. The $CO_2$ concentration in the atmosphere of the chamber was maintained at approximately 5% by the addition of 1 mol $dm^{-3}$ $Na_2CO_3/NaHCO_3$ buffer. Polychromic radiation was provided by a quartz lamp and photon flux density was altered by moving the position of the light source. Oxygen consumption was measured after darkening the chamber. The tissue types under investigation were healthy leaves, infected regions and uninfected regions of diseased leaves, and uninfected leaves of otherwise rusted leek plants. Four replicates of each tissue type were examined. ↕ (See Appendix III)

To measure the rate of $O_2$ evolution from localized regions of rusted leaves in the presence and absence of Pi, samples of the tissue types described above were incubated in a solution containing (mol $m^{-3}$) either MES, 50; KCl, 30; $CaCl_2$, 1; pH 6.0 or MES, 50; $KH_2PO_4$, 30; $CaCl_2$, 1; pH 6.0. The samples were incubated at 18°C for 1 h at a photon flux density of 350 $\mu$mol photons $m^{-2}s^{-1}$. Oxygen evolution was then measured at 600 $\mu$mol photons $m^{-2}s^{-1}$.

### RuBPcase Activity in Discrete Regions of Rusted Leaves

The method for extraction and assay of RuBPcase is described in Section 3. Total soluble protein was determined according to Lowry **et al** (1951) and chlorophyll was determined in acetone as before. The stomatal resistance of leaves was measured using a diffusive resistance

porometer (Delta T Devices).

**Extraction of Cytokinins and ABA**

Tissue samples (healthy leaves, infected and uninfected regions of rusted leaves, uninfected leaves from otherwise rusted plants and roots from healthy and rusted plants) were harvested and frozen in liquid nitrogen. The frozen samples were then ground in a mortar and pestle and extracted with 5 ml of 80% methanol containing 10 mg $l^{-1}$ BHT at 4-10$^{o}$C for 1 h. Samples of sap from healthy and rusted plants were collected by means of filter paper being placed on top of plants when their stems had been removed. The filter paper was weighed before and after sap collection, and was then placed in a flask for extraction as with other tissue samples. The samples were shaken in flasks placed on an orbital mixer, and the radio labelled internal hormone standard was added at this stage. The samples were then centrifuged at 8000 rpm for 5 minutes, the supernatant stored on ice and the pelleted sample extracted two more times as described above. The extracts were combined and water (4 ml) was added (Weiler, Eberle and Mertens, 1986).

Aliquots were passed through reversed-phase C18 cartridges (SepPak, Alltech) which removed lipids and pigments. The methanol in the samples was removed under a stream of nitrogen. Plant extracts were stored in a refrigerator until required (usually within the hour). The quantitative determination of the cytokinins trans-zeatin riboside (t-ZR), dihydrozeatin riboside (DHZR) and of ABA was conducted using an enzyme immunoassay (Idetek's Phytodetek kits, San Bruno, California) which utilised a monoclonal antibody to each of the growth regulators in question. The assay method used the competitive antibody binding method to measure concentrations of

each hormone in plant extracts. Each hormone was labelled with alkaline phosphatase (tracer) and then added along with plant extract to the antibody coated microwells. A competitive binding reaction was set up between a constant amount of the tracer, a limited amount of the antibody and the unknown sample containing the hormone. The intensity of the colour produced, read at 405 nm with a platereader (Titertek Multiscan MCC, Flow Labs), was inversely proportional to the amount of hormone in the sample. The colour was related to the sample hormone concentration by means of standard curves (see Appendix I).

## RESULTS

### Photosynthetic $O_2$ Evolution and Dark Respiration

The rate of oxygen evolution by discrete regions of infected leek leaves and of uninfected leaves of rusted plants was measured as a function of photon flux density. Oxygen evolution, expressed on both a chlorophyll and a unit area basis, was significantly decreased in infected regions at 14 and 21 days after inoculation (Figures 11 and 12). However, uninfected regions of infected leaves exhibited rates of oxygen evolution similar to control leaves, on a chlorophyll basis, and yet reductions were apparent in these regions when expressed on a unit area basis (Figures 11 and 12).

Rates of oxygen evolution were significantly stimulated in uninfected leaves of otherwise rusted leek plants. This stimulation was apparent at both 14 and 21 days after inoculation, when expressed as per unit chlorophyll and per unit area (Figures 11 and 12). At 21 days after inoculation (photon flux density of 800

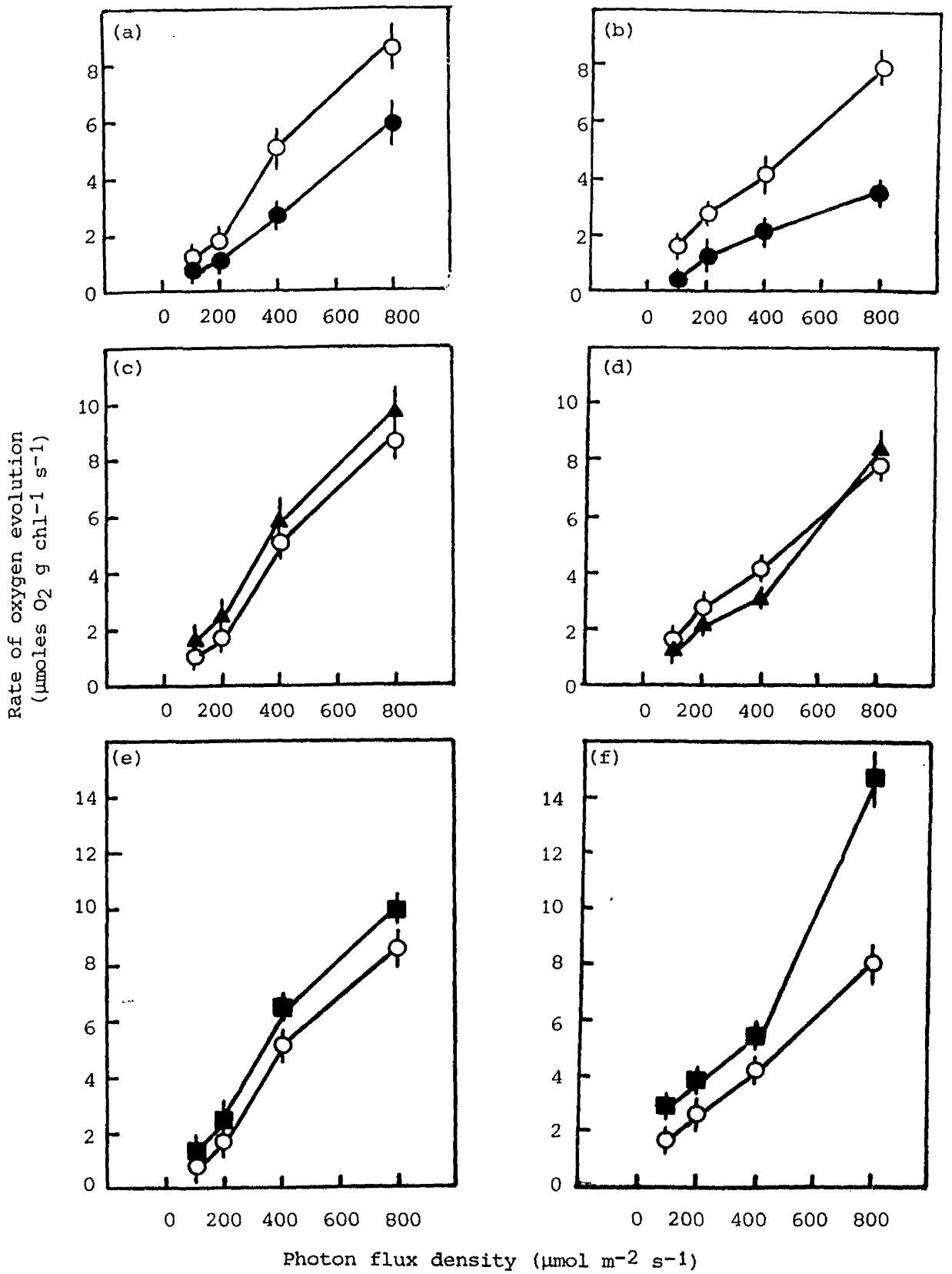



Figure 11. Change in the rate of net photosynthesis (μmol $O_2$ g chlorophyll-1 $s^{-1}$) as a function of photon flux density for controls (○), infected regions (●) and uninfected regions (▲) of rusted leaves and uninfected leaves of otherwise rusted plants (■). (a), (c), (e) 14 days after inoculation and (b), (d), (f) 21 days after inoculation. Standard errors of the means of four determinations are given.

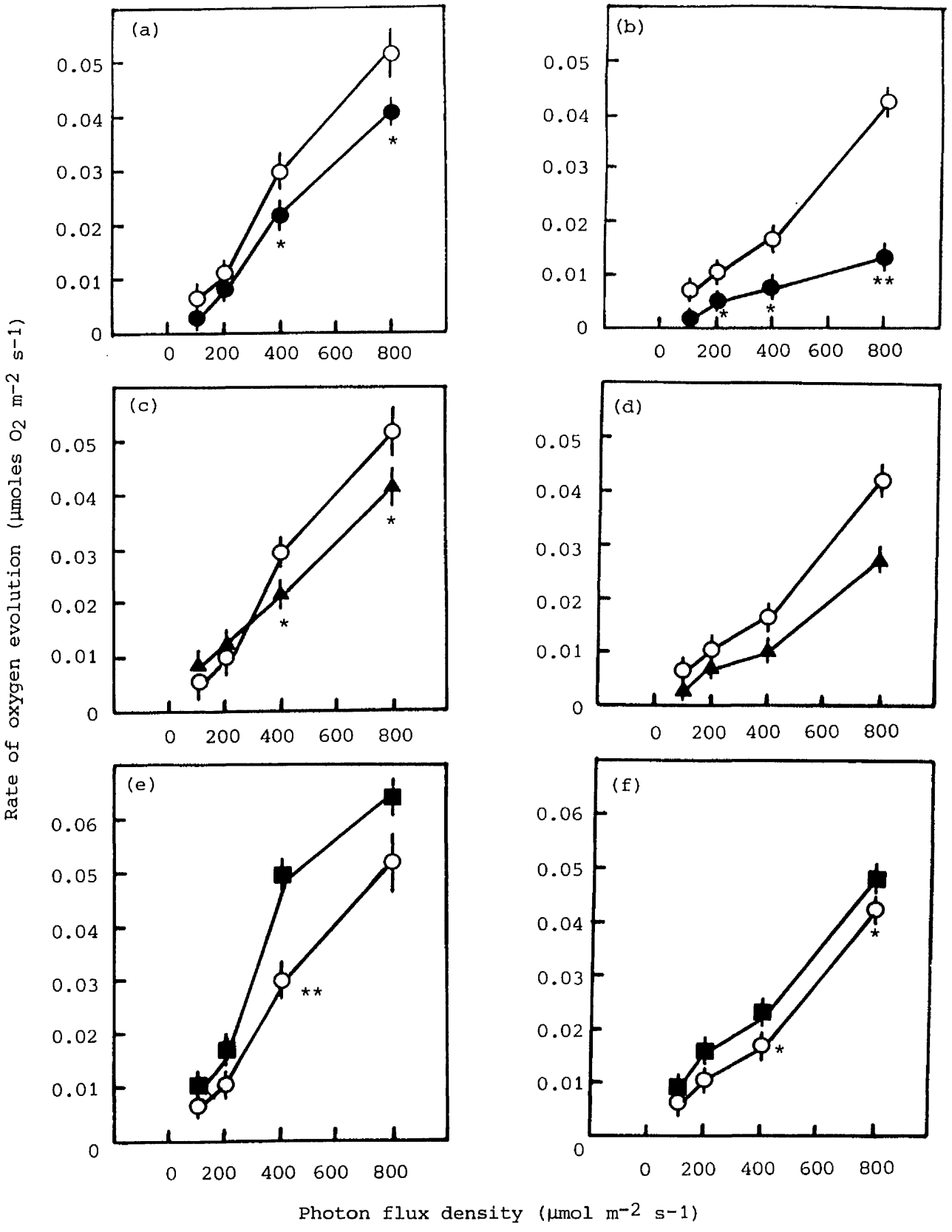



Figure 12. Change in the rate of net photosynthesis (μmoles $m^{-2}$ $s^{-1}$) as a function of photon flux density for controls (○), infected regions (●) and uninfected regions (▲) of rusted leaves and uninfected leaves of otherwise rusted plants (■). (a), (c), (e) 14 days after inoculation and (b), (d), (f) 21 days after inoculation. Standard errors of the means of four determinations are given.

| Tissue | Respiration rate ($\mu$mol $O_2$ $m^{-2}$ $s^{-1}$) | | | |
|---|---|---|---|---|
| | Days after inoculation | | % of control | |
| | 14 | 21 | 14 | 21 |
| Control | 0.0041 ± 0.0007 | 0.0026 ± 0.0003 | - | - |
| Infected regions | 0.0142 ± 0.0014 | 0.0155 ± 0.0021 | 346% | 586% |
| Uninfected regions | 0.0024 ± 0.0003 | 0.0025 ± 0.0002 | 58% | 96% |
| Uninfected leaves | 0.0024 ± 0.0005 | 0.0024 ± 0.0002 | 58% | 92% |

Table 9. Rates of dark respiration ($\mu$mol $O_2$ $m^{-2}$ $s^{-1}$) of control leaves, infected regions and uninfected regions of rusted leaves, and uninfected leaves of otherwise rusted leek plants. Values are the mean of four measurements with standard error.

$\mu$ moles $m^{-2}$ $s^{-1}$) oxygen evolution was almost 100% higher in uninfected leaves compared to controls.

Rust infection led to an increase in the rate of dark respiration per unit area in infected regions of the diseased leaf for the duration of the experiment (Table 9). Dark respiration was lower in regions between pustules and in uninfected leaves of diseased plants compared to control values. This reduction was only apparent at 14 days after inoculation because when the experiment was concluded oxygen consumption by healthy leaves was similar to that of both uninfected leaves and regions between pustules of diseased leaves (Table 9).

**Effects of Exogenous Phosphate**

Rates of oxygen evolution by discrete regions of rusted leaves, uninfected leaves of rusted plants and healthy plants were measured in the presence and absence of inorganic phosphate. The addition of inorganic phosphate had no appreciable effect on oxygen evolution by any of the tissue types described above, expressed either on a chlorophyll basis (Table 10) or on a unit area basis (Table 11). Photosynthesis per unit area was slightly enhanced in pustule regions following phosphate addition at 14 days after inoculation, but the increase was not significant.

**RuBPcase Activity in Localized Regions of Diseased Leaves**

The activity of RuBPcase was significantly reduced in infected regions and uninfected regions of rusted leaves compared to control leaves at 14 days after inoculation. No significant alteration in RuBPcase activity was detected at this time in uninfected leaves of otherwise rusted leeks when compared to controls (Figure 13). By

| Tissue | ± Pi | Photosynthetic rate ($\mu$mol $O_2$ g $chl^{-1}$ $s^{-1}$) | |
|---|---|---|---|
| | | Days after inoculation | |
| | | 14 | 21 |
| Control | + | 6.36 ± 0.160 | 5.83 ± 0.047 |
| | - | 6.28 ± 0.181 | 5.64 ± 0.057 |
| Infected regions | + | 3.24 ± 0.073 | 2.38 ± 0.118 |
| | - | 3.31 ± 0.132 | 2.28 ± 0.091 |
| Uninfected regions | + | 6.32 ± 0.056 | 5.95 ± 0.095 |
| | - | 6.26 ± 0.056 | 5.92 ± 0.154 |
| Uninfected leaves | + | 8.93 ± 0.036 | 8.63 ± 0.065 |
| | - | 8.93 ± 0.034 | 8.52 ± 0.053 |

Table 10. Rate of photosynthesis ($\mu$mol $O_2$ g $chl^{-1}$ $s^{-1}$) of control leaves, infected and uninfected regions of rusted leaves, and uninfected leaves of otherwise rusted leek plants. Values are the mean of four measurements with standard error.

| Tissue | ±Pi | Photosynthetic rate ($\mu$mol $O_2$ $m^{-2}$ $s^{-1}$) | |
|---|---|---|---|
| | | Days after inoculation | |
| | | 14 | 21 |
| Control | + | 0.034 ± 0.0017 | 0.027 ± 0.0011 |
| | - | 0.035 ± 0.0018 | 0.024 ± 0.0011 |
| Infected regions | + | 0.026 ± 0.0022 | 0.005 ± 0.0001 |
| | - | 0.021 ± 0.0023 | 0.005 ± 0.0003 |
| Uninfected regions | + | 0.031 ± 0.0022 | 0.024 ± 0.0020 |
| | - | 0.030 ± 0.0010 | 0.023 ± 0.0016 |
| Uninfected leaves | + | 0.044 ± 0.0020 | 0.033 ± 0.0021 |
| | - | 0.043 ± 0.0013 | 0.034 ± 0.0013 |

Table 11. Rate of photosynthesis ($\mu$ mol $O_2$ $m^{-2}$ $s^{-1}$) of control leaves, infected and uninfected regions of rusted leaves, and uninfected leaves of otherwise rusted leek plants. Values are the mean of four measurements with standard error.

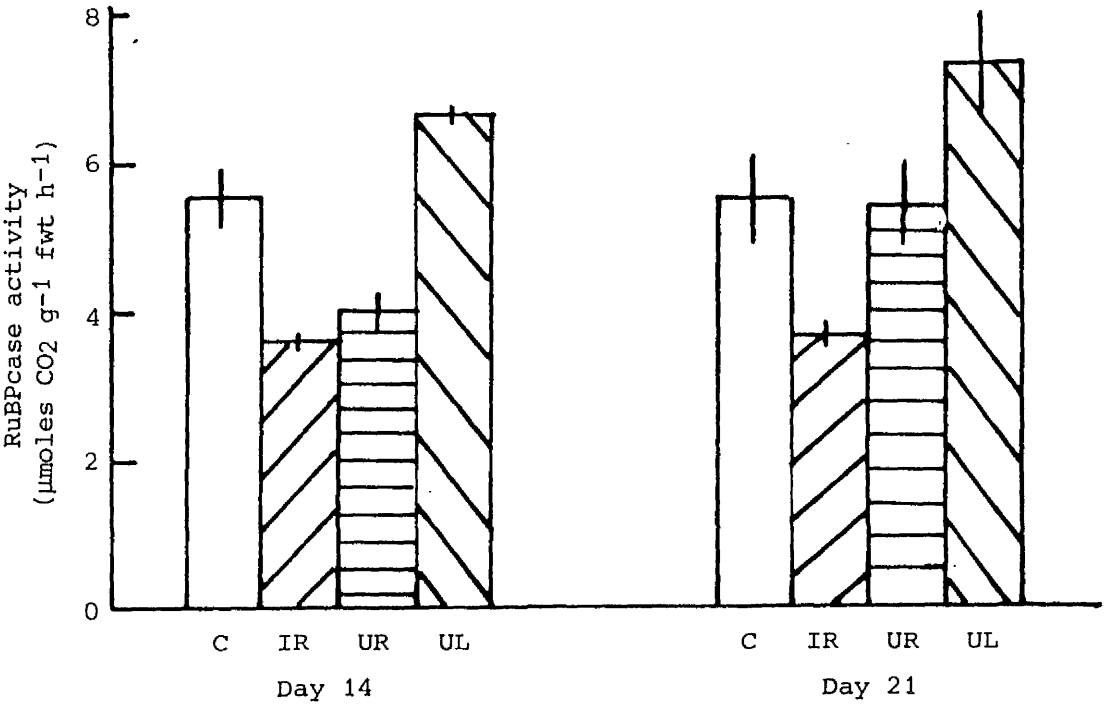



Figure 13. RuBPcase activity in discrete regions of rusted leek leaves (IR - infected regions, UR - uninfected regions), and in uninfected leaves of otherwise rusted plants (UL). Healthy controls (C). Each bar represents the mean of four replicates with standard error.

the end of the experiment, activity of the enzyme was still reduced in pustule areas compared to control levels and to uninfected regions of the diseased leaf. However, RuBPcase activity was significantly stimulated in uninfected leaves of rusted plants by 21 days after inoculation (Figure 13).

**Protein, Chlorophyll and Stomatal Resistance**

Rust infection of leek leaves led to losses of total soluble protein and chlorophyll within pustule areas. In regions between pustules and in uninfected leaves of otherwise rusted plants, protein and chlorophyll levels remained at near control values. This pattern was apparent at both 14 and 21 days after inoculation (Table 12).

Stomatal resistance was significantly lower in infected regions for the duration of the experiment but remained unchanged in uninfected regions of diseased leaves compared to controls. However, although stomatal resistance in uninfected leaves of rusted plants was similar to control values at 14 days after inoculation, it was considerably higher in these leaves by 21 days after inoculation (Table 12).

**Cytokinin and ABA Concentration in Plant Extracts**

The concentration of t-ZR, DHZR and ABA was determined in a variety of plant tissues using a monoclonal antibody immunoassay. In all cases, the concentration of cytokinins and ABA was much greater at 21 days after inoculation than at 14 days after inoculation. The recovery of both cytokinins and ABA, as determined by the addition of an internal hormone standard, was usually between 80% and 90%.

| (a) Tissue | Total soluble protein (mg $g^{-1}$ fwt) | |
|---|---|---|
| | Days after inoculation | |
| | 14 | 21 |
| Control | 21.25 ± 0.059 | 20.68 ± 0.179 |
| Infected regions | 18.72 ± 0.157 | 17.30 ± 0.114 |
| Uninfected regions | 21.00 ± 0.157 | 20.07 ± 0.098 |
| Uninfected leaves | 21.52 ± 0.183 | 20.53 ± 0.229 |

| (b) Tissue | Chlorophyll content (mg $g^{-1}$ fwt) | |
|---|---|---|
| | Days after inoculation | |
| | 14 | 21 |
| Control | 3.50 ± 0.043 | 3.19 ± 0.022 |
| Infected regions | 2.51 ± 0.065 | 2.33 ± 0.082 |
| Uninfected regions | 3.52 ± 0.089 | 3.20 ± 0.015 |
| Uninfected leaves | 3.51 ± 0.085 | 3.37 ± 0.056 |

| (c) Tissue | Resistance (s/cm) | |
|---|---|---|
| | Days after inoculation | |
| | 14 | 21 |
| Control | 12.32 ± 0.217 | 12.50 ± 0.129 |
| Infected regions | 8.92 ± 0.179 | 4.82 ± 0.390 |
| Uninfected regions | 13.17 ± 0.111 | 12.90 ± 0.108 |
| Uninfected leaves | 12.15 ± 0.299 | 15.07 ± 0.193 |

Table 12. (a) Total soluble protein, (b) chlorophyll concentration and (c) stomatal resistance values in control leaves, infected and uninfected regions of rusted leaves, and uninfected leaves of otherwise rusted leek plants.

The concentrations of both t-ZR and DHZR were considerably higher in pustule areas compared to uninfected regions of diseased leaves, uninfected leaves of rusted plants and healthy controls, by 14 days after inoculation (Figures 14 and 15). With respect to ABA, concentrations were significantly higher in both infected regions and in uninfected leaves of otherwise rusted plants by 14 days after inoculation (Figure 16). At the end of the experiment the concentration of t-ZR in pustule regions was similar to that found in healthy controls (Figure 14). However, t-ZR concentration was significantly increased in uninfected regions and in uninfected leaves of rusted plants compared to controls (Figure 14). DHZR concentration was slightly, although significantly, increased in all tissue types compared to healthy controls by 21 days after inoculation (Figure 15). The concentration of ABA was significantly less in infected regions compared to uninfected areas of the same leaf and healthy controls by the end of the experiment. However, there was almost eight times more ABA present in uninfected leaves of otherwise rusted plants than in healthy leaves by 21 days after inoculation (Figure 16).

The concentrations of both cytokinins and ABA were always consistently lower in roots of infected plants than in healthy ones at 21 days after inoculation, and with the exception of DHZR, the concentration was also lower at 14 days after inoculation (Table 13). At 14 days after inoculation the concentrations of all compounds studied were significantly higher in sap collected from healthy plants than in sap from diseased plants. No difference was detected in t-ZR concentration of healthy and rusted sap at 21 days after inoculation, although the concentration of DHZR was reduced in

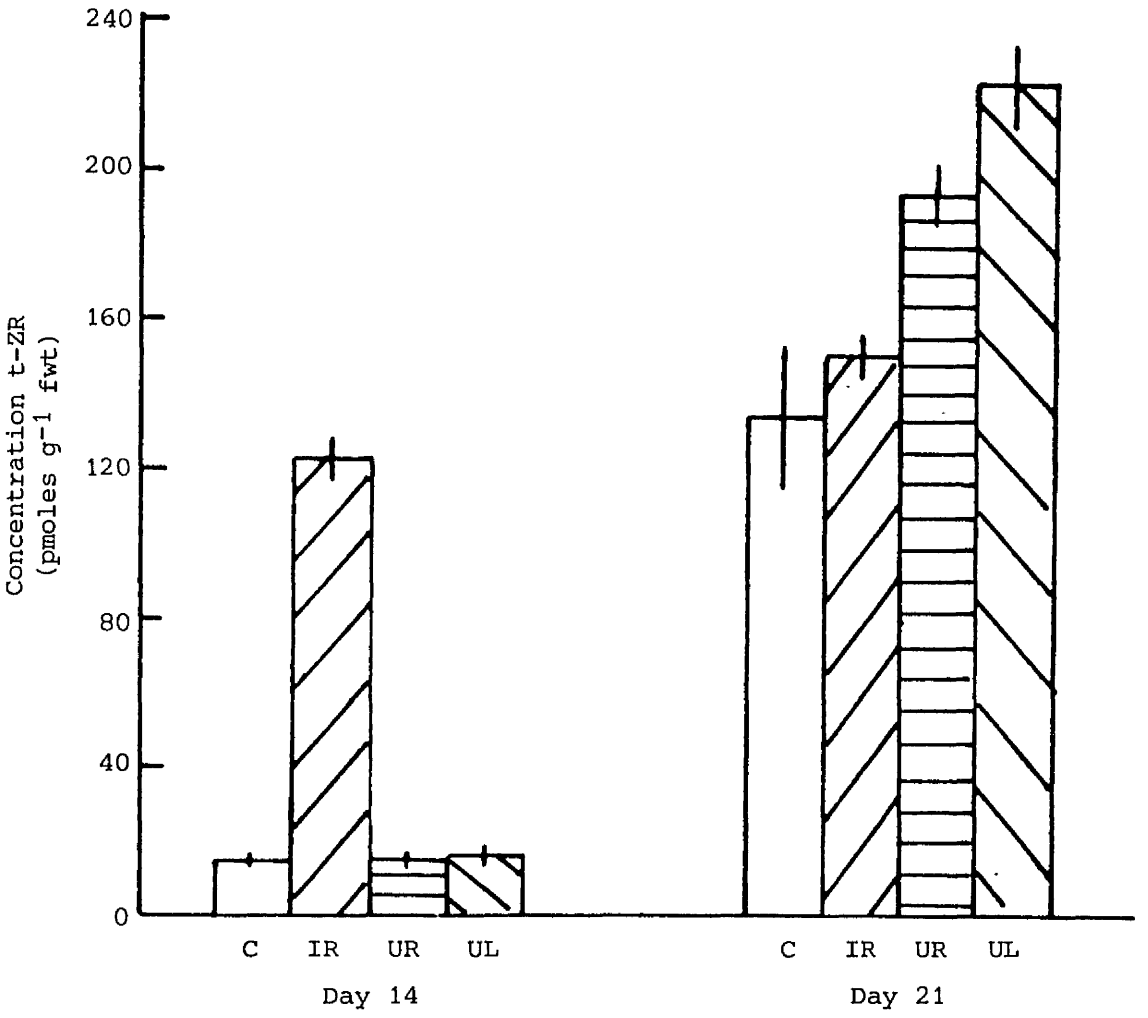



Figure 14. Concentrations of t-ZR in discrete regions of rusted leek leaves (IR - infected regions, UR - uninfected regions), and in uninfected leaves of otherwise rusted leaves (UL). Healthy controls (C). Each bar represents the mean of four replicates with standard error.

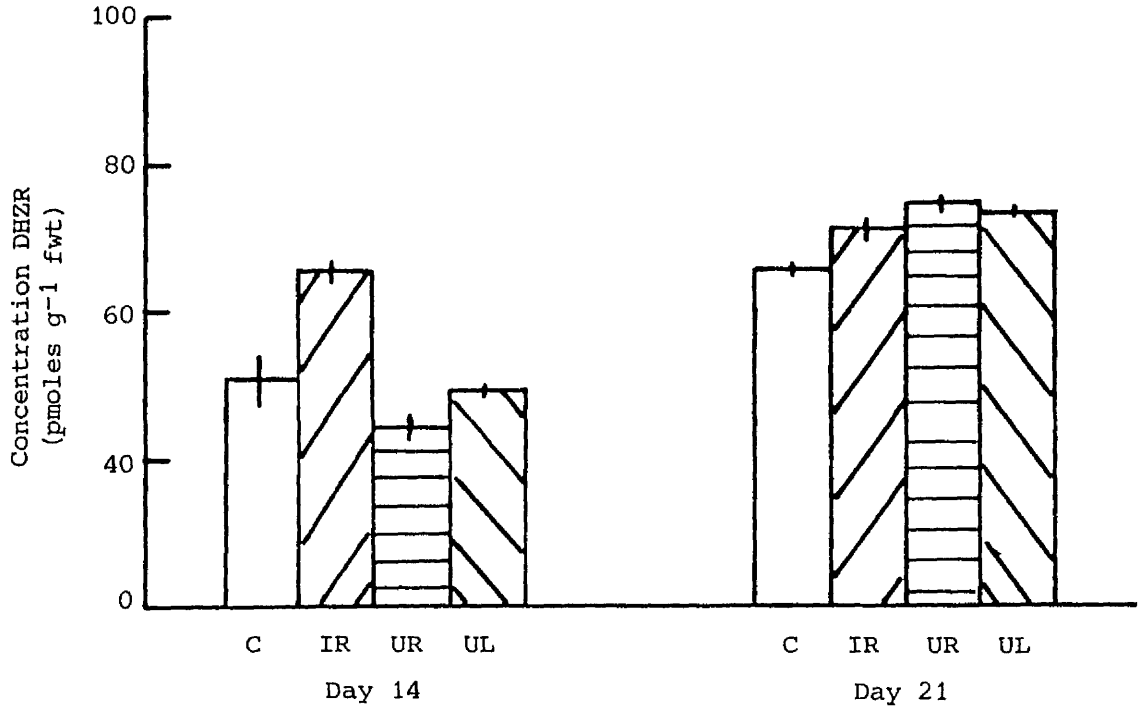



Figure 15. Concentrations of DHZR in discrete regions of rusted leek leaves (IR - infected regions, UR - uninfected regions), and in uninfected leaves of otherwise rusted plants (UL). Healthy controls (C). Each bar represents the mean of four replicates with standard error.

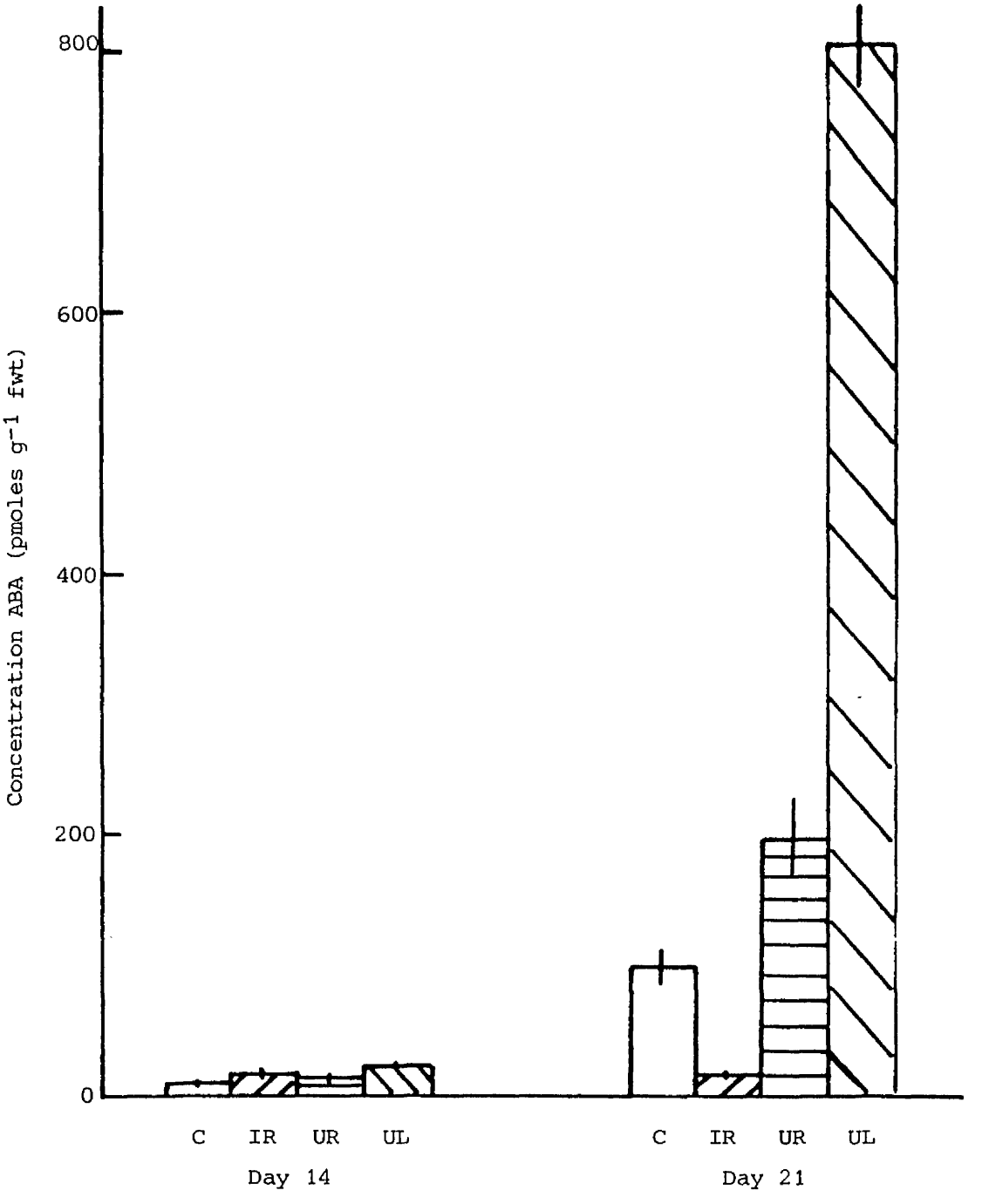



Figure 16. Concentrations of ABA in discrete regions of rusted leek leaves (IR - infected regions, UR - uninfected regions), and in uninfected leaves of otherwise rusted plants (UL). Healthy controls (C). Each bar represents the mean of four replicates with standard error.

| Tissue type | t-ZR concentration (pmoles g-1 fwt) | | DHZR concentration (pmoles g-1 fwt) | | ABA concentration (pmoles g-1 fwt) | |
|---|---|---|---|---|---|---|
| | Days after inoculation | | Days after inoculation | | Days after inoculation | |
| | 14 | 21 | 14 | 21 | 14 | 21 |
| Healthy roots | 21.60 ± 0.174 | 37.75 ± 1.168 | 13.87 ± 0.230 | 25.11 ± 0.500 | 29.88 ± 0.518 | 38.46 ± 2.846 |
| Rusted roots | 15.86 ± 0.409 | 28.02 ± 1.204 | 13.53 ± 0.239 | 21.65 ± 0.586 | 0.64 ± 0.079 | 2.17 ± 0.401 |
| *Healthy sap | 740.62 ± 66.43 | 1537.69 ± 241.000 | 941.66 ± 39.861 | 2004.95 ± 61.709 | 52.77 ± 1.203 | 215.75 ± 11.130 |
| *Rusted sap | 71.08 ± 12.58 | 1695.75 ± 70.160 | 72.58 ± 1.750 | 1485.53 ± 61.165 | 12.16 ± 3.193 | None detected |

Table 13. Concentration of t-ZR, DHZR and ABA in sap and roots of healthy and rust infected plants. Each value is the mean of four replicates with standard error.
* Values for sap measurements are given by pmoles $g^{-1}$ sap.

rusted sap at this time. No ABA was detected in any sample of sap collected from diseased plants at 21 days after inoculation (Table 13).

## DISCUSSION

Much research over many years has been conducted to examine the effects of foliar pathogens on photosynthesis by host plants (see Walters, 1985). Much of this research has been carried out on whole infected leaves, and although a lot of valuable information has been obtained from these experiments many of the results have been conflicting. One major disadvantage of whole leaf studies is that the amount of infection per leaf will probably be highly variable in any one pathosystem, and certainly there will be large differences in percentage infection between different pathogens. Heavy rust infections of leek leaves, for example, may only cover about half of the available green leaf area, whereas barley leaves heavily infected with powdery mildew may lose almost all of their green leaf area. Previous experiments with rusted leek leaves (see Section 3) indicated that rates of photosynthesis were reduced in diseased leaves, coupled with reductions in the activity of RuBPcase, chlorophyll content and total soluble protein. However, all the results obtained were an average of both infected and uninfected areas on the diseased leaf. In an attempt to gain a clearer understanding of the leek/rust interaction, it was decided to examine localized regions of diseased leaves. In view of the interesting results obtained for uninfected leaves of rusted plants (see Section 3), such leaves were included in all experiments conducted here.

Oxygen evolution was measured under conditions of saturating $CO_2$, so that any observed changes in photosynthetic rates could be attributed to changes within chloroplasts rather than to altered diffusion of $CO_2$ into the leaf (Scholes and Farrar, 1986). Any changes due to photorespiration were also suppressed in this experimental system, since it is thought that at 5% $CO_2$, photorespiration is almost completely inhibited (Delieu and Walker, 1981). It is important to note, therefore, that if experiments were carried out at non-saturating $CO_2$ conditions, the results could be very different to those reported here.

Rates of oxygen evolution, expressed on both a chlorophyll and a leaf area basis, were considerably reduced in pustule areas of diseased leaves for the duration of the experiment. This reduction must be due to changes within chloroplasts, rather than to altered $CO_2$ diffusion within the pustule, for the reasons described above. As the concentration of chlorophyll is greatly reduced in pustule areas, this may be a contributory factor in the decline in photosynthesis. Much research has involved possible relationships between chlorophyll loss due to fungal infection and the subsequent reduction in photosynthesis, although many of the results are contradictory. Thus Allen (1942) found that photosynthesis per mole of chlorophyll in mildewed barley increased in the early stages of infection then declined. So and Thrower (1976) found a significant correlation between reduced photosynthesis and chlorophyll levels in **Vigna sesquipedalis** following infection with rust, although photosynthesis declined more rapidly than chlorophyll levels in the wheat/**Puccinia striiformis** interaction (Doodson **et al**, 1965).

Scholes and Farrar (1986) reported that gross photosynthesis per unit chlorophyll was increased in pustule areas of brown-rusted barley leaves, which contrasts with the results obtained for pustule areas of rusted leek leaves. However, within the barley/brown rust pathosystem, the largest loss of chlorophyll was in areas between pustules, whereas chlorophyll was retained in pustule areas. These authors also suggested that within brown rust pustules, non-cyclic photophosphorylation was not inhibited but rather was greatly accelerated. In this respect, their results resemble those provided by Wynn (1963) and Ahmad **et al** (1983) who, although using whole leaves, showed that no reduction in non-cyclic electron transport occurred in chloroplasts isolated from rust-infected oats and barley respectively.

The results presented here for infected regions of rusted leek leaves are supported by data from various researchers (Montalbini and Buchanan, 1974; Scholes and Farrar, 1985), who suggested that rust of broad bean and rust of bluebell effected a preferential inhibition of non-cyclic electron transport. It is also interesting to note that the activity of RuBPcase is substantially reduced in pustule areas of rusted leek leaves. Reductions in the activity of RuBPcase in mildewed and rusted plants have been reported before (Wrigley and Webster, 1966; Gordon and Duniway, 1982b; Walters and Ayres, 1984). However, as far as we are aware, this is the first report of changes in RuBPcase activity in discrete regions of an infected leaf. Gordon and Duniway (1982b) found that the reduction in total soluble protein was somewhat less than the reduction in RuBPcase, and they suggested that infection with sugar beet powdery mildew engendered a preferential degradation of RuBPcase protein.

Although we can provide no data for concentration of RuBPcase protein, it is interesting to note that in infected areas of rusted leek leaves, a 12% reduction in total soluble protein is associated with a 37% reduction in RuBPcase activity. Further, in uninfected regions of rusted leaves, a 2% reduction in total soluble protein is associated with a 6-28% reduction in RuBPcase activity. It seems therefore, that there is a greater loss of RuBPcase activity (and total soluble protein) in pustule regions than in uninfected regions away from pustules. These changes in RuBPcase within different regions of a rusted leaf are very interesting in view of the alterations in chloroplast ultrastructure which occur within a few days of rust or powdery mildew infection (Dyer and Scott, 1972; Chakravorty and Scott, 1982; Higgins, Manners and Scott, 1985). Several mechanisms have been suggested to account for the reduction in the amount and activity of RuBPcase in diseased plants, and these have been fully discussed in Section 3. The reduction in RuBPcase activity in infected regions of leek leaves will undoubtedly account, in part, for the reduction in photosynthesis.

Oxygen evolution by uninfected regions of diseased leek leaves was similar to controls when expressed on a chlorophyll basis. However, photosynthesis per unit area was significantly reduced in these areas. Although chlorophyll was not reduced in these regions, the activity of RuBPcase was slightly lower than in control plants. This would probably contribute to the lowered photosynthetic rates observed here. It is also possible that, although total chlorophyll concentration was unaffected in areas between pustules, the composition of the pigment system could be altered. Research has shown that the ratio of chlorophylls a:b declined in diseased tissue

whilst the carotenoid concentration remained relatively high, suggesting that although some of the light-harvesting complex was lost, a proportion of chlorophyll a must also have been lost from the photosystem II antenna matrices, leading to a reduction in the rate of non-cyclic photophosphorylation (Scholes and Farrar, 1985). These authors also suggest that alteration in chloroplast size may be indicative of changes in the osmotic environment within infected cells. It is of interest therefore, that we reported in this section increased stomatal resistance and ABA accumulation in regions between pustules on rusted leek leaves, suggesting the development of water stress in these areas. It is feasible that changes in the osmotic environment of infected cells, perhaps due to fungal sink activity, might drain water away from inter-pustule regions and so lead to water stress. It is possible that such a situation exists in uninfected areas of diseased leaves of leek, although in the absence of relevant data this must remain mere speculation. Photosynthesis was stimulated in uninfected leaves of otherwise rusted leek plants. This is in agreement with previous results obtained from whole leaf studies (Roberts and Walters, 1986; Section 3). The large stimulation is undoubtedly correlated with the increased RuBPcase activity in these leaves. Other causes of this increased photosynthetic rate and its potential importance in host survival have been discussed in Section 3. Whipps and Lewis (1981) have suggested that sequestration of phosphate by fungal pathogens could contribute to the observed reductions in photosynthetic rates and in the enhanced starch synthesis caused by biotrophs. If this hypothesis is correct, then supplying Pi to leaves should alleviate (at least partially) this Pi limitation and lead to an increased rate of photosynthesis. Scholes and Farrar

(1986) tested this hypothesis with brown rust-infected barley, and found very little effect of exogenous phosphate on photosynthesis by discrete regions of diseased leaves. Our results with regions of rust-infected leek leaves agree with those of Scholes and Farrar (1986), since we could find no enhancement of photosynthesis in infected and uninfected regions of rusted leek leaves following the addition of exogenous phosphate.

Rates of dark respiration are known to be increased in rust-infected tissue (Mitchell, 1979; Owera **et al**, 1981). More recently, Scholes and Farrar (1986) showed that rates of dark respiration within brown rust pustules on barley leaves were four times greater than values for control leaves. Our results show that 14 days after inoculation, almost coincident with fungal sporulation in this system, dark respiration was 3.5 times greater than controls. However, unlike Scholes and Farrar (1986), who found only a two-fold stimulation of dark respiration in pustule areas at the green-island stage, we found that at 21 days after inoculation, rates of dark respiration in pustule regions had increased to almost six times the values obtained from controls. It seems possible that most of the increases in rates of dark respiration in both systems were of fungal origin, since in both leek/rust and barley/brown rust the fungi were either approaching sporulation or were actively sporulating, and so fungal activity would have been intense.

Uninfected regions of infected leek leaves were found to have substantially reduced rates of dark respiration, 14 days after inoculation, while seven days later, dark respiration in these areas had returned to near normal control values. This contrasts with the results obtained for barley/brown rust, where rates of dark

respiration were much increased in regions between pustules until the green-island stage, when dark respiration rates fell below rates obtained for controls. Scholes and Farrar (1986) suggested that the increase in dark respiration in regions between brown-rust pustules on barley leaves could be associated with the loss of chlorophyll and the degeneration of chloroplasts that occurs in these areas. It is interesting to note, therefore, that although rates of dark respiration were reduced in regions between pustules in our system, these regions show no reduction in chlorophyll or total soluble protein. They do, however, show a reduction in rates of photosynthesis (on a leaf area basis) and a loss of RuBPcase activity. A loss of RuBPcase activity without a loss of chlorophyll seems difficult to imagine, unless there is no chloroplast degeneration in these regions of rusted leek leaves. Perhaps loss of RuBPcase between pustules is thus associated with, for example, a derangement in the synthesis of the small RuBPcase subunit precursor on cytoplasmic 80S ribosomes.

Growth regulators play an important part in the regulation of plant growth and development. Although reduced photosynthetic rates may be solely responsible for reductions in the growth of diseased plants, alterations in the concentration of specific plant growth regulators should not be ignored. It was decided, therefore, to make a quantitative examination of two cytokinins and ABA in discrete regions of diseased leek plants. Many studies by other authors have involved the use of bioassay techniques, yielding results which are open to criticism. Many attempts to demonstrate a clear correlation between endogenous growth substance levels and developmental processes have proved indecisive. One possible

explanation of the lack of correlation appears to be attributable to the crudity of the techniques available for the determination of endogenous growth substance levels in plant tissues (Wareing, 1986).

The technique employed in the experiments reported here involved the use of monoclonal antibodies, which have several advantages over other techniques. The appeal of immunoassays in plant hormone analysis is based on their low limits of detection, the fact that they require little sophisticated equipment and their potential for processing large numbers of samples (Crozier, Sandburg, Monteird and Sundberg, 1986). It is also possible that the selectivity of immunoassays is such that they can be used to analyse trace quantities of hormones in plant extracts in circumstances where it would be impossible to use alternative procedures without extensive sample purification, although this view is still open to question (Weiler, 1984). The use of monoclonal antibodies has contributed substantially to increased assay specificity, allowing a further simplification of assay protocols. They are easily compatible with enzyme-labelled plant growth regulators, and are more precise (Weiler **et al**, 1986).

A review of the literature shows that no attempts have previously been made to quantify individual plant growth regulators in particular areas of diseased leaves, which leads to difficulties in discussing the results reported here in relation to other studies. It is interesting to note that by 14 days after inoculation the concentration of both cytokinins was significantly higher in infected regions of rusted leek leaves compared to uninfected areas of the same leaf and to healthy controls. This is interesting in view of the suggestion of Scholes and Farrar (1986)

that increased rates of photosynthesis in pustule regions of brown rust-infected barley might be due to changes in cytokinins in these regions. Although Scholes and Farrar (1986) provided no data for cytokinin concentrations in pustule areas on rusted barley leaves, the increases in cytokinin concentration formed in our leek/rust system were associated with substantial reductions in photosynthesis and chlorophyll in pustules. However, it should be noted that increased cytokinin concentrations were detected in uninfected areas between leek rust pustules where chlorophyll was retained and photosynthetic rates were maintained at near control values. Cytokinins activate several enzymes, probably via **de novo** synthesis, and induce the metabolism and transportation of the various important components of cell nutrition concomitant with a general stimulation of metabolic activities and cell division. In contrast to abscisic acid and ethylene, which often induce symptoms of senescence, the cytokinins might be called "juvenility factors" in several cases (Moore, 1979; Skoog, 1980). Differentiated cells around the sites of infection may undergo division accompanied by increased protein synthesis and respiration. These areas with new metabolic activities become "metabolic sinks" and thus exhibit a reduced expression of senescence symptoms. This would appear to be the case in pustule regions of rusted leek leaves, despite reduced photosynthetic rates in these areas. Protein content was also reduced in these areas but this could be attributed to increased breakdown of protein as opposed to a reduction in protein synthesis.

Separate studies using whole leek plants indicated that the concentration of selected minerals was higher in rusted plants (see Section 6) and it is possible that these minerals may have

accumulated at infection sites. Furthermore, research on at least two hemibiotrophs, **Helminthosporium** sp. and **Colletotrichum lindemuthianum**, has shown that these pathogens are capable of accumulating $^{14}C$-labelled carbohydrates (Shaw and Samborski, 1956; Wong and Thrower, 1978). In the former, this occurs around older lesions and in the latter, in the very young infected regions. Although we can provide no evidence of carbohydrate accumulation at infection sites of rusted leek leaves, the evidence from the hemibiotroph studies lends credence to the notion of increased metabolic sink activity in pustule regions. It is tempting to suggest that the increased cytokinin concentration in rusted leek leaves might be linked with such metabolic sink activity.

In uninfected regions of diseased leaves, cytokinin levels were near control values at 14 days after inoculation, suggesting perhaps that the pathogen can only exert an influence on plant hormones in areas of colonization. However, this situation was not maintained and by the end of the experiment the concentration of both cytokinins was higher in uninfected regions than in pustule areas or in healthy leaves. Furthermore, photosynthetic rates were higher in uninfected regions compared to pustule areas, coupled with increases in chlorophyll and total soluble protein content. It may be useful to compare these uninfected regions to green islands, which are residual areas of chlorophyllous tissue surrounded by chlorotic tissue associated with lesions caused by biotrophic pathogens. They may be mimicked by the application of cytokinins to leaves, in which case chlorophyll retention is increased and chlorophyllase activity arrested (Pegg, 1981). Uninfected regions between pustules on leek leaves show very close similarities to green islands, with respect

to chlorophyll retention and enhanced photosynthesis. However, it should be noted that due to a limited supply of monoclonal antibodies measurements could not be made when "real" green islands began to form around rust pustules, i.e. approximately 24 days after inoculation. It is possible therefore, that cytokinin levels may return to near control values in regions between pustules as green islands are formed around infection sites.

It is interesting to note that the concentration of ABA was greatly increased in uninfected regions of diseased leaves compared to pustule regions towards the end of the experiment. This may seem surprising since metabolism in these areas, illustrated by photosynthetic rates was maintained at a reasonable level and cytokinin levels were high. However, the high levels of ABA in uninfected regions of diseased leek leaves may be linked to water stress, for although we provide no measurements of leaf water potentials, the values for stomatal resistance in these areas were almost three times those of pustule areas by 21 days after inoculation. There is much evidence to suggest that the concentration of ABA rises dramatically in response to water stress. Wright and Hiron (1969) demonstrated that radiant heating of leaves of several species (bean, **Phaseolus vulgaris**; tomato, **Lycopersicon esculentum**; and wheat, **Triticum aestivum**) led to increased ABA content. Wright (1977) and Bearsdell and Cohen (1975) later suggested that the concentration of ABA was increased when leaf water potentials fall below a critical value. An examination of the water relations of rusted faba bean revealed that rust caused increased transpiration from infected tissues after sporulation but, even in well-watered plants, infection of proximal halves lowered

the leaf water potentials and inhibited transpiration from distal, uninfected regions of the same leaf (Tissera and Ayres, 1986). It is possible therefore, that a similar situation exists in rusted leek plants, which may account, in part, for the large increases in ABA concentration in uninfected regions of rusted leek leaves.

In view of the large stimulation in the rate of net photosynthesis by uninfected leaves of otherwise rusted leek plants (Roberts and Walters, 1986), an examination was made of growth regulators in these leaves. In many respects uninfected leaves resembled uninfected regions of diseased leaves. The concentrations of both cytokinins were similar to control levels at 14 days after inoculation, although by the end of the experiment cytokinin levels were significantly higher in uninfected leaves of rusted leeks. It is possible that the high cytokinin levels in uninfected tissues of rusted leeks may be related directly or indirectly to the very large stimulation in photosynthetic rates in these leaves. It is known that cytokinins can direct the transport, accumulation and retention of metabolites in tissues and organs, and that these properties are undoubtedly related to their capacity to delay senescence (Mittelheuser and Van Stevenick, 1971). The evidence suggests that cytokinins in some way help the plant to sustain an effective level of protein synthesis (Goodman **et al**, 1986), and an examination of protein content of uninfected leaves of rusted leek plants suggests that this may be the case.

Research by Osborne (1967) suggested that senescence in the leaf could be linked to a "progressive turning off of genetic information in the nucleus" of cells normally engaged in anabolic processes. As fewer genes remain functional as templates for RNA

synthesis, the cells progressively reduce their protein synthesis activities and pass into a state of dormancy. The genes may be reactivated by cytokinins, e.g. kinetin, and cells will then resume synthesis of both protein and RNA. Hence, it would appear that cytokinins may retard senescence by sustaining chloroplast nucleic acid synthesis (Osborne, 1967), and by sustaining an effective level of protein synthesis. Certainly, protein content is not altered in uninfected leaves of rust-infected leeks. Shibaoka and Thimann (1970) contend that cytokinins maintain membrane integrity by inhibiting proteolysis rather than promoting protein synthesis. It seems reasonable that either an increase in chloroplast nucleic acid synthesis or a reduction in chloroplast breakdown will have beneficial effects for the plant, and may account, in part, for the stimulation of photosynthesis in uninfected leaves of rusted leek leaves.

It is difficult to reconcile an eight-fold increase in ABA concentration in uninfected leaves of rusted leeks with delayed senescence, stimulation of photosynthesis and maintenance of juvenility. However, the large increase in ABA content in these leaves may be a transient response to water stress. An examination of the water relations of rusted leek plants was beyond the scope of these experiments, although measurements of stomatal resistance allowed us to make some tentative assumptions. Transpiration from rusted areas of diseased plants reflects the development of the fungus. Before sporulation, transpiration which potentially is by a mainly stomatal pathway, is inhibited, probably by stomatal closure (Tissera and Ayres, 1986). Following sporulation, transpiration is usually increased because a third pathway is introduced, in parallel

with stomatal and cuticular pathways as the epidermis is ruptured (Tissera and Ayres, 1986). Stomatal resistance in pustule areas of rusted leek leaves was 4.82 s/cm by 21 days after inoculation, compared to 12.50 and 15.07 s/cm in healthy controls and uninfected leaves respectively. The very large increase in stomatal resistance in uninfected leaves suggests that these leaves are indeed undergoing some water stress. ABA plays a major role in regulating stomatal closure, the basis for this being in the guard cell turgor-regulating system, which depends upon a $H^+/K^+$ exchange process. ABA inhibits $K^+$ uptake by the guard cells, which in turn keeps them flaccid and hence closed (Walton, 1980). Under conditions of water stress, increases in ABA content are usually coupled with reductions in cytokinin concentration. This does not appear to be the case for uninfected leaves of rusted leek plants, at least at 21 days after inoculation. However, experimental limitations meant that no measurements could be made after this time, and therefore there is no reason to suppose that reductions in cytokinins did not occur after 21 days.

The concentrations of t-ZR and ABA were significantly lower in roots of rusted plants for the duration of the experiment, and DHZR concentration was reduced in diseased roots at the end of the experiment. These results are in direct contrast to those provided by Vizarova and Minarcic (1974) who, using bioassay methods, reported an increase in cytokinin levels in roots of mildewed barley up to and including the fourth day after inoculation. Cytokinin levels in the roots then returned to normal values. It should be stressed again that direct comparisons cannot be made between different pathosystems, and that techniques such as bioassays are

not always completely reliable. The concentrations of cytokinins and ABA could be reduced in roots of rusted plants due to a reduction in the biosynthesis of these compounds in the roots or to an increase in translocation of these compounds to other parts of the plant. An examination of levels of cytokinins and ABA in sap extracts revealed that rust infection led to reductions in the concentration of all compounds studied, and no ABA was detected in any sap from rusted leek plants by the end of the experiment. Again, if the synthesis of these compounds in the roots is reduced following rust infection, less will be available for transport in the xylem. It is interesting that evidence has been presented to suggest that ABA is exported from leaves of vine to the roots and that this then moves back to the leaves (Loveys, 1984). It is possible, therefore, that the high ABA content observed in uninfected leaves of rusted leeks, coupled with the absence of this compound in xylem sap from rusted plants, is the result of a retention of ABA within the leaf. It is tempting to suggest that the compound could be compartmentalised within these particular leaves, although we have no direct evidence for this. However, research by Meidner (1975) and work with tracers added to the transpiration stream (Maier-Maercker, 1980) suggest that the walls of the epidermal cells adjacent to the guard cells and the guard cells themselves may be important evaporation sites. It seems, therefore, that a proportion of ABA arriving from the roots will arrive directly in the apoplast adjacent to the guard cell, which is now recognised as the site of action of ABA on stomata (Hartung, 1983; Hornberg and Weiler, 1984). The large increases in ABA in uninfected tissues of rusted leek plants may, therefore, be a consequence of accumulation and retention of the compound within the

tissue, as opposed to an increased biosynthesis of the compound **in situ.**

A detailed examination of discrete regions of a diseased plant is important for a variety of reasons. Generally, little attention has been paid in the past to healthy tissues of infected plants, and many studies have not distinguished between healthy and diseased parts when measurements were made. Since even heavily infected leek plants have some uninfected tissues, and individual rusted leaves have some uninfected regions, examinations of the type described in this section are essential for a better understanding of this particular pathosystem.

SECTION 6

# Shoot:root Interrelationships in Leeks Infected With Rust: Growth and Nutrient Relations

## INTRODUCTION

All green plants require the same basic set of mineral nutrients and the various elements are used by different plants for essentially similar ends. These elements are present in the soil solution, quite often in very low concentrations, and the fact that roots are able to accumulate these ions to comparatively high concentrations in their tissues illustrates their efficiency as absorbing organs. The plant cell is not only capable of absorbing nutrients against a concentration gradient, but also of preferentially absorbing selected ions, this selectivity being a property of the plasma membrane (Clarkson, 1974).

### Metabolic Functions of Minerals

Nitrogen is central to normal plant growth and development, and is an essential constituent of many organic compounds. It s importance will be examined in Section 7. Of all the other nutrients, $K^+$, generally speaking, is the most mobile element in plants, and does not occur as part of any stable organic compound. Research has shown that both $K^+$ and $Na^+$ are present as free ions, and are not bound to any protoplasmic constituents (Palmer and Gulati, 1976). Potassium serves at least two roles: it is an important osmotic constituent of cell sap and is an activator or cofactor of many enzymes including ATPases. The transport of $K^+$ appears to be essential for the motor activity in opening and closing of guard cells (Fujino, 1967; Humble and Raschke, 1971).

Although $Ca^{2+}$ must be considered as a macronutrient, the concentration of free ions in the protoplast may be quite low (Jones and Lunt, 1967). A large proportion of the $Ca^{2+}$ accumulated may be

as pectate salts in cell walls or as insoluble salts within cells, e.g. Ca-oxalate crystals. Since these crystals are membrane-bound (Schotz, Diers and Bathelt, 1970) they may represent a process regulating the amount of free $Ca^{2+}$. Calcium is quite immobile, not being readily translocated in the phloem, and consequently can have a very uneven distribution in the plant (Loneragan and Snowball, 1969). Perhaps the most important function of $Ca^{2+}$ is in stabilizing cell membranes, although the exact mechanism by which this is achieved is unknown. It has been shown that $Ca^{2+}$ may promote the absorption of other ions, e.g. $K^{+}$ (Vietts, 1944). With the exception of the enzymes involved in the hydrolysis of ATP and phospholipids, $Ca^{2+}$ tends to inhibit some enzymatic processes which require $Mg^{2+}$ nonspecifically (Evans and Sorger, 1966).

Magnesium appears to be a non-specific activator of many enzymes (Evans and Sorger, 1966), including most ATPases and other enzymes involved in the transfer of phosphate (Epstein, 1972). It is also a constituent of chlorophyll, and about half the $Mg^{2+}$ in a leaf is in the chloroplasts (Stocking and Ongun, 1962). Phosphorous is an exceptionally mobile element being transported in both xylem and phloem. Distribution within the plant is determined by properties of the source and sink rather than those of the transport system (Bieleski, 1973). Like nitrogen, phosphorous is a major component of many organic compounds, including sugar phosphates, nucleic acids, phospholipids and co-enzymes (Evans and Sorger, 1966).

**Uptake and Translocation of Minerals**

Elements reach the root surface from the soil following ionization in the soil solution. These ions can then move to the

root surface in the soil solution by means of either mass flow or by diffusion (Barber, 1962; Drew, Vaidyanathan and Nye, 1967). Whereas mass flow can supply the plant's need for both magnesium and calcium (Barber, Walker and Vasey, 1962), the supply of other ions including potassium could well be mainly by diffusion processes (Tinker, 1969).

An appreciable volume of the root exterior to the endodermis is accessible to ions which enter by diffusion or mass flow. This "free space" is normally regarded as comprising the cell walls and any water-filled intercellular spaces (apoplast), but not the cytoplasm (symplast). The cell walls bear fixed negative charges that cause an asymmetric distribution of ions within the free space as a whole. The ready physical movement of ions into the free space explains why a rapid initial uptake of ions occurs for a period, when roots are transferred from a solution of lower to higher concentration. This is followed by a slower steady uptake, the rate of which is controlled by movement into the symplast.

Active ion transport has been described as the movement of ions against their electrochemical gradient, whereas passive transport occurs when ions are moving down the electrochemical gradient (Nobel, 1970; Hodges, 1973; Luttge, 1973). The two possible physical driving forces involved in ion transport are (1) concentration gradients and (2) electrical gradients, since the ions carry an electrical charge (Clarkson, 1974). The origin of the electrical potential across membranes is probably due to a combination of the processes of diffusion, absorption by fixed charges and active electrogenic transport. Diffusion potentials may arise from a difference in mobility of ions in a membrane or by

differences in the relative permeability of a membrane to various ions, whereas fixed charges are due to a dissociation of organic molecules being held within the cell. Electrogenic transport involves active transport whereby a net charge is transferred across a membrane at the expense of metabolic energy.

An important question to be asked is how ions move from cell to cell across the root. In penetrating the root centripetally, the first tissues crossed are the epidermis and the cortex. The apoplast of cortical cells can be penetrated by water and ions from the external medium as an apparent free space, and thus the plasmalemmas of all cortex cells provide a highly enlarged surface for active ion uptake into the root symplast. The rate-limiting step for ion transport in the symplast is thought to be the plasmodesmatal transfer of ions. It has been demonstrated that symplastic transport is sufficiently rapid to account for the bulk of cortical cell transport, and that the transport of ions in the symplast involves a two-way exchange of symplastic and vacuolar ions (Arisz, 1956).

The way in which ions move into the xylem vessels has been debated for some time. Early research indicated that ions in the symplast diffuse into the apoplast and from there to the xylem vessels. This was linked with a reduction in the oxygen tension within the stele inducing a "leakiness" of the parenchyma cell to ions (Crafts and Boyer, 1938). Electrochemical measurements have also been used to support "leakiness" as the mechanism for release from the symplast. Dunlop and Bowling (1978) determined the electrochemical activity of $K^+$ in cells across the cortex and stele, and found no evidence for active transport into the xylem vessels.

Their results were criticized on technical grounds, and it is now generally accepted that ion transfer from xylem parenchyma to xylem vessels is due to the active release of ions (Lauchli, Pitman, Luttge, Kramer and Ball, 1978). This is further supported by evidence that plasmalemmae of xylem parenchyma cells show potassium-stimulated ATPase activity (Pitman, 1977; Winter-Sluiter, Lauchli and Kramer, 1977).

Several factors will probably influence the regulation of ion uptake and transport. It has been suggested that potassium transport in roots of barley (Glass and Dunlop, 1979) is regulated by allosteric control of potassium-carrier activity by the concentration of cytoplasmic potassium. It has been shown that the influx of potassium into barley roots is adjusted rapidly as the internal concentration of potassium rises (Glass and Dunlop, 1979). The rate of entry of nitrate and phosphate into the plant may depend on feedback control of the transport system by an organic rather than an inorganic pool, since these ions are assimilated, to a large extent, into organic compounds in the cytoplasm (Clarkson and Hanson, 1980). The absorption of phosphate, for example, is in the form of the dihydrogen ion ($H_2PO_4^-$) and is metabolically dependent, involving three stages: (1) esterification at root entry; (2) utilization in metabolism of root tissues; (3) hydrolysis of organic phosphates to inorganic phsophates before or at entry into the xylem (Bieleski, 1973; Loughman, 1976; Loughman and Russell, 1957).

There are numerous, although often contradictory, reports that growth regulators influence the rates of absorption and transport of ions either directly, because of some interaction with membranes (e.g. Lea and Collins, 1979), or indirectly, through effects on

metabolism. Cram and Pitman (1972) provided evidence for a regulatory mechanism in plants under water stress. Thus, abscisic acid (ABA) inhibited the transport of potassium and chloride from roots to shoots in maize and barley and the authors suggested that ABA levels in the leaf may be increased by water stress. This was shown to be the case (Wright and Hiron, 1969; Wright, 1977) and, coupled with the fact that ABA can be translocated from the shoot to the root (Hocking **et al**, 1972), suggests that ABA could control a negative feedback system regulating ion levels in the shoot. Cytokinins may also help in the regulation of ion uptake by roots and the subsequent transport of these ions into the xylem. Many of the reports, however, yield contradictory evidence, and it appears that cytokinins may both promote and inhibit ion uptake (Van Steveninck, 1972, 1974; Ilan, 1971; Collins, 1974).

**Uptake and Utilization of Minerals by Diseased Plants**

Pathogens are known to alter host nutrient status and metabolism in a variety of ways (Durbin, 1967; Walters, 1985). A familiar alteration in the solute distribution pattern in infected leaves is an accumulation of nutrients at infection sites, which ensures an abundant supply of nutrients for fungal development (Shaw and Samborski, 1956; Shaw, 1961; Thrower, 1965). In many cases, e.g. rusted bean, it appears that host nutrients are translocated out of younger, uninfected leaves to infected leaves (Zaki and Durbin, 1965; Poszar and Kiraly, 1966). However, in rusted bean, calcium accumulation at infection sites is the result of decreased movement away from the infection site as opposed to increased movement toward it. It was also shown that infection led to a decrease in the amount of calcium moving into the leaf (Durbin,

1967). Wieneke, Covey and Benson (1971) reported accumulation of $^{35}S$ at the infection sites of **Podosphaera leucotricha** on apple leaves, although no $^{45}Ca$ was detected. Since the conidia produced were radioactive, the $^{45}Ca$ was available to the fungus. On the other hand, levels of nitrogen, phosphorous and potassium were decreased in leaves of **Coccinea cordifolia** infected with powdery mildew (Jamal and Khan, 1976).

The uptake and distribution of minerals is affected by several factors, including the water relations of the host plant. It is generally agreed that rust diseases eventually cause an increase in transpiration per unit area of leaf (Ayres, 1978). This is due to non-stomatal water loss because it only occurs after the rust pustule erupts. Ahmad, Owera, Farrar and Whitbread (1982) indicated that there was an increase in the content of various cations in barley infected with brown rust, and suggested that this may have been due to an increased transpiratory flux following sporulation. These authors concluded that the accumulation of cations in the barley/rust combination appeared to be due primarily to a reduction in the export of phloem-mobile ions. However, this does not preclude the possibility of a local redistribution within the leaf, including ion accumulation in pustules. Similar results were reported by Paul and Ayres (1987a,b), where increased ion uptake and concentrations were found in the groundsel - **Puccinia lagenophorae** system.

Although the effects of rust and mildew on host physiology are not directly comparable, much of the literature is concerned with the effects of powdery mildew on the nutrient relations of the host plant. Several workers (Comhaire, 1963; Fric, 1978; Walters and

Ayres, 1981) have shown that in compatible host-mildew combinations, the phosphate concentration of infected tissues increases. However, there are still contradictions in the evidence. Whereas Comhaire (1963) found that in roots of mildewed wheat more $^{32}P$-labelled phosphate was **absorbed** than in uninfected controls, Fric (1978) observed reduced rates of phosphate absorption in mildewed barley. More recently, however, Walters and Ayres (1981) showed that uptake and translocation of $^{32}P$-labelled phosphate, by roots of mildewed barley, was much increased compared to healthy controls.

In work on rust-infected leeks (Roberts and Walters, 1987; Section 7), increases were found in the concentrations of total nitrogen and nitrate-nitrogen, although ammonium-nitrogen concentration was decreased. Although an increased nitrate concentration was also found in leaves of rye infected with **Puccinia recondita** (Piening, 1972), total nitrogen concentration decreased in leaves of brown rust-infected barley (Ahmad **et al**, 1982). The increased ion uptake and concentrations found in several pathosystems (e.g. Ahmad **et al**, 1982; Paul and Ayres, 1987a,b; Walters, 1981), occurred despite increased shoot:root ratios. This has prompted an examination of the relationship between shoots and roots, and the possibility that a functional equilibrium may exist in diseased plants.

**Root:Shoot Interrelationships**

In terms of both structure and function, roots and shoots are so different that they may be considered as two separate systems within the intact plant. Despite this, these two apparently different systems are integrated physiologically, with the shoot collecting resources as the basis for energy supply, while the root

system absorbs mineral ions and water (Brouwer, 1963). Our understanding of this system is poor, but recent research has highlighted the existence of a functional equilibrium between the root and shoot systems within the intact plant. Such a relationship was originally proposed by Davidson (1969), who suggested that the relationship could be expressed by:

root mass x rate (absorption) $\propto$ leaf mass x rate (photosynthesis) (1)

This equation was rearranged by Hunt (1975) to:

$$Wr/Ws \propto 1/(SAR/USR) \quad (g\,g^{-1}dw^{-1}d^{-1}) \tag{2}$$

$$\text{Mass Ratio} \propto 1/\text{Activity Ratio} \tag{3}$$

where Wr and Ws are the dry weights of the root and shoot respectively, and SAR and USR are the specific absorption rate and unit shoot rate.

Subsequently, Thornley (1975) simplified Eqn (2) to:

$$\Delta M = fm\Delta W \tag{4}$$

where, during a given time period, $\Delta M$ is an increment in weight of a given element M, $\Delta W$ is an increment in total plant dry weight and fm is a constant if a functional equilibrium does exist. It should be noted that M can be a single element or compound, or a group of elements or compounds.

Following Thornley's (1975) approach, a functional equilibirum has been demonstrated for both nutrient and water uptake in young peach trees (Richards, 1977; 1978). Richards (1977) artificially increased the shoot:root ratio (S:R) of young peach trees and found

that there was a corresponding increase in the efficiency of water uptake per unit root length, suggesting that the root seemed to respond to shoot demand. These results agreed with Thornley's (1975) hypothesis that a decrease in root mass should be accompanied by an increase in specific activity of the roots.

Information hinting at the possible maintenance of a functional equilibrium in plants infected with biotrophic pathogens is scant. However, based on the results of several authors, Walters (1985) suggested that plants infected with biotrophic fungi might be able to maintain a functional equilibrium with respect to shoot and root activity.

In view of the contradictions in the literature concerning nutrient uptake and metabolism an examination was made of the uptake, content and concentration of nitrogen, phosphate, potassium and calcium in rust-infected leek plants. Because of the paucity of data on functional equilibria in infected plants, it also seemed prudent to examine the possibility that in these plants, a functional equilibrium with respect to nutrient uptake may be maintained.

## MATERIALS AND METHODS

### Plant Material

Leeks (**Allium porrum** L. var. Autumn Mammoth) were grown from seed and inoculated as described in Section 2 - General Materials and Methods. Plants to be infected were transferred to a separate glasshouse at the sixth leaf stage, where they were inoculated to give different levels of infection, i.e. 10, 20 and 30%

approximately. These intensities of infection were obtained by inoculating varying areas of one, two or three of the lower leaves with rust. Differences in intensity of infection were used as a means of altering shoot:root ratio in order to allow an examination of shoot:root functional equilibria. Previous workers (e.g. Richards, 1977) used root excision as a means of changing shoot:root ratio prior to investigating functional equilibria. Plants were fed twice weekly with Long Ashton solution, and were reinoculated throughout the experiment to maintain the different intensities of infection.

**Ionic Content of Tissues**

Plants were harvested at intervals between two and eight weeks after inoculation, and divided into shoot and root material. Plant material was dried to constant weight in a forced-draught oven (Gallenkamp). The material was then ground in a knife mill, followed by digestion in nitric, perchloric and sulphuric acids. Cations were determined using a sequential inductively coupled plasma emmision spectrophotometer (Thermoelectron Plasma 100). Phosphate was determined using a mixed reagent of the following composition: 125 $cm^3$ $5NH_2SO_4$; 37.5 $cm^3$ ammonium molybdate (40 g $l^{-1}$); 75 $cm^3$ ascorbic acid and 12.5 $cm^3$ potassium antimonyl tartrate. An aliquot of the sample was diluted to 40 $cm^3$ in a 50 $cm^3$ volumetric flask and 8 $cm^3$ of the mixed reagent was added. The volume was then made up with distilled water. The flasks stood for 10 min before the optical density was read at 882 nm on an LKB spectrophotometer. Standard curves were prepared from potassium phosphate (see Appendix I).

Nutrient uptake was calculated as specific absorption rate (SAR) on the basis of root dry weight (see Evans, 1972). Functional equilibria with respect to nutrient uptake were examined using the simplified equation of Thornley (1975);

$$\triangle M = fm \triangle W \qquad (4)$$

where, during a given time period, $\triangle M$ is an increment in weight of element M, $\triangle W$ is an increment in total plant dry weight and fm is a constant if an equilibrium does exist.

## RESULTS

### Growth of Healthy and Rust-infected Leeks

All levels of rust infection led to significant reductions in the dry weights of both shoot and root and hence in total plant dry weight over the entire experimental period (Figure 17). Root dry weight was reduced more than shoot dry weight in rusted leeks two weeks after inoculation, leading to an increase in the shoot:root ratio (S:R). However, from three weeks after inoculation until the end of the experiment, shoot dry weight in infected plants was reduced slightly more than root dry weight and hence S:R fell to below control values (Figure 18).

### Uptake and Distribution of Nitrogen

The nitrogen contents of plants with 20% and 30% rust infection were significantly reduced in comparison with healthy controls from two to five weeks after inoculation ($P < 0.01$; Table 14). In plants with 10% infection, nitrogen contents were initially slightly higher than control values, but by four and five weeks after inoculation, rusted leeks contained less nitrogen than control plants ($P<0.01$).

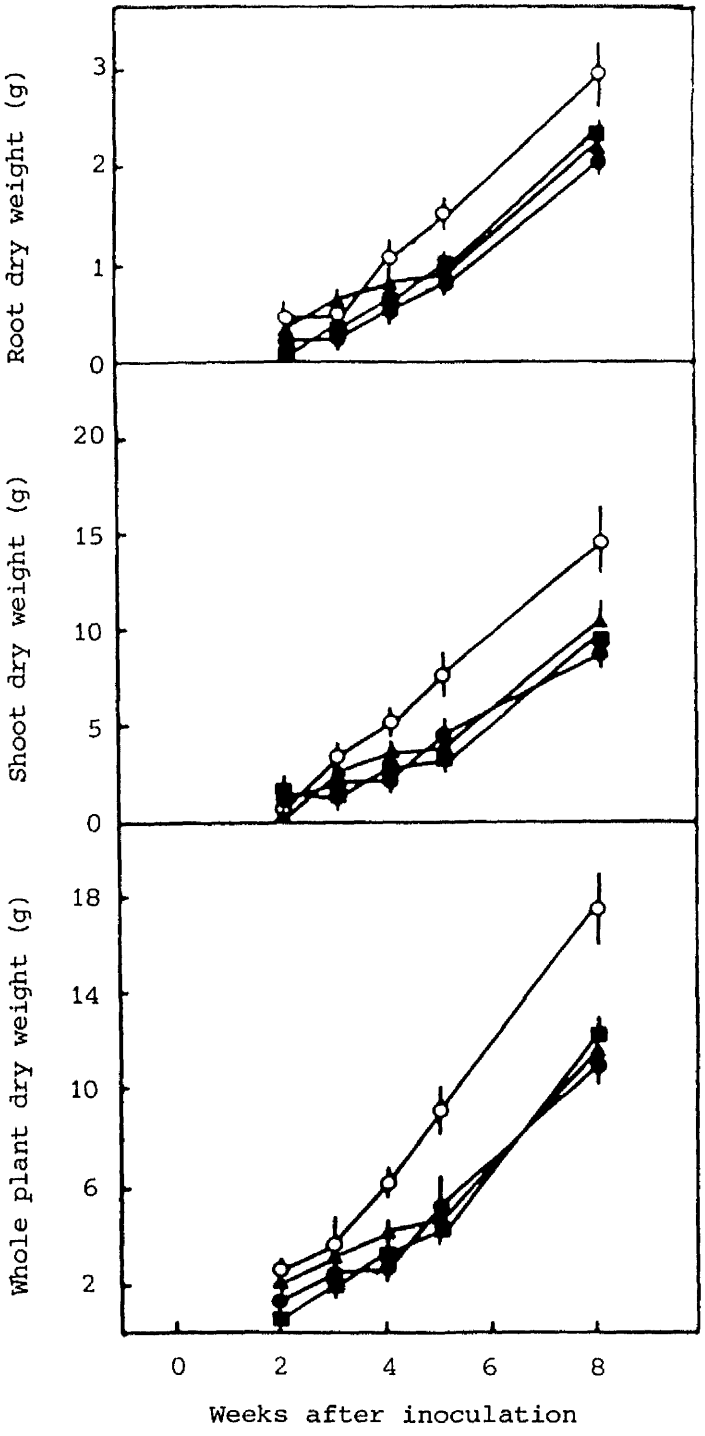



Figure 17. The effect of different levels of rust infection on root, shoot and whole plant dry weight in leeks. Infection levels given by (▲) 10%; (■) 20%; (●) 30%. Healthy controls (○). Each point is the mean of four replicates with standard error.

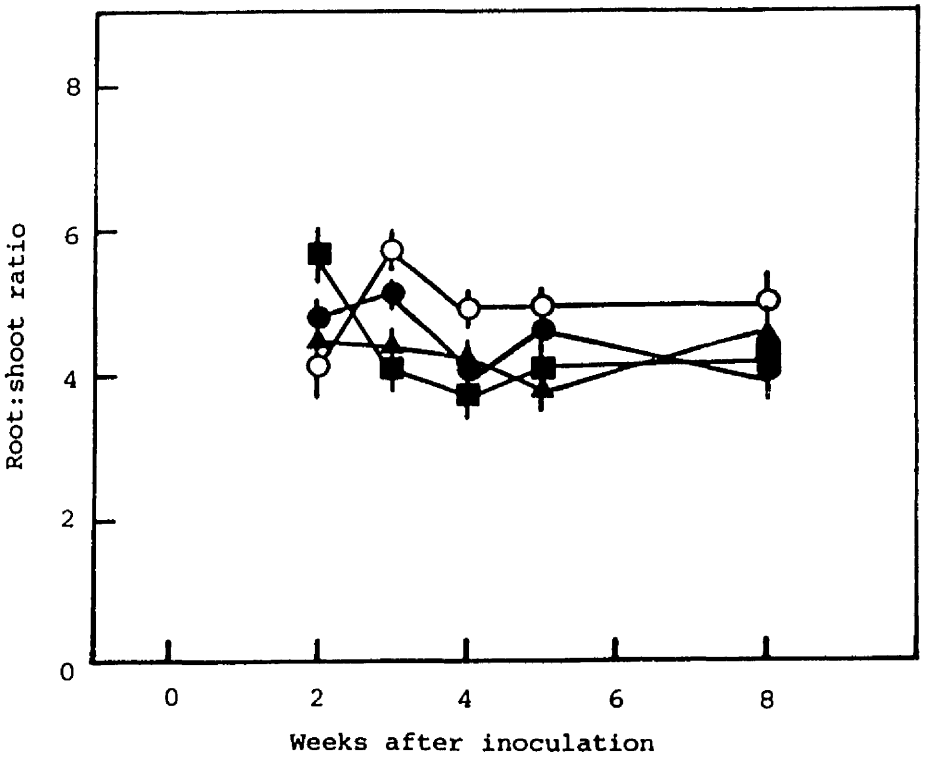



Figure 18. The effect of different levels of rust infection on shoot:root ratio in leeks. Infection levels given by (▲) 10%; (■) 20%; (●) 30%. Healthy controls (○). Each point is the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 96.9 ± 7.73 | 126.7 ± 12.22 | 210.0 ± 12.64 | 261.9 ± 18.15 | 334.2 ± 23.86 |
| 10% rust | 100.4 ± 7.00 | 133.5 ± 6.64 | 176.8 ± 6.92 | 214.7 ± 8.55 | 457.6 ± 22.92 |
| 20% rust | 57.3 ± 5.70 | 110.1 ± 10.54 | 148.5 ± 9.01 | 180.9 ± 9.50 | 440.7 ± 17.49 |
| 30% rust | 65.5 ± 3.93 | 112.2 ± 7.32 | 150.2 ± 13.26 | 200.8 ± 14.43 | 331.6 ± 15.78 |
| Concentration (mg/g) | | | | | |
| Control | 84.7 ± 6.90 | 65.0 ± 3.01 | 62.2 ± 4.11 | 50.7 ± 3.55 | 41.6 ± 3.01 |
| 10% rust | 84.0 ± 5.73 | 80.8 ± 3.13 | 80.0 ± 3.13 | 77.8 ± 3.07 | 60.3 ± 3.03 |
| 20% rust | 87.8 ± 10.05 | 93.3 ± 9.20 | 84.0 ± 5.43 | 72.0 ± 3.72 | 65.3 ± 2.57 |
| 30% rust | 89.0 ± 5.13 | 80.4 ± 5.18 | 89.9 ± 8.12 | 70.3 ± 5.82 | 64.9 ± 3.15 |
| SAR (mg $g^{-1}$ $d^{-1}$) | | | | | |
| Control | 15.5 ± 1.21 | 7.8 ± 0.62 | 11.2 ± 0.69 | 4.8 ± 0.33 | 1.2 ± 0.08 |
| 10% rust | 19.2 ± 0.96 | 7.8 ± 0.38 | 7.9 ± 0.32 | 5.8 ± 0.24 | 5.1 ± 0.23 |
| 20% rust | 22.6 ± 2.03 | 17.5 ± 1.57 | 8.2 ± 0.57 | 4.9 ± 0.24 | 5.4 ± 0.21 |
| 30% rust | 19.1 ± 1.15 | 16.5 ± 0.99 | 8.7 ± 0.78 | 7.8 ± 0.62 | 2.8 ± 0.14 |

Table 14. The effect of different levels of rust infection on the amount, concentration and specific absorption rate (SAR) of nitrogen in whole leek plants. All values are the mean of four replicates with standard error.

However, by the end of the experiment, at eight weeks after inoculation, rusted plants (10% and 20% infection) contained significantly more nitrogen than controls ($P<0.01$); no significant difference in the nitrogen content of plants with 30% infection could be found at eight weeks after inoculation (Table 14).

A very similar pattern of changes was observed for shoot nitrogen content of rusted and healthy plants (see Appendix II, Table 1). Thus, two weeks after inoculation, shoot nitrogen content was significantly ($P<0.01$) reduced in plants with 20% and 30% infection, while a significant increase in the amount of nitrogen was observed in shoots of plants with 10% rust infection. By eight weeks after inoculation however, the nitrogen content of rusted shoots was greater than values for controls. Significant reductions in the nitrogen contents of roots of all rusted plants were observed two weeks after inoculation ($P<0.001$). For the remainder of the experiment, the nitrogen contents of roots of both rusted and control plants were similar and not significantly different (see Appendix II, Table 2).

Two weeks after inoculation, whole plant nitrogen concentrations of infected leeks were not significantly different from values observed in healthy controls (Table 14). Thereafter, nitrogen concentrations in all infected plants were significantly greater than controls ($P<0.001$, Table 14). Shoot nitrogen concentrations for the different rust treatments were significantly greater than controls for the entire experiment ($P<0.001$), see Appendix II, Table 1). Apart from the first harvest at two weeks after inoculation, where nitrogen concentrations in the roots of all of the rusted plants were reduced compared to controls, nitrogen

concentrations in roots of infected plants were always significantly greater than controls ($P<0.001$; see Appendix II, Table 2).

Leeks infected with different intensities of rust showed significantly greater SAR's for nitrogen than did healthy plants for most of the experimental period ($P<0.001$; Table 14). The only deviation from this trend occurred at four weeks after inoculation, when SAR's for nitrogen in rusted plants were slightly lower than, but not significantly different from, values obtained for controls (Table 14).

**Uptake and Distribution of Phosphate**

The amount of phosphate found in rust-infected leeks was usually significantly less than found in healthy controls ($P<0.01$; Table 15). However, at two, three and eight weeks after inoculation, the phosphate contents of plants with 10% infection were slightly greater than controls (Table 15). A greater phosphate content was observed in shoots of rusted plants for most of the experiment ($P<0.001$; see Appendix II, Table 3), although at two and three weeks after inoculation phosphate contents in plants with 10% infection were very similar to controls (Table 15) and at eight weeks after inoculation, a slight increase in phosphate content was detected in shoots of these plants (see Appendix II, Table 3).

The amount of phosphate found in roots of all rusted plants was significantly less than values found in controls for most of the experiment ($P<0.01$; see Appendix II, Table 4). Only at three weeks after inoculation was there a deviation from this trend; here, roots of plants showing 10% rust infection contained very similar amounts of phosphate when compared to controls (see Appendix II, Table 4).

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 12.3 ± 0.65 | 18.6 ± 1.60 | 30.7 ± 1.87 | 43.7 ± 4.52 | 85.8 ± 9.24 |
| 10% rust | 12.0 ± 1.48 | 18.8 ± 1.12 | 25.3 ± 1.70 | 29.2 ± 1.97 | 98.6 ± 8.51 |
| 20% rust | 6.7 ± 0.96 | 12.1 ± 1.00 | 18.9 ± 0.86 | 27.8 ± 2.01 | 72.7 ± 3.82 |
| 30% rust | 7.4 ± 0.70 | 13.9 ± 0.85 | 17.8 ± 2.67 | 31.9 ± 1.94 | 68.3 ± 4.13 |
| Concentration (mg/g) | | | | | |
| Control | 104.1 ± 1.87 | 100.7 ± 2.18 | 100.0 ± 3.17 | 93.6 ± 5.12 | 96.1 ± 1.70 |
| 10% rust | 112.3 ± 2.41 | 113.9 ± 0.97 | 118.0 ± 1.44 | 115.1 ± 1.46 | 112.9 ± 0.92 |
| 20% rust | 113.9 ± 3.64 | 108.3 ± 2.19 | 113.4 ± 1.46 | 114.1 ± 2.43 | 114.4 ± 0.97 |
| 30% rust | 108.8 ± 1.29 | 108.8 ± 1.14 | 109.0 ± 2.19 | 114.6 ± 2.68 | 113.4 ± 1.21 |
| SAR (mg $g^{-1}$ $d^{-1}$) | | | | | |
| Control | 1.9 ± 0.16 | 1.7 ± 0.16 | 1.6 ± 0.09 | 1.2 ± 0.07 | 0.7 ± 0.05 |
| 10% rust | 2.3 ± 0.16 | 1.6 ± 0.06 | 1.2 ± 0.05 | 0.6 ± 0.02 | 1.5 ± 0.07 |
| 20% rust | 2.6 ± 0.26 | 1.8 ± 0.16 | 1.5 ± 0.08 | 1.3 ± 0.06 | 0.9 ± 0.03 |
| 30% rust | 2.2 ± 0.13 | 2.3 ± 0.14 | 0.9 ± 0.07 | 2.2 ± 0.17 | 0.8 ± 0.04 |

Table 15. The effect of different levels of rust infection on the amount, concentration and specific absorption rate (SAR) of phosphate in whole leek plants. All values are the mean of four replicates with standard error.

Phosphate concentrations in rusted leeks were significantly greater than controls for the entire experiment ($P<0.001$; Table 15). A similar trend was obtained for shoot and root phosphate concentrations (see Appendix II, Tables 3 and 4). The trends in SAR's for phosphate, however, were not so clear cut (Table 15). Here, whereas SAR's for phosphate in rusted plants were greater than controls at two, three and eight weeks after inoculation, a reduced SAR for phosphate was observed in infected plants compared to controls, in the middle of the experimental period, four and five weeks after inoculation ($P<0.01$, Table 15). However, it should be noted that at five weeks after inoculation, plants with 30% rust infection exhibited a much greater SAR for phosphate ($P<0.001$) than did controls (Table 15).

**Uptake and Distribution of Potassium**

The potassium content of rusted plants was usually significantly reduced in comparison with values for healthy controls ($P<0.01$; Table 16). However, two and eight weeks after inoculation, the potassium content of plants with 10% infection were greater than controls (Table 16). A similar trend was obtained for shoot potassium content (see Appendix II, Table 5). The situation for root potassium content was more complex. Two, four and eight weeks after inoculation, the potassium content of roots of infected plants were significantly lower than controls ($P<0.001$; see Appendix II, Table 6). However, significant increases in root potassium content were observed in rusted plants compared to controls, three and five weeks after inoculation ($P<0.01$).

Two weeks after inoculation, the potassium concentrations in rusted plants were not significantly different from control values.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 176.1 ± 13.65 | 209.7 ± 20.69 | 368.7 ± 21.70 | 442.6 ± 31.27 | 781.1 ± 57.17 |
| 10% rust | 169.4 ± 11.52 | 243.2 ± 10.14 | 310.7 ± 9.87 | 344.9 ± 12.79 | 920.7 ± 46.12 |
| 20% rust | 88.5 ± 9.57 | 187.7 ± 16.89 | 267.5 ± 16.98 | 310.2 ± 15.14 | 691.9 ± 27.11 |
| 30% rust | 99.8 ± 5.93 | 183.3 ± 11.12 | 234.3 ± 21.30 | 347.3 ± 27.19 | 464.1 ± 21.07 |
| Concentration (mg/g) | | | | | |
| Control | 168.8 ± 13.64 | 123.7 ± 12.70 | 135.5 ± 8.29 | 88.2 ± 6.25 | 95.4 ± 6.96 |
| 10% rust | 168.8 ± 10.93 | 158.9 ± 7.00 | 152.7 ± 5.96 | 144.2 ± 5.70 | 122.5 ± 6.20 |
| 20% rust | 163.3 ± 17.23 | 170.3 ± 16.76 | 170.2 ± 10.97 | 140.3 ± 7.44 | 111.5 ± 4.95 |
| 30% rust | 161.3 ± 9.46 | 142.7 ± 9.01 | 161.5 ± 14.53 | 136.6 ± 11.27 | 102.5 ± 4.95 |
| SAR (mg $g^{-1}$ $d^{-1}$) | | | | | |
| Control | 28.1 ± 2.25 | 8.8 ± 0.88 | 21.4 ± 1.28 | 6.8 ± 0.47 | 5.5 ± 0.38 |
| 10% rust | 32.4 ± 2.27 | 17.4 ± 0.69 | 12.4 ± 0.48 | 5.2 ± 0.16 | 12.1 ± 0.61 |
| 20% rust | 34.9 ± 2.79 | 32.9 ± 2.96 | 17.1 ± 1.16 | 6.4 ± 0.34 | 7.9 ± 0.31 |
| 30% rust | 29.2 ± 1.46 | 29.5 ± 1.94 | 11.6 ± 0.69 | 17.4 ± 1.39 | 2.5 ± 0.09 |

Table 16. The effect of different levels of rust infection on the amount, concentration and specific absorption rate (SAR) of potassium in whole leek plants. All values are the mean of four replicates with standard error.

However, for the rest of the experimental period, potassium concentrations in rusted plants were significantly greater than controls ($P<0.001$; Table 16). Shoot and root potassium concentrations exhibited similar trends (see Appendix II, Tables 5 and 6), although potassium concentrations in roots of rusted plants were not always significantly greater than controls.

Increased SAR's for potassium were observed in rusted leeks, two three and eight weeks after inoculation when compared with uninfected controls. A large increase in SAR for potassium was also found in leeks with 30% infection intensity, five weeks after inoculation (Table 16). SAR's for potassium were reduced in rusted plants four weeks after inoculation and in plants with 30% infection, eight weeks after inoculation (Table 16).

**Uptake and Distribution of Calcium**

The calcium contents of all rust-infected plants were reduced compared with controls, over the entire experimental period (Table 17). The only departure from this pattern was an increase in calcium content of plants with 10% and 30% rust infection, eight weeks after inoculation ($P<0.01$). A similar trend was found for the amount of calcium in shoots of rusted plants (see Appendix II, Table 7). Although root calcium content changed in a similar manner to shoot and total plant calcium, some deviation was evident. Thus, an increased calcium content was observed in plants with 10% infection three and four weeks after inoculation ($P<0.001$; see Appendix II, Table 8).

Calcium concentrations in rusted plants were significantly reduced in comparison with controls two weeks after inoculation

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 16.0 ± 1.27 | 26.0 ± 2.58 | 41.4 ± 2.49 | 57.9 ± 4.01 | 118.2 ± 8.59 |
| 10% rust | 14.8 ± 0.86 | 25.2 ± 2.27 | 29.0 ± 0.87 | 42.9 ± 1.35 | 172.2 ± 8.60 |
| 20% rust | 8.3 ± 0.80 | 19.0 ± 1.79 | 23.3 ± 1.41 | 39.0 ± 2.05 | 109.5 ± 3.44 |
| 30% rust | 8.5 ± 0.50 | 19.9 ± 1.21 | 21.0 ± 1.88 | 45.1 ± 3.61 | 135.9 ± 6.71 |
| Concentration (mg/g) | | | | | |
| Control | 15.5 ± 1.25 | 14.0 ± 1.36 | 11.9 ± 0.78 | 11.0 ± 0.76 | 11.7 ± 0.84 |
| 10% rust | 13.1 ± 0.87 | 15.7 ± 0.78 | 16.3 ± 0.64 | 15.4 ± 0.61 | 17.9 ± 0.90 |
| 20% rust | 13.2 ± 1.38 | 16.2 ± 1.57 | 13.7 ± 0.80 | 14.9 ± 0.79 | 16.3 ± 0.66 |
| 30% rust | 11.8 ± 0.64 | 16.8 ± 1.06 | 12.2 ± 0.92 | 16.1 ± 1.31 | 22.7 ± 1.06 |
| SAR (mg $g^{-1}$ $d^{-1}$) | | | | | |
| Control | 2.6 ± 0.20 | 2.6 ± 0.24 | 2.1 ± 0.12 | 1.5 ± 0.10 | 0.9 ± 0.07 |
| 10% rust | 2.8 ± 0.19 | 2.5 ± 0.12 | 0.7 ± 0.02 | 2.1 ± 0.08 | 2.7 ± 0.14 |
| 20% rust | 3.3 ± 0.37 | 3.8 ± 0.37 | 0.9 ± 0.05 | 2.4 ± 0.12 | 1.5 ± 0.04 |
| 30% rust | 2.5 ± 0.14 | 4.0 ± 0.24 | 0.3 ± 0.02 | 3.7 ± 0.29 | 1.9 ± 0.07 |

Table 17. The effect of different levels of rust infection on the amount, concentration and specific absorption rate (SAR) of calcium in whole leek plants. All values are the mean of four replicates with standard error.

($P<0.01$; Table 17). However, for the remainder of the experiment, the calcium concentrations in rusted plants were significantly greater than controls ($P<0.001$). Calcium concentrations in shoots of rusted plants were similar to controls from two to four weeks after inoculation, and significant increases in calcium concentrations in infected shoots were only observed towards the end of the experiment, five and eight weeks after inoculation ($P<0.01$, 0.001; see Appendix II, Table 7). The pattern of changes in root calcium concentrations was similar to that found in whole plants. Thus, the concentration of calcium in roots of infected plants was significantly greater than controls for most of the experiment ($P<0.01$). Only at two weeks after inoculation were calcium concentrations in roots of infected leeks less than controls ($P<0.001$; see Appendix II, Table 8).

SAR's for calcium were greater than healthy control values particularly towards the end of the experiment, five and eight weeks after inoculation ($P<0.001$; Table 17). However, two weeks after inoculation, although some SAR's for calcium were slightly greater than controls, the differences were not significant. Four weeks after inoculation, significant decreases in SAR's for calcium were observed in rusted plants compared to controls ($P<0.001$, Table 17).

**Functional Equilibria with Respect to Nutrient Uptake**

Figures 19-23 represent the relationship between $\Delta W$ and $\Delta M$, where $\Delta W$ is the increment in total plant dry weight and $\Delta M$ is the increment in the weights of individual minerals or a group of minerals, for the different rusted treatments and for controls. The relationship between dry weight accumulation and the uptake of individual and total nutrients was linear for plants of all

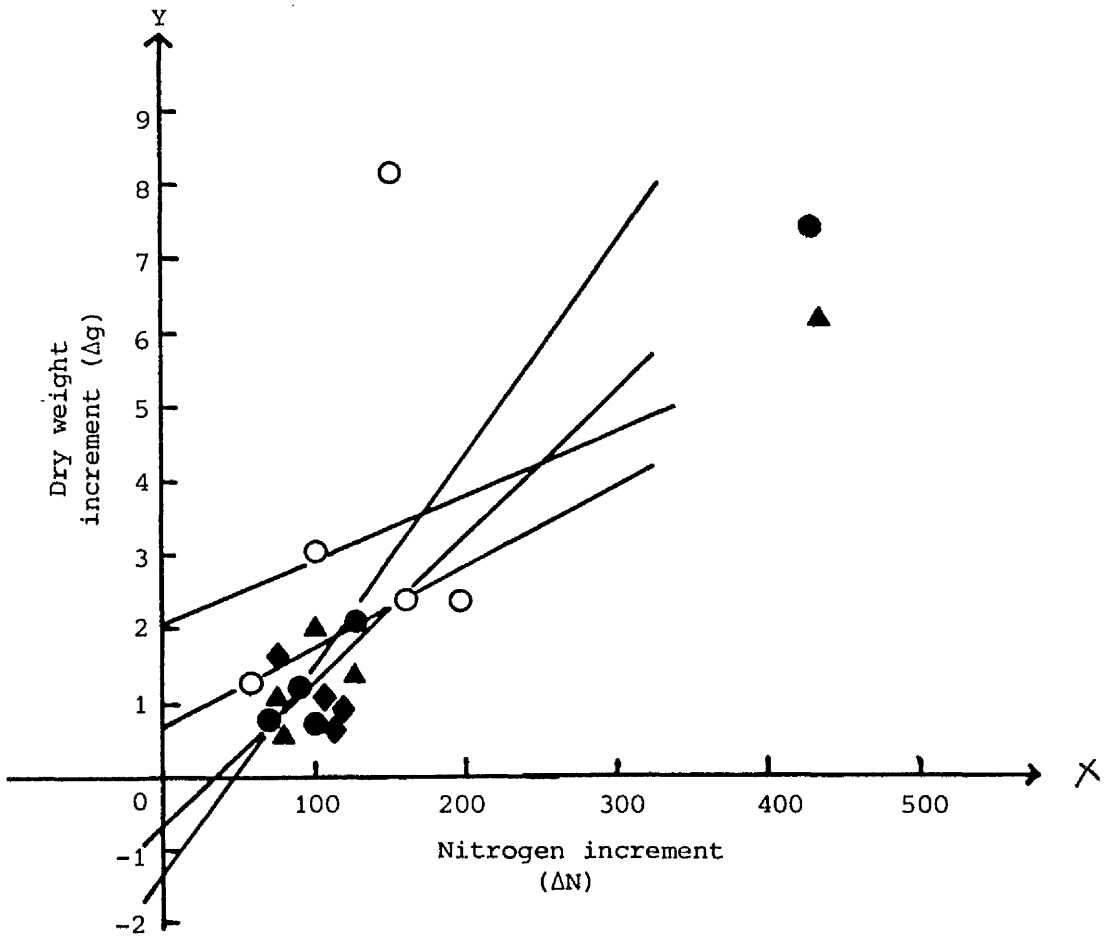



Figure 19. Functional equilibrium for nitrogen uptake in leeks infected with 10% rust (●), 20% rust (■) and 30% rust (▲). Controls (○). Equations of lines shown are 10%: y = 0.06x - 1.427; r = 0.87; 20%: y = 0.02x + 0.070, r = 0.99; 30%: y = 0.04x - 0.743; r = 0.99; Control: y = 0.02x + 2.085, r = 0.20.

**Regressions calculated for individual values**

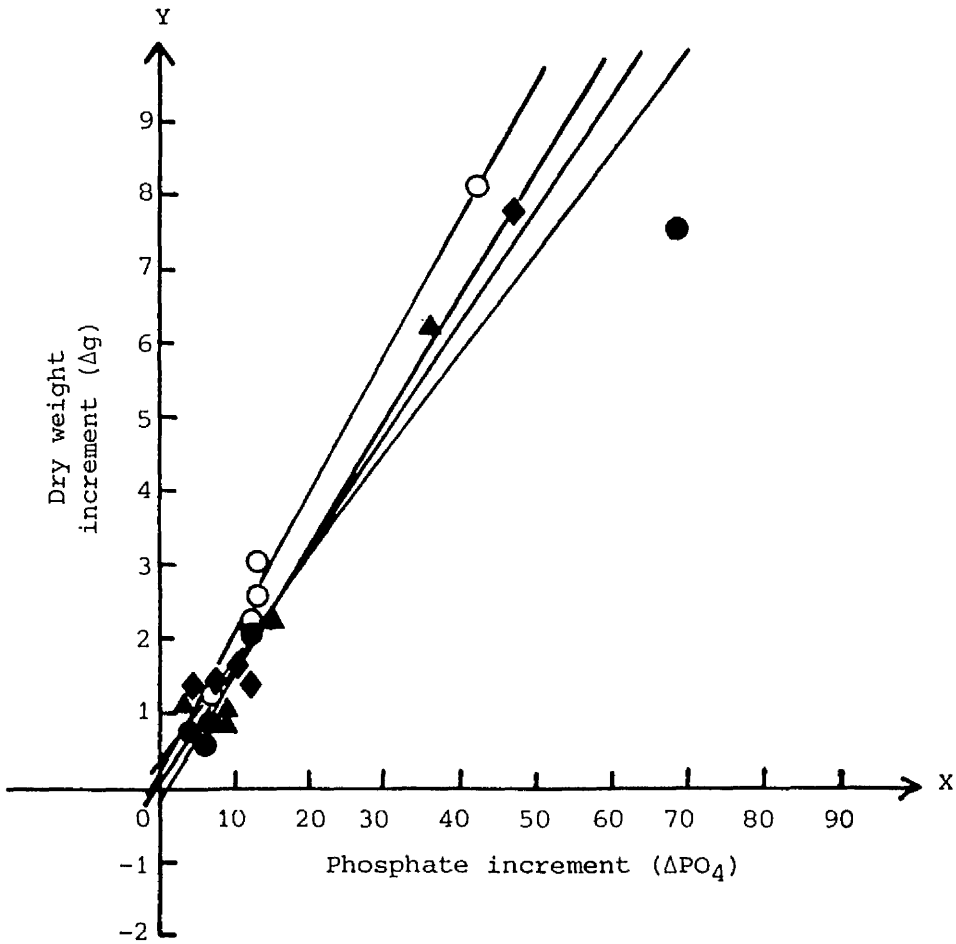



Figure 20. Functional equilibrium for phosphate uptake in leeks infected with 10% rust (●), 20% rust (■) and 30% rust (▲). Controls (○). Equations of lines shown are 10%: y = 0.10x + 0.492, r = 0.99; 20%: y = 0.16x + 0.033, r = 0.99; 30%: y = 0.17x - 0.037, r = 0.99; Control: y = 0.19x + 0.167, r = 0.99.

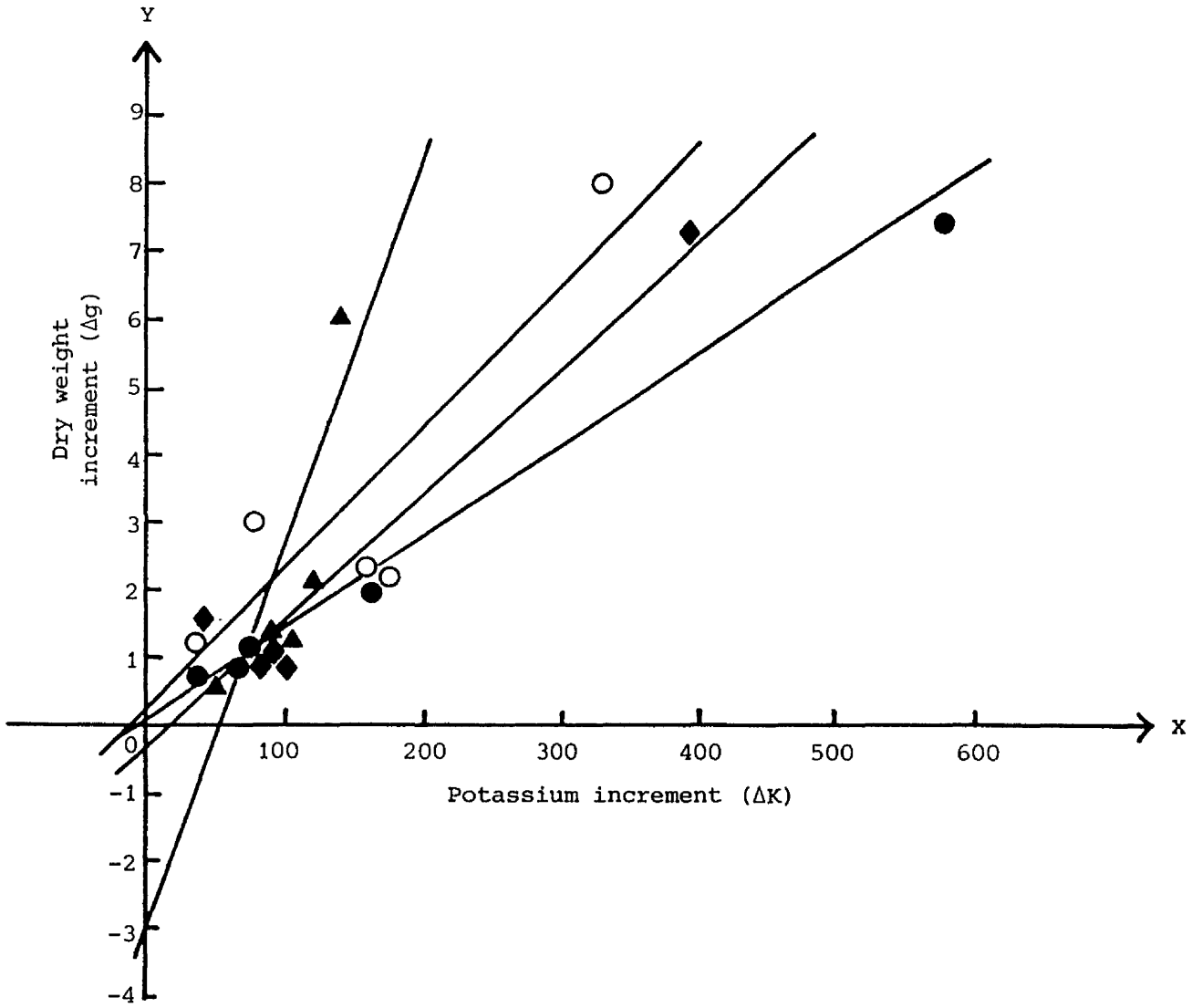



Figure 21. Functional equilibrium for potassium uptake in leeks infected with 10% rust (●), 20% rust (■) and 30% rust (▲). Controls (○). Equations of lines shown are 10%: y = 0.013x + 0.173, r = 0.99; 20%; y = 0.019x - 0.31, r = 0.98; 30%: y = 0.058x - 3.07, r = 0.67; Control: y = 0.021x + 0.252, r = 0.88.

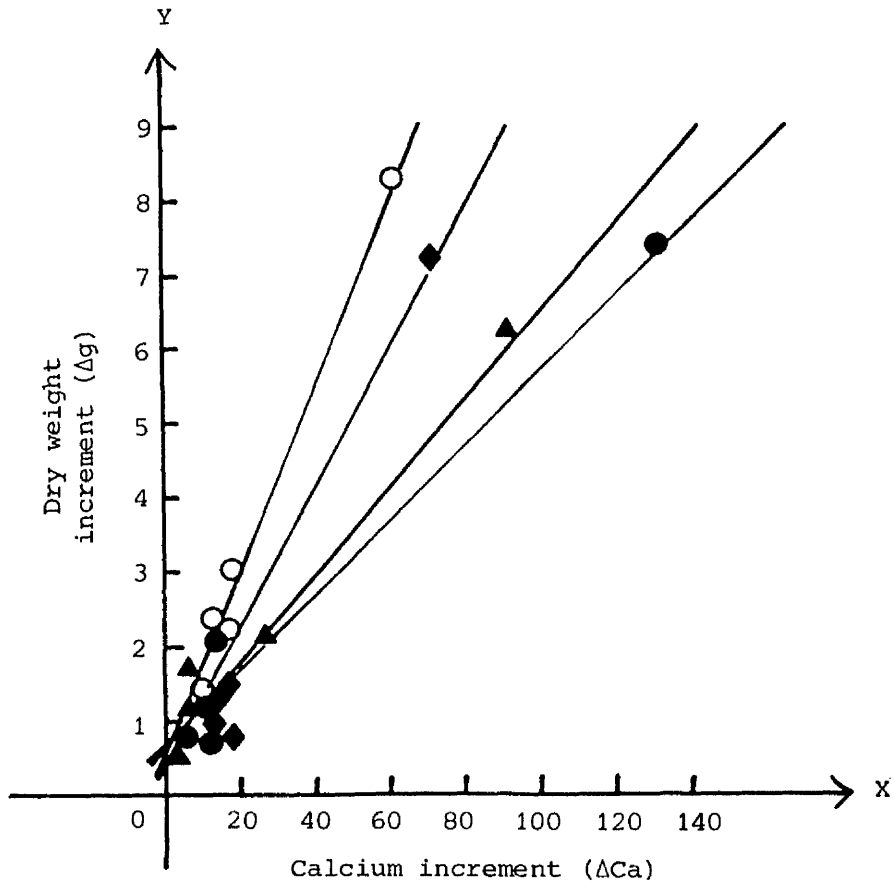



Figure 22. Functional equilibrium for calcium uptake in leeks infected with 10% rust (●), 20% rust (■) and 30% rust (▲). Controls (○). Equations of lines shown are 10%: y = 0.05x + 0.674, r = 0.98; 20%: y = 0.10x + 0.200, r = 0.99; 30%: y = 0.06x + 0.627, r = 0.99.

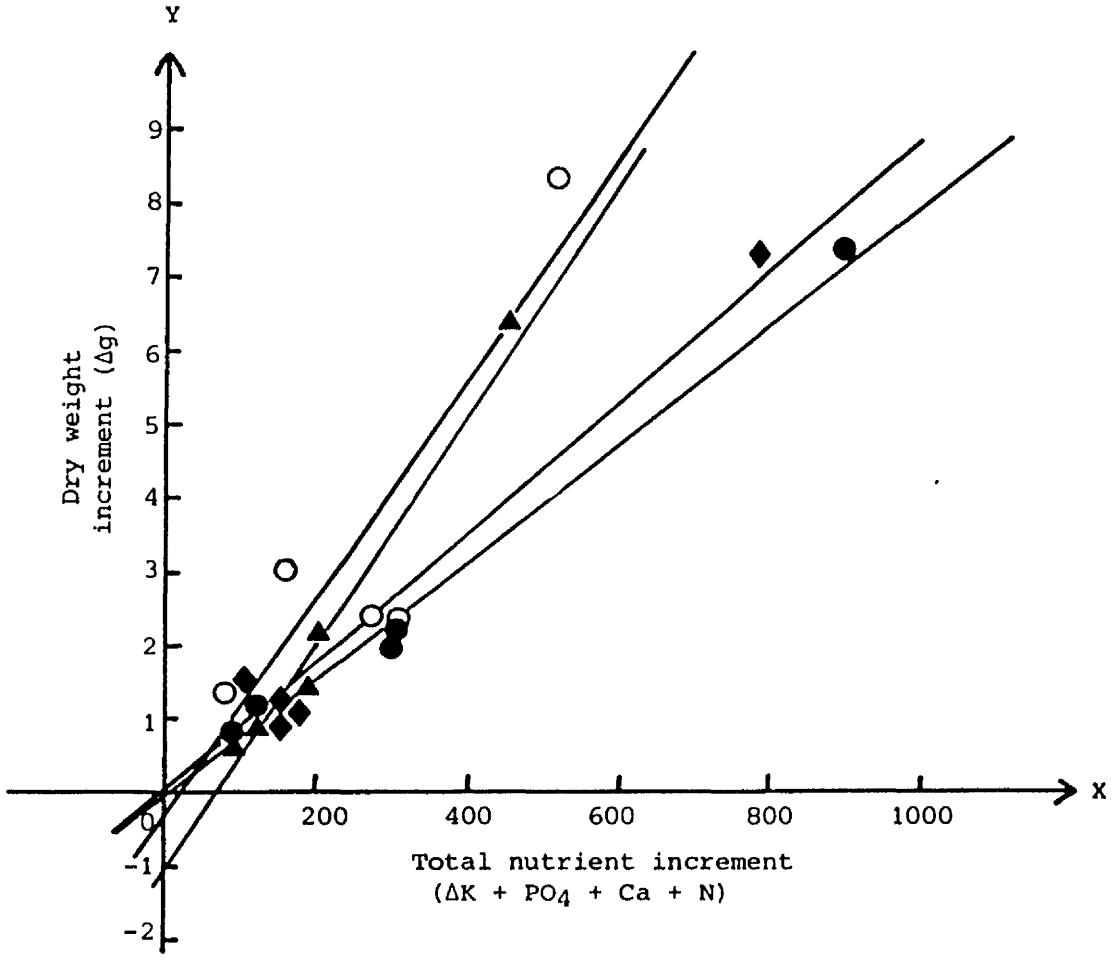



Figure 23. Functional equilibrium for total nutrient uptake in leeks infected with 10% rust (●), 20% rust (■) and 30% (▲). Controls (○). Equations of lines shown are 10%: $y = 0.008x - 0.043$, $r = 0.99$; 20%: $y = 0.009x - 0.119$, $r = 0.99$; 30%: $y = 0.016x - 1.213$, $r = 0.99$; Control: $y = 0.015x - 0.351$, $r = 0.87$.

treatments, and the fitted regressions were highly significant for both rusted and control plants (Figures 19-23). Thus, the graphs suggest that a functional equilibrium existed between the shoot and root systems for nutrient uptake in these experiments. Furthermore, it seems clear that nutrient uptake was not a limitation to growth under the conditions of these experiments.

However, comparison of the slopes of lines fitted to each of the different treatments showed that although rust infection had no significant effect on the functional equilibrium between shoot and root for potassium uptake and, in general, for nitrogen uptake, rust infection did significantly alter the equilibrium between shoot and root for the uptake of phosphate, calcium and total nutrients ($P<0.001$)

## DISCUSSION

It is commonly found that rust disease reduces the dry weight of the infected plant (e.g. Manners and Myers, 1973; Owera **et al**, 1981). The results presented here also show that rust infection of leeks resulted in substantial reductions in dry weights of both root and shoot, and hence the total plant, from as early as two weeks after inoculation. Such reduction in the growth of plants infected with biotrophs is often attributed to the severe reductions in photosynthesis that occur in infected leaves, coupled with an increasing loss of host assimilates via increased rates of dark respiration (e.g. Walters, 1985). This is discussed in detail in Section 3.

The data on nutrient contents and uptake presented here are similar to those obtained for brown rust infection of barley by Ahmad **et al**,(1982) and for groundsel infected with rust (Paul and Ayres, 1987a). The results of the former authors show that concentrations of calcium, magnesium and potassium were substantially increased by 16 days after inoculation with brown rust. Similar results were obtained by Paul and Ayres (1987a), who found increases in the concentrations of potassium and nitrogen in rusted groundsel plants over a seven week experimental period. In rusted leek plants, the concentrations of these ions, with the addition of phosphate and calcium, were generally increased following infection, compared to healthy controls. However, in contrast to the results presented by Ahmad **et al**,(1982), which showed that the actual content of these minerals was increased, despite reductions in dry weight, the results presented for rusted leeks show that probably because of reductions in dry weight, the amounts of most of these minerals were reduced in plants with 20% and 30% infection. In leeks with 10% infection, the mineral contents were usually not significantly different from controls. It should be noted that for the rusted treatments, nutrient contents will have been underestimated. Ahmad **et al**,(1982) reported that the loss of phosphorus in spores accounted for about 25%, and of nitrogen for about 55%, of the amount of these nutrients being imported into these plants. Since nutrient loss in fungal spores was not estimated for rusted leeks, it seems reasonable to assume that nutrient losses in leek rust uredospores could be very substantial over the course of an eight week experimental period.

The increased mineral ion content in rusted leeks might be expected to be of some benefit to the host plant. However, in spite of these increases in ion concentrations, growth was substantially reduced following rust infection. In the light of these results, it may prove instructive to examine pertinent data from experiments on mycorrhizal plants. Thus, it is known that mycorrhizal plants frequently show much increased phosphorus concentrations (e.g. Harley and Smith, 1983). Such plants are commonly larger than their uninfected counterparts. However, a closer examination of the relevant data reveals that growth of mycorrhizal plants does not keep pace with the increase in phosphorus concentration. It is obvious, therefore, that in this situation, phosphorus is not limiting growth, and that some other factor must be limiting. Stribley, Tinker and Rayner (1980) suggested that the increased utilization of photosynthate in mycorrhizal plants leads to such plants becoming carbon-limited.

A similar situation appears to exist in many plants infected with obligate parasites, where carbon assimilation and translocation are greatly disturbed. Livne and Daly (1966) showed that when unifoliate leaves of French bean (**Phaseolus vulgaris**) were infected by the rust, **Uromyces appendiculatus**, there was a substantial reduction in the amount of newly fixed carbon exported from that leaf. Import from the next trifoliate leaf increased, but this diversion of assimilates to the rust was at the expense of the roots and the newly emerging leaves. Siddiqui and Manners (1971), using the wheat/**Puccinia striiformis** system, showed that the amount of $^{14}C$-labelled assimilates moving to infected leaves was increased, whilst that going to roots was greatly decreased. Altered patterns

of assimilate movement were also found in powdery mildew infected plants. Reductions in the percentage of assimilates translocated to roots have been noted by a variety of authors (Edwards, 1971; Lupton and Sutherland, 1973; Fric, 1975), and Farrar (1980) went on to suggest that since the reserves of insoluble materials, such as starch and fructosans, seem to be small, the availability of energy for active processes may be greatly affected by small changes in assimilate supply resulting from mildew infection. It is possible then, that a similar situation exists in leeks infected with rust, and because nutrient supply is not limited in diseased plants, carbon is probably the major limitation to plant growth.

Ahmad **et al**, (1982) showed that the increased cation contents were accompanied by increased cation uptake per unit of root tissue, following sporulation of the rust. They suggested that the simplest explanation of their results was an increase in transpiration following sporulation, due to a decreased leaf diffusive resistance to water vapour loss. Water relations are known to be affected in many plants infected with obligate biotrophic pathogens. Rust and powdery mildew infections result in an inhibition of stomatal opening in the light (Walters, 1985). In rusts, stomatal apertures eventually remain smaller (Duniway and Durbin, 1971), and, once fungal sporulation has ruptured the cuticle, stomatal resistance ceases to be the primary factor determining whole leaf resistance, as cuticular resistance is much reduced. Cuticle rupture results in an increase in non-stomatal transpiration per unit leaf area (Johnson and Miller, 1934, 1940; Murphy, 1935). Paul and Ayres (1984) have shown that, after sporulation, groundsel plants infected with the rust, **Puccinia lagenophorae**, transpired more (expressed on

a per plant basis) than did uninfected controls, and, in addition, rust infection resulted in an increased transpiration ratio (mg water lost/mg $CO_2$ fixed), indicating a much decreased water use efficiency.

The results reported here showed that SAR's for nitrogen, phosphate, potassium and calcium in rusted plants were usually significantly greater than controls over the entire experimental period. Sporulation in the leek-rust system occurs around 12-14 days after inoculation, and certainly in these experiments, sporulation two weeks after inoculation was a fraction of that occurring some weeks later. Although no measurements of transpiration could be made in these experiments, an examination was made of stomatal resistance in pustule areas of rusted leek leaves (see Section 5). This revealed that resistance was substantially reduced in rusted areas of leaves, probably due to the rupture of the cuticle.

It seems reasonable to assume, therefore, that rusted leeks, like other rust-infected plants, will show an increased transpiration rate (per unit leaf area) following sporulation and rupture of the epidermis. It is possible that the transpiration rate of leeks two weeks after inoculation, might be considerably less than the transpiration rate some weeks later when sporulation was greater. Such behaviour may help us to explain (1) the greater differences in SARs and nutrient concentrations in control and infected leeks eight weeks compared to two weeks after inoculation and (2) the increase in SAR and concentration of calcium in rusted plants eight weeks after inoculation and the lack of any substantial effect two weeks after inoculation.

Because the data presented here represent changes in nutrient concentrations in whole shoot and root systems and not in individual leaves, any possible changes in nutrient distribution between infected and uninfected leaves will be masked. Ahmad **et al**,(1982) showed that retranslocation of potassium from brown-rusted barley leaves was substantially reduced in comparison to controls. These authors concluded that the accumulation of cations in infected leaves was due, in part, to a reduction in assimilate export in the phloem. Furthermore, they showed that in rusted barley leaves, the rate of phosphorous accumulation was increased above, and it's retranslocation reduced below, that expected from changes in xylem and phloem transport alone. They suggested that this implied a further effect of retranslocation of phosphorous, due either to alterations in phloem loading of phosphate or to sequestration of phosphate by the fungus as organic phosphates (Mukherjee and Shaw, 1962) or polyphosphate (Bennett and Scott, 1971). Whereas the phosphorus accumulation noted in the work of Ahmad **et al**, (1982) was detected in infected first leaves as indicated above, no data is provided here for individual leaves. However, phosphorus was accumulated in the whole plant, and it is likely that this accumulation is partly due to a more substantial accumulation of phosphorus in infected leaves. Studies with discrete regions of infected leek leaves revealed that although photosynthesis was significantly reduced in pustule areas of rusted leaves, the addition of inorganic phosphate to the medium did not have any appreciable effect on photosynthetic rates, suggesting that phosphate was not a limiting factor in photosynthesis by rusted areas of diseased leek leaves (see Section 5). Furthermore, preliminary measurements for different experiments indicated that

phosphorous concentration did not change in uninfected leaves of rusted leeks (results not shown in this thesis). It is also interesting to note that uninfected leaves of rusted leek plants exhibit greatly increased stomatal resistance, coupled with very high concentrations of ABA, suggesting that these leaves are water-stressed (see Section 5). As mentioned earlier, increases in the concentration of ions in rusted plants may be due, in part, to increased rates of transpiration. If this is the case, it is doubtful that ions could accumulate in uninfected leaves of rusted leek plants since transpiration appears to be reduced in these leaves. Nevertheless, we should not dismiss the possible role of transpiration-linked flux of ions into uninfected leaves until there is more comprehensive data on transpiration in these leaves. Although phosphorous is also phloem-mobile, alterations in the patterns of translocation in infected plants may lead to a translocation of compounds out of uninfected leaves to lower infected leaves, and experiments examining photosynthesis in individual leaves of rusted leek plants suggests that this may be the case. A similar situation may exist for the translocation of phosphorous in rusted leek plants, although this is highly speculative.

It should be noted that in rust-infected groundsel, the primary mechanism by which elevated SARs for various nutrients were achieved was through increased "specific root length" (SRL) (Paul and Ayres, 1987b). Increases in SRL resulted in more functional root per unit dry weight. Paul and Ayres (1987b) found that rust-induced increases in SARs for various nutrients coincided with the onset of flowering in rusted groundsel, when rust-induced changes in SRL were

also most pronounced.

In the economy of the whole plant, specialized organs such as the root and shoot are in constant competition for available energy for their development (Walters, 1985). A measure of the resultant pattern of differential growth of the two organs, expressed as the shoot:root ratio (S:R) can thus provide an index for the performance of each organ in a certain growth environment. Among the external factors known to affect S:R are individual factors such as nitrogen, water, light intensity and temperature (see Brouwer, 1966). Rates of root pruning (Brouwer, 1963; Troughton, 1963), daylength (Troughton, 1960) and root temperature (Davidson, 1969) are also known to influence S:R. An important question to be asked is whether S:R is affected by disease, and if so, what the implications will be for the host plant.

It has been suggested that plants infected with rusts and powdery mildews may be able to maintain a functional equilibrium between root and shoot with respect to nutrient uptake, providing the intensity of infection is not too severe (Walters, 1985). These suggestions were based on the observations that although total plant dry weight (and root weight especially) was reduced following rust or mildew infection, the concentrations of several minerals were generally increased compared with controls (e.g. Ahmad **et al**, 1982; Walters, 1981; Walters and Ayres, 1981). Indeed, the increased uptake and concentrations of minerals observed in infected leeks, in spite of substantial growth reductions, suggest the maintenance of a functional shoot:root equilibrium with respect to nutrient uptake in these plants. Analysis of the results presented here after Thornley (1975) and Richards (1977), involving a plot of dry weight increment

against incremental nutrient weight, produced graphs showing highly significant linear regressions for both control and rusted plants. Thus, for every increase in plant dry weight there was a corresponding increase in nutrient uptake, suggesting that nutrient uptake was not a limitation to growth and that a functional equilibrium existed between root and shoot for nutrient uptake. However, when lines were fitted to each of the different treatments and a comparison was made of the different slopes, rust infection did appear to significantly alter the functional equilibrium between shoot and root with respect to the uptake of phosphate, calcium and total nutrients. Rust infection did not alter the equilibrium between root and shoot with respect to the uptake of potassium and, in general, nitrogen. Perhaps this should not be surprising since, in spite of a reduction in plant dry weight, the concentrations of many ions increased above controls. Indeed in a plot of incremental dry weight versus incremental nutrient uptake (after Richards, 1977), functional equilibrium is given by the slope of the line (i.e. $\Delta W/ \Delta M$). In rusted leeks, increments in dry weights are smaller than in controls, but there is not a proportional fall in incremental nutrient content and so $\Delta W/ \Delta M$, or functional equilibrium, declines.

Paul and Ayres (1987b) have suggested that the increased SARs for nitrogen, phosphorous and potassium in rusted groundsel could largely be attributed to increased shoot demand per unit root, resulting from the higher S:R's of infected plants. They also suggested that because of specific rust-related changes in demand for soil water and nutrients, and rust-induced changes in whole plant nutrient concentrations, a functional equilibrium (sensuo

Davidson, 1969) between shoot and root with respect to the uptake of individual nutrients did not exist in their system. However, when they examined total root activity for a range of nutrients, it appeared that an overall equilibrium was maintained.

The situation for leeks infected with rust is somewhat different. Thus, although an equilibrium with respect to potassium uptake did appear to exist (or at least was not significantly altered), an equilibrium did not appear to exist for the uptake of calcium, phosphate and total nutrients. The increases in ion concentrations and changes in $\triangle W/\triangle M$ in rusted plants would appear to indicate that shoot demand was greater in these plants than in controls. However, S:R was actually reduced in infected plants for most of the experiment, making it difficult to explain increased shoot demand in terms of S:R. There is evidence to suggest that pathogen requirements for certain nutrients exceed those of the total host plant (Ahmad **et al**, 1984). Furthermore, it is possible that infection may cause marked increases in the requirements for a particular nutrient. However, such contributions to increased shoot demand may be quite small, since even under conditions of heavy pathogen colonization, fungal biomass will be small compared to host biomass (Ahmad **et al**, 1984).

Fungal infection is also known to radically alter the physiology and biochemistry of the host plant (e.g. Farrar, 1984; Walters, 1985) and it is possible that such alterations in host metabolism, e.g. transpiration and nitrogen metabolism, may also play a role in increased shoot demand. The role of growth regulators in altering the nutrient relations of rust-infected plants was not examined, although it is possible that, in view of

large changes in abscisic acid and cytokinins found in roots and shoots of rusted leeks (see Section 5), they may have an important role in the maintenance of a functional shoot:root equilibrium with respect to nutrient uptake.

SECTION 7

# Nitrogen Assimilation and Metabolism in Healthy and Rust-Infected Leeks

## INTRODUCTION

Nitrogen is a major component of a number of compounds which are essential for the structure and functioning of biological organisms, and, apart from water, nitrogen is the key substance that limits the primary productivity of plant life in many parts of the world. The major transfer of nitrogen between plants, microflora and soil takes place in a series of steps. Thus, (a) microbial decomposition of organic materials in the soil leads to the production of ammonia (ammonification), (b) this ammonia is then converted via micro-organisms to first nitrite and then nitrate (nitrification), and (c) ammonium and nitrate are assimilated by microflora and higher plants.

### Uptake, Assimilation and Metabolism of Nitrogen in Healthy Plants

The uptake of both ammonium and nitrate is energy dependent (Mengel and Viro, 1978). There appears to be a similarity between ammonium uptake and the uptake of other monovalent cations, particularly potassium. However, although Haynes and Goh (1978) suggested that the uptake of ammonium appears to be strictly competitive with potassium, in contrast with these findings, Mengel, Viro and Hehl (1976) demonstrated that the uptake of ammonium nitrogen is not affected by potassium under reducing conditions. These workers suggested that under the reducing conditions of their experiment, ammonium may have been absorbed mainly in the form of ammonia by diffusion. Separate research has suggested that ammonia is able to diffuse through biological membranes (Heber, Kirk, Gimmler and Schafer, 1974; Moore, 1974).

Nitrate absorption by nitrate depleted plants has been shown to exhibit an early lag period followed by a rapid rate of absorption. This has been demonstrated in barley (Blevins, Hiatt and Lowe, 1974; Rao and Rains, 1976a,b), wheat (Minotti, Williams and Jackson, 1968, 1969a) and maize (Jackson, Flesher and Hageman, 1973). The accelerated rate after induction appears to be dependent on a critical internal concentration, which is in turn a function of the external concentration (Neyra and Hageman, 1975).

Research suggests that under normal conditions, nitrate uptake and nitrate-reductase activity are closely correlated (Brunetti, Picciurro and Boniforti, 1972). Neyra and Hageman (1975) suggested that the rate of nitrate-reductase induction was regulated by the nitrate flux into the plant. A functional similarity appeared to exist between nitrate-reductase induction and nitrate uptake, both processes being induced by nitrate and nitrite and inhibited by ammonium and amino acids (Goldsmith, Livoni, Norbeg and Segel, 1973; Schloemer and Garret, 1974a,b).

The enhanced absorption of nitrate which occurs after the illumination of plants may be due to an increased supply of energy for the transport of ions through ATP supplied by photophosphorylation (Rao and Rains, 1976a). In addition to light, internal factors may affect nitrate uptake. Smith (1973) showed that the influx of nitrate and chloride was subject to feedback control from the internal content of these ions. Also, organic anions could play an important role in controlling nitrate and chloride flux.

Repression of nitrate uptake by ammonium was found in ryegrass (Lycklama, 1963), wheat (Minotti **et al**, 1969a; Breteler and Smit, 1974), maize (Schrader, Domska, Jung and Peterson, 1972) and apples (Frith, 1972; Frith & Nichols, 1975). Several theories have been proposed to explain the inhibitory effect ammonium has on nitrate uptake. Minotti **et al** (1969b) suggested that high acidity adjacent to cellular boundary membranes, caused by ammonium uptake, may have been responsible for the inhibition in nitrate uptake. A mechanism involving protein-protein interactions was postulated in **Lemna** (Orebamjo and Stewart, 1975). Ammonium adapted plants contain a protein which inhibits nitrate reductase (Stewart, Lee, Orebamjo and Javill, 1974). It was suggested that in the presence of high ammonium levels, the inhibiting protein bound to nitrate reductase, thus inactivating it (Orebamjo and Stewart, 1975).

Nitrate must be reduced before it is assimilated but ammonium, once absorbed, may immediately be used in the synthesis of amino acids, and other organic compounds. The process of nitrate reduction to ammonium is mediated by two enzymes, nitrate reductase (NR), which converts nitrate to nitrite, and nitrite reductase (NiR), which in turn converts nitrite to ammonium. These reductions require a great deal of energy, as shown in Eq. 1.

$$NO_3^- + H_2O + 2H^+ \xrightarrow{\text{NR and NiR}} NH_4 + 2O_2 \qquad -83\text{Kcal} \qquad \text{Eq. 1}$$

Hence, energy is conserved when ammonium is supplied to the plant, less reduction being necessary during assimilation. Nitrate reductase is a molybdenum flavohaemo protein which appears to specifically utilize NADH in physiological conditions but may also

utilize reduced flavins (see Hewitt, 1975). Formation of NR occurs as a nitrate-inducible response involving protein synthesis (Afridi and Hewitt, 1964; Zeilke and Filner, 1971). Continuous nitrate flux may be necessary for the inducing reaction to occur and for continuing enzyme formation. The enzyme is normally in a state of natural turnover, being formed and degraded simultaneously and is dependent on the presence of mRNA (Oaks, Wallace and Stevens, 1972). A specific degrading enzyme has been found and isolated from maize (Wallace, 1973; Wallace and Johnson, 1978).

Nitrite reductase is an iron protein containing both sirohaem (an iron-chlorine tetrapyrole) and a non-haem iron and acid labile sulphide centre and is located in the chloroplasts of green tissue (Ritenour, Joy, Bunning and Hageman, 1967; Magalhaes, Neyra and Hageman, 1974). Reduced ferredoxin is the natural electron donor for nitrite reductase, and may be necessary for its induction (Sluiters-Scholten, 1975). The reducing power necessary to reduce ferredoxin may be supplied either photochemically during non-cyclic photophosphorylation or by NADPH in the presence of ferredoxin NADP reductase (Haynes and Goh, 1978).

Analysis of both ammonium and nitrate assimilation has revealed that glutamic acid and glutamine are the primary products of ammonium assimilation. For many years, the reductive amination of 2-oxoglutarate to glutamate, catalyzed by the enzyme glutamate dehydrogenase (GDH), was considered to be the main reaction of ammonium assimilation in plants. However, the $K_M$ (Michaelis constant) of ammonium for GDH is extremely high, in the range where $NH_4$ toxicity would result (Chou and Splittstoesser, 1972). Although ammonium was known to be assimilated into glutamine via glutamine

synthetase (GS), this was not considered to be of importance in the formation of $\alpha$-amino nitrogen. However, Miflin (1974) demonstrated that in chloroplasts, GS, which had a much lower $K_M$ for ammonia, had a much greater activity. Mann, Fentem and Stewart (1980) have shown the existence of two isoenzymes of GS (I and II), one form being predominant in roots (GSI) and both forms being found equally in leaves. Lea and Miflin (1974) proposed that ammonia was assimilated in higher plants through the combined operation of GS and glutamate synthase (GOGAT), in which glutamate acts as the first recipient of reduced nitrogen.

Glutamate synthase (GOGAT) is widespread in micro-organisms and has been identified in **Pisum sativum** roots (Miflin and Lea, 1975). The enzyme was found to be essentially specific for glutamine ($K_M$330 mol/l), 2-oxo-glutarate ($K_M$ 150 mol/l) and ferredoxin ($K_M$ 2 mol/l). GOGAT was inhibited by the glutamine analogues azaserine and albizziine, which are known to inhibit other glutamine-amide transferases (Lea and Norris, 1976; Meister, 1974). Rhodes, Rendon and Stewart (1976) studied the changes in levels of the enzyme in **Lemna** in relation to some exogenously applied substrates and products, and concluded that there was no simple relationship between GOGAT and the endogenous pools of glutamate or any other of the metabolites they studied. The localization of the enzymes of nitrogen assimilation are shown in Figure 24 and the GS-GOGAT pathway is shown in Figure 25. Nitrogen, once assimilated, may be either directly utilized in the formation of amino acids for the synthesis of metabolic proteins, or transported to another part of the plant where it can be used for metabolism or long term storage. Briefly, in the former, nitrogen is incorporated into

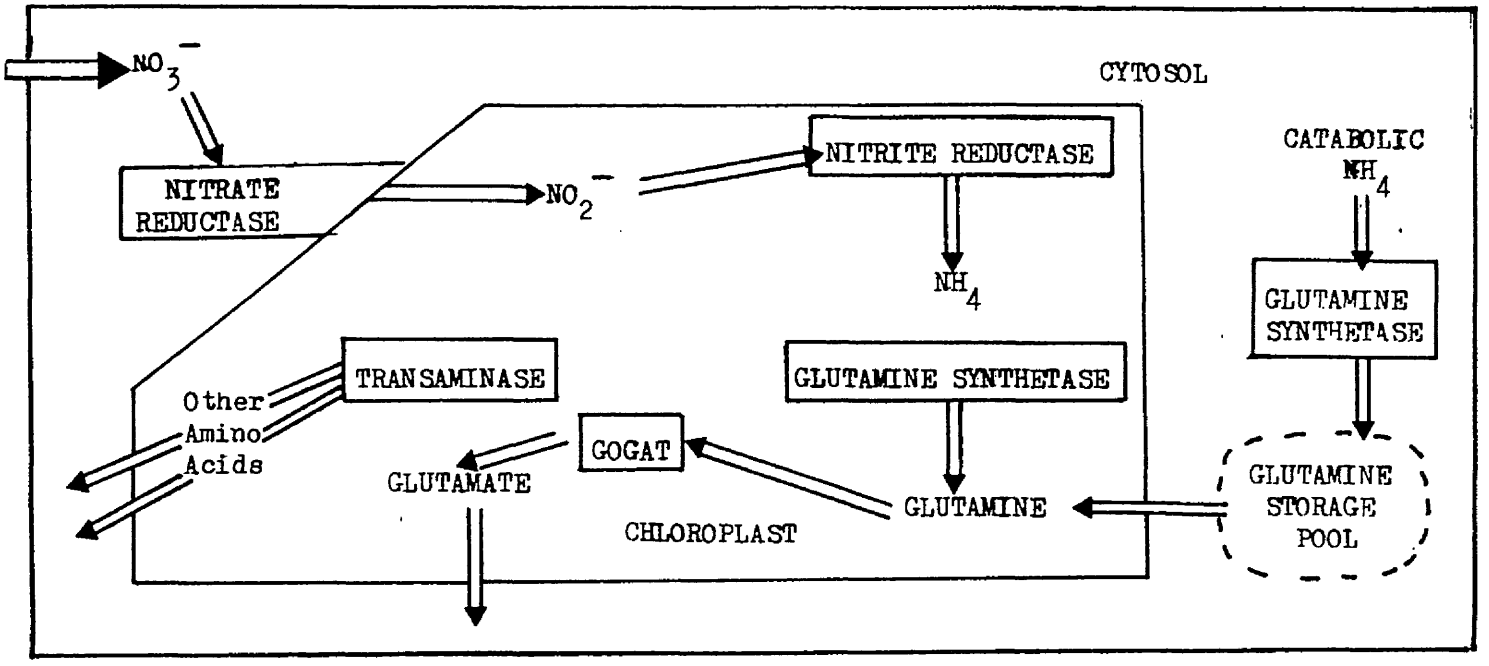



Figure 24. Localization of enzymes involved in nitrogen assimilation.

amino acids via transamination reactions, except that one of the N atoms in each arginine, tryptophan and histidine is derived by transfer of the amide-amino group of glutamine. When assimilated nitrogen is transported, a number of compounds may be formed, but in the assimilating tissue, reactions need to occur to maintain the level of glutamate as the $NH_4^+$ acceptor. On arrival at the other end of the transport chain, the nitrogen is transformed into a complete range of amino acids. Following long-term storage, usually in the form of protein, the nitrogen is remobilized and used for metabolic protein synthesis (Miflin and Lea, 1976).

**The Nitrogen Economy of Diseased Plants**

As discussed above, nitrogen plays a central role in plant growth and metabolism, and it is surprising therefore that information concerning the effects of infection by obligately biotrophic pathogens on nitrogen is scant. Powdery mildew infection of barley resulted in a reduction in the nitrate content of all tissues (Walters and Ayres, 1980; Murray and Ayres, 1986a). On the other hand, no change in nitrate content of leaf tissues of rusted wheat or rye could be detected (Rohringer, 1957; Piening, 1972). However, Ahmad **et al**, (1982) indicated that although uptake of nitrate by roots of brown rust-infected barley plants was much increased, total nitrogen content of rusted plants was much lower than in controls. These authors suggested that a large amount of nitrogen was lost in fungal spores.

In barley infected with powdery mildew, Walters and Ayres (1980) suggested that the reduction in nitrate content of roots was probably the result of reduced rates of uptake of the ion. Murray and Ayres (1986a) demonstrated that the reduction in nitrate content

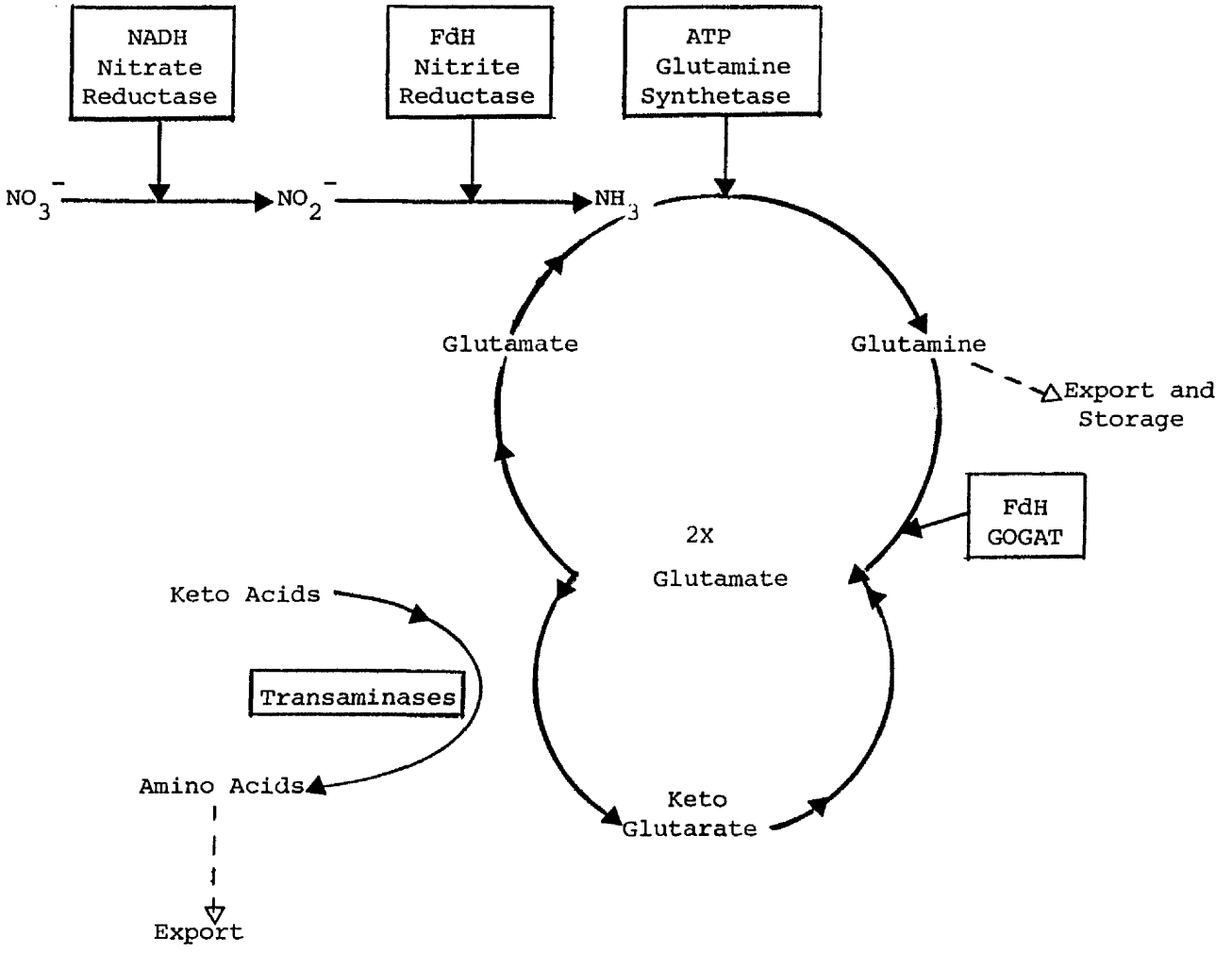



Figure 25. The GS - GOGAT pathway of nitrogen assimilation

of roots and shoots of mildewed barley were reflected in the lower rates of nitrate accumulation shown by roots and shoots both before and during sporulation. Reduced nitrate uptake may have been caused by the accumulation of ammonium ions in roots of mildewed barley (Hewitt, 1975; Walters and Ayres, 1980), but the most likely cause of reduced uptake was that the root was starved of photosynthetic products, since net photosynthesis and translocation of assimilates were reduced following mildew infection (Edwards, 1971; Lupton and Sutherland, 1973; Fric, 1975; Ayres, 1978; Walters and Ayres, 1982) and $NO_3$ uptake is known to be highly energy dependent.

The increased uptake of nitrate by roots of barley infected with brown rust reported by Ahmad **et al** (1982) may have been linked with potassium recirculation from the shoot (Kirkby and Armstrong, 1980). Indeed, not only was potassium uptake by roots of rusted barley increased in comparison with controls, but it s re-translocation and exudation from infected leaves was much greater than for uninfected control plants (Ahmad **et al**, 1982).

Increases in ammonium nitrogen in leaves of cereals infected with rusts and powdery mildews have been demonstrated (see Walters, 1985) and Walters and Ayres (1980) showed that roots of mildewed barley also accumulated ammonium ions. The increases in ammonium ions in leaves of diseased plants may have been due to an increased breakdown of amino acids and amides (Sadler and Scott, 1974), since the activity of GS in mildewed and rusted leaves was much increased upon infection (Farkas and Kiraly, 1961; Sadler and Scott, 1974). The increased ammonium content of roots of mildewed barley, on the other hand, appeared to be the result, not only of an enhanced degradation of amino-acids and amides, but also to a much decreased

assimilation of ammonium via both the GS-GOGAT and GDH pathways (Walters and Ayres, 1980).

During the course of experiments on shoot:root equilibria (see Section 6) changes in ammonium nitrogen were detected, which did not agree with previously published reports of ammonia on mildewed or rusted tissues. It was decided therefore, to examine the mechanisms underlying this alteration in host metabolism and perhaps as a result, to add some consistency to the few differing reports in the literature.

## MATERIALS AND METHODS

The growth and inoculation of leeks (**Allium porrum** L. var. Autumn Mammoth) are described in Section 2 - General Materials and Methods.

### Determination of Total Nitrate, Ammonium and Nitrogen

The total nitrate content of fresh tissues was determined by the method of Woolley, Hicks and Hageman (1960). One gram of fresh material was crushed in 20 $cm^3$ hot distilled water with a mortar and pestle and the extract was filtered. To 2.5 $cm^3$ of the extract in a stoppered tube was added 9 $cm^3$ of acetic acid (20%) and 0.8 g of a powder containing the following: 100 g barium sulphate, 75 g acetic acid, 10 g manganese sulphate, 4 g sulphanilic acid, 2 g powdered zinc and 2 g L-naphthylamine. The contents of the tube were then shaken for 2 min, allowed to settle, then shaken again, this process being repeated twice. The contents were then filtered, and the absorption of the filtrate was measured at 520 nm using an LKB spectrophotometer. Standard curves were prepared from stock

solutions of potassium nitrate (see Appendix I).

Total ammonium contents of water extracts of fresh tissues were determined by nesslerisation (Jackson, 1962). One gram of fresh material was crushed in 20 $cm^3$ hot distilled water with a mortar and pestle and the extract was filtered. The filtrate was then made up to volume in a 100 $cm^3$ volumetric flask. Aliquots of the filtrate were placed in a 50 $cm^3$ volumetric flask together with 1 $cm^3$ of 10% sodium tartrate solution and 1 $cm^3$ of acidified sodium chloride solution (acidified to pH 2.5 with hydrochloric acid). A little gum acacia was added followed by 2.5 $cm^3$ Nessler's reagent (Sigma Chemicals, Poole). The solution was brought to volume and after 25 mins the % transmission was measured at 410 nm using an LKB spectrophotometer. Standard curves were prepared from stock ammonium chloride solutions (see Appendix I). Total nitrogen was measured as ammonia after Kjeldahl digestion with a selenium catalyst.

**Determination of Ammonia Evolution**

Leaves (10 g fresh weight) were harvested, the basal ends removed under water and the remainder of the leaves placed upright at the bottom of a desiccator, with their basal ends only in 20 $cm^3$ of distilled water. A petri-dish containing 15 $cm^3$ of a 5% aqueous solution of boric acid was placed on a fine mesh support above the leaves, the desiccator sealed with high vaccuum grease and incubated under constant illumination for 24 h. After incubation, ammonia was determined in aliquots of the water in which the leaves had been placed, and in the boric acid solution, by nesslerisation (Jackson, 1962).

**Degradation of Amino Acids and Amides**

Leaves (500 mg fresh weight) were harvested, cut into 1 cm segments and floated on (but not submerged in) 10 mM solutions of either glutamine, glutamic acid, asparagine or aspartic acid in petri-dishes. The dishes were incubated in the light for 18 h, and liberation of ammonium into the bathing medium was determined by nesslerisation (Jackson, 1962).

**Enzyme Assays**

Nitrate reductase ($NADH_2$:nitrate oxidoreductase, E.C. 1.6.6.2) activity **in vivo** was determined by the method of Deane-Drummond, Clarkson and Johnson (1979). The incubation medium contained 50 mM phosphate buffer, 1% propanol (v/v) and 100 mM nitrate at pH 5.0. Leaves (0.1 g fresh weight) were transferred to glass bottles containing cold incubation medium (4 $cm^3$/0.1 g leaves) and vacuum infiltrated for 4 minutes. Incubation was for 20 minutes at 27°C in the dark and the reaction was terminated by boiling for 10 minutes. After cooling, nitrite was measured in a 1 $cm^3$ aliquot by adding 1 $cm^3$ sulphanilamide (1% in 3N HCl) and then 1 $cm^3$ N-1-naphthylethylenediamine dihydrochloride (0.05%) (Jaworski, 1971). The optical density was determined at 540 nm. Standard curves were prepared from stock solutions of potassium nitrite (see Appendix I).

Nitrite reductase (Donor:nitrite oxidoreductase, E.C. 1.6.6.4) activity **in vivo** was measured using a method modified from that described by Sadler and Scott (1974). Leaves (1 g fresh weight) were harvested and cut into 5 mm sections and placed in vials containing 10 $cm^3$ of 2 x $10^{-5}$M sodium nitrite and 0.4 $cm^3$ of dimethyl sulphoxide. The pH was adjusted to 7.8 with a few drops of M phosphate buffer. The leaf segments were vacuum infiltrated with

this reaction medium for 3 minutes, prior to incubation at 27°C under constant illumination for 1 h. Aliquots of the medium were taken both before and after incubation, the nitrite content of these being determined by diazotisation with sulphanilamide and N-1-naphthylethylene diamine hydrochloride (Jaworski, 1971).

The **in vivo** activity of glutamine synthetase (L-glutamate:ammonia ligase (ADP), E.C. 6.3.1.2) was determined by the method of Walters and Ayres (1980). Approximately 100 mg fresh weight of leaf material was freeze-thawed by six successive periods of immersion in liquid nitrogen for 30 s followed by thawing at 30°C for 2 min. Tissue was then placed in a reaction mixture comprising, L-glutamate (25 $\mu$mol), ATP (25 $\mu$mol), $MgSO_4$ (100 $\mu$mol), hydroxylamine (100 $\mu$mol), and Tris-HCl (400 $\mu$mol, pH 7.3), final volume 2 $cm^3$. The reaction mixture was incubated at 27°C for 30 minutes before the reaction was stopped by the addition of 1 $cm^3$ of a mixture of 10 g TCA plus 8 g $FeCl_3$ in 250 $cm^3$ of 0.5 N HCl. After centrifugation, the optical density of the solution was measured at 500 nm. L-Glutamic acid-monohydroxymate was used as a standard for calibration (see Appendix I).

## RESULTS

### Inorganic Nitrogen Content of Leaves

All measurements were made at 12 days after inoculation, immediately prior to sporulation. No measurements were conducted after this time, due to the difficulties in trying to separate host and pathogen. Although rust infection of leek leaves resulted in a significant increase in nitrate concentration, ammonium

concentration in these tissues was decreased (Figure 26). A small, although not significant increase in total nitrogen was detected in rusted leaves (Figure 26). Evolution of ammonia gas by rusted leaves was significantly greater than that of healthy plants (Table 18).

**Amino Acid and Amide Degradation**

The results shown in Figure 27 suggest that all the amino acids and amides tested may serve as precursors of ammonium in both healthy and rust-infected leaves. However, although no significant difference in the degradation of aspartic acid, glutamine or asparagine was apparent in control and rusted tissue, infection significantly stimulated the degradation of glutamic acid in leaves of leek.

**Enzymes of Nitrogen and Ammonium Assimilation**

The activities of various enzymes involved in nitrogen and ammonium assimilation were studied because of the significant alterations in nitrate and ammonium contents in rusted leaves. Infection led to significant increases in the activities of nitrate reductase (NR) and glutamine synthetase (GS), but had no effect on the activity of nitrite reductase (NiR) (Figure 28).

## DISCUSSION

Leaves infected with rust showed a significant accumulation of nitrate ions, while the ammonium content was substantially reduced. Although the results presented here represent measurements made at 12 days after inoculation, previous experiments (see Section 6) showed that this pattern of ion distribution remained constant for

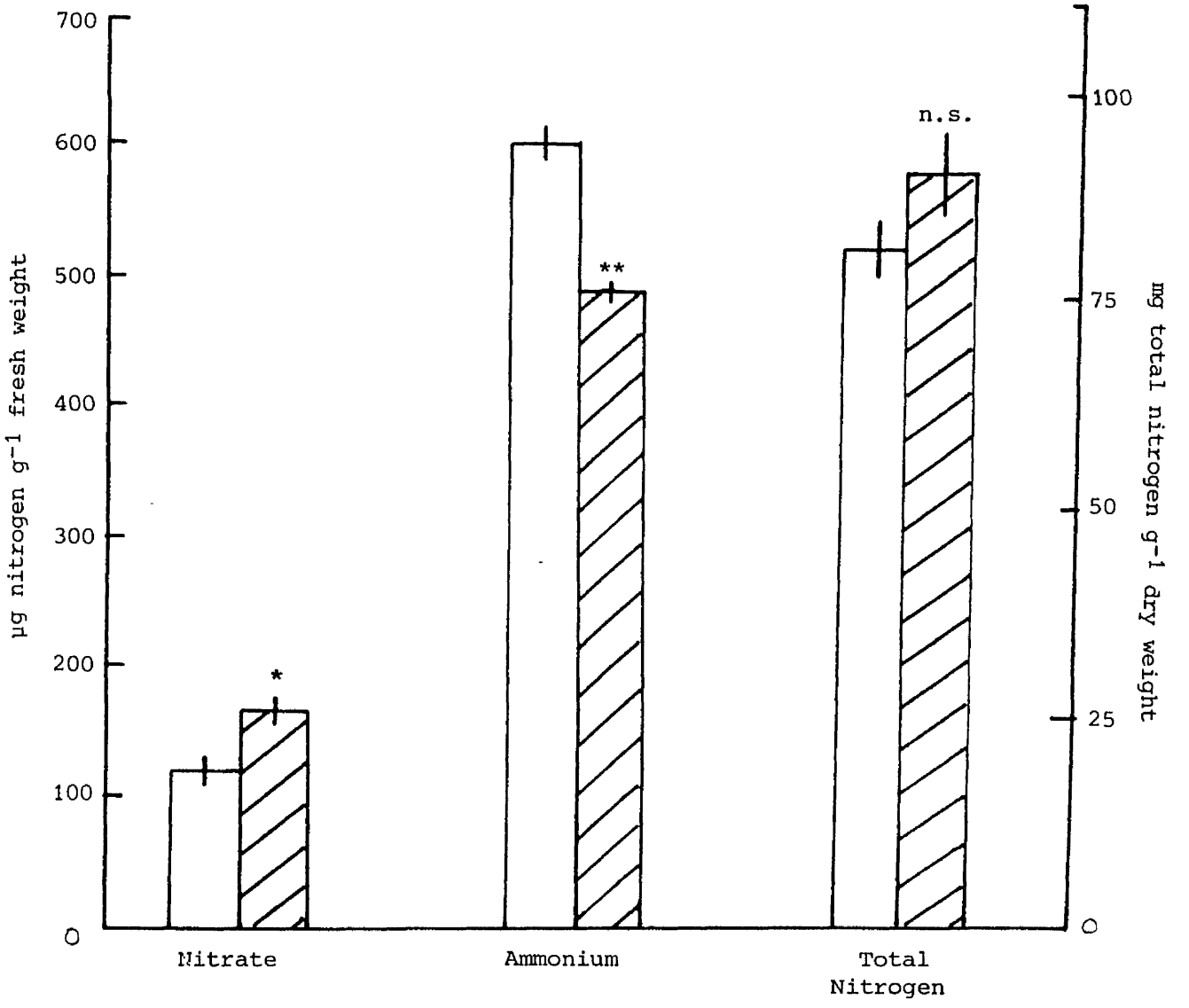



Figure 26. Inorganic nitrogen content in healthy leek leaves (□) and in leaves infected with rust (▨). Measurements were made at 12 days after inoculation. Each value is the mean of four replicates with standard error. Differences between means significant at $P<0.02$ (*), $P<0.001$ (**), n.s., not significant.

| Treatment | Ammonia evolution<br>mg $NH_3$ evolved $g^{-1}$ fresh weight$^{-1}$ $h^{-1}$ |
|---|---|
| Healthy | 0.26 ± 0.006 |
| Rusted | 0.54 ± 0.0008 ** |

Table 18. Evolution of ammonia gas by healthy leek leaves and by leaves infected with rust. Measurements were made at 12 days after inoculation. Each value is the mean of four replicates with standard error. Differences between means significant at $P<0.001$ (**).

up to eight weeks after inoculation. This indicates that such alterations in inorganic nitrogen content do not represent a transient response limited to the early stages of the host-parasite interaction.

Accumulation of nitrate in fungal spores may be a contributory factor to the increase in the total nitrate content of rusted leaves, since although many fungi display slow rates of nitrate reduction, they have not totally lost the ability to utilize nitrate as a source of nitrogen (McLean, 1982). On the other hand, Murray and Ayres (1986a) found relatively small amounts of nitrogen in mycelium and conidia of the powdery mildew fungus **Erysiphe graminis hordei.** Interestingly however, although the barley leaf itself contained the majority of organic nitrogen, the concentration of nitrogen, on a fresh weight basis, was much higher in the mildew mycelium than in the leaf fraction. Nevertheless, these authors concluded that direct losses of nitrogen from leaves did not markedly affect the nitrogen budget constructed for the barley/powdery mildew interaction. It is interesting to note that Ahmad **et al**,(1982) reported that although uptake of nitrate by roots of brown-rusted barley plants was much increased, the total nitrogen content of rusted plants was much lower than controls, and suggested that as much as 55% of nitrogen was lost in the rust spores. This serves to illustrate the danger of generalizing about results obtained from different pathosystems.

Interestingly, a small increase in the total nitrogen concentration was detected in leaves of rusted leeks. This agreed with the recent work of Paul and Ayres (1987a), who found somewhat larger increases in total nitrogen in rusted groundsel. Also,

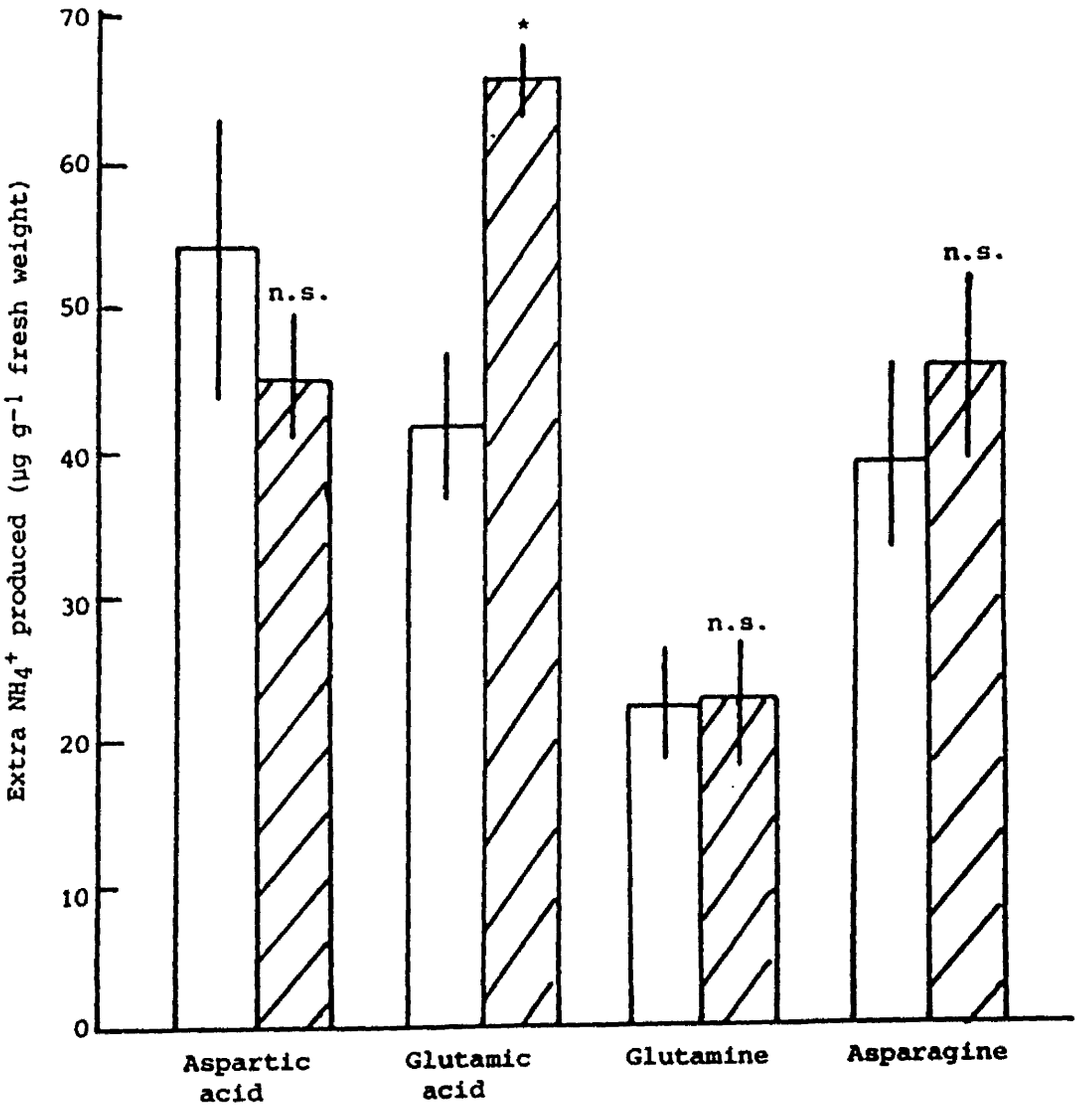



Figure 27. Degradation of amino acids and amides by healthy leek leaves (□) and by leaves infected with rust (▨). Measurements were made at 12 days after inoculation. Each value is the mean of four replicates with standard error. Differences between means significant at $P<0.05$ (*), n.s., not significant

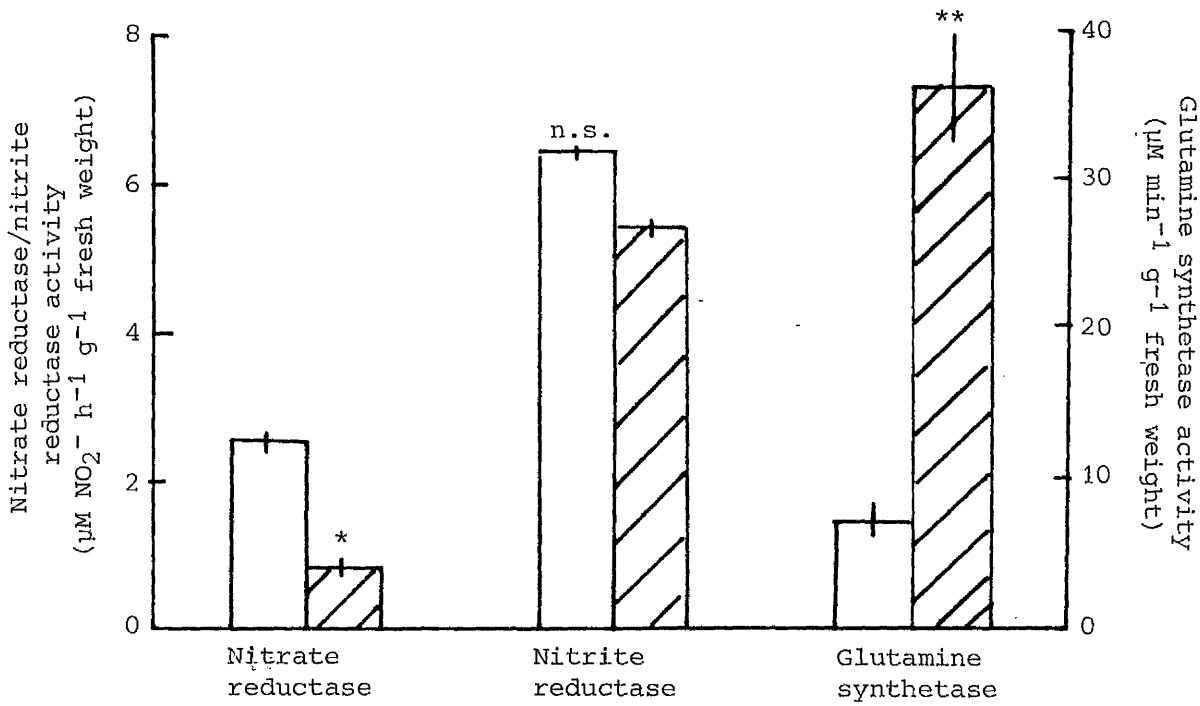



Figure 28. Activities of enzymes involved in nitrate and ammonium assimilation in healthy leek leaves (□), and in leaves infected with rust (▨). Measurements were made at 12 days after inoculation. Each value is the mean of four replicates with standard error. Differences between means significant at $P<0.05$ (*); $P<0.01$ (**), n.s, not significant.

previous research by Calonge (1967) indicated that following infection of barley with brown rust, the total nitrogen content was initially increased in infected leaves. After a series of fluctuations, nitrogen concentration maintained a value of more than twice that in the healthy leaf by around six weeks after inoculation. In rusted leek leaves, the increased nitrate content is not enough to balance the much larger decrease in ammonium nitrogen in the tissue. Perhaps changes in the concentration of amino acids and polyamines, known to occur in other host/pathogen systems (Murray and Ayres, 1986a; Greenland and Lewis, 1984) may play a role in the alterations in total nitrogen found in the leek/rust system.

Previous research by other workers (Walters and Ayres, 1980) showed that infection by powdery mildew caused a reduction in the nitrate content in all tissues of barley plants, while ammonium content increased. These authors suggested that the reduction in nitrate was probably the result of reduced uptake of the nitrate ion. Nitrate represents about 45% of all nitrogenous materials translocated to the shoot in barley plants (Pate, 1973), and therefore reduced nitrate uptake was probably also the cause of the reduction in nitrate in infected and uninfected leaves (Walters and Ayres, 1980). Depression of nitrate uptake **in vivo** occurred during the period 2 to 6 days after inoculation of barley with powdery mildew (Murray and Ayres, 1986a). These workers suggested that such responses may have resulted from changes in the morphology of the root system caused by infection (Walters and Ayres, 1982), although they stressed that increased shoot demand for nitrogen was probably the more likely cause. A substantial reduction in nitrate reductase

(NR) activity was also reported in mildewed barley (Murray and Ayres, 1986b). On the other hand, Sadler and Scott (1974) measured the nitrate content of mildewed leaves but found no effects of infection. In this respect, their results resemble those from investigations of the nitrate content of rust-infected leaves of wheat (Rohringer, 1957) and rye (Piening, 1972). The activity of NR was reduced in leek leaves infected with rust, which could account, in part, for the accumulation of nitrate reported here.

In the light of all the apparent inconsistencies in the literature regarding nitrate content of diseased tissues, it is important to note that the leek/rust and particularly the barley/mildew systems are probably not directly comparable, since the groups studying mildewed barley used young seedlings while the experiments reported here were performed on four month old leeks.

The reduced ammonium content in leaves of leek conflicts with the increases in ammonium nitrogen in leaves of various cereals infected with powdery mildews (Sadler and Scott, 1974; Walters and Ayres, 1980) and rusts (Murphy, 1936; Farkas and Kiraly, 1961; Blumenbach, 1968). Sadler and Scott (1974) concluded that the incorporation of ammonium ions into amino acids was limited by the supply of $\alpha$-ketoglutarate. These authors also suggested that at least two separate pools of ammonium occur in infected tissues and that increased ammonium production was derived from an increased breakdown of amino acids and amides. Walters and Ayres (1980) showed that roots of mildewed barley also accumulated ammonium ions, which they suggested was the result, not only of an enhanced degradation of amino-acids and amides, but also to a much decreased assimilation of ammonium via both the GS-GOGAT and glutamate

dehydrogenase pathways. The breakdown of amino acids and amides in mildewed and rusted plants may have two common causes. First, it is known that high levels of ammonium ions in tissues can lead to the breakdown of amino acids and amides (Lea and Fowden, 1975). Second, in infected plants there may be the need for the redirection of more carbon skeletons to the tricarboxylic acid cycle.

It is difficult to reconcile the results obtained for rusted leek leaves with many of the observations presented by other authors. However, the reduction in ammonium content in leaves of rusted leeks could be explained in terms of decreased ammonium production or by increased ammonium utilization. The activities of some of the enzymes involved in ammonium production and utilization were examined in an attempt to explain the observed reductions in ammonium content in rusted leeks. Our results showed that nitrite reductase (NiR) was not signficantly altered following infection. Also, with the exception of glutamic acid, amino acid and amide breakdown was not substantially altered. This suggested that increased ammonium utilization may have been responsible for the reduction in ammonium content in rusted leeks, perhaps partly via the activity of glutamine synthetase.

Glutamine synthetase (GS) activity was substantially increased in leek leaves infected with rust. The origin of this increased GS activity (i.e. host, fungal or both) is not known. It is tempting to suggest that the increases reported here derive mainly from the host, since very little fungal material was present at the time of the experiment. Furthermore, it has been suggested that glutamate dehydrogenase (GDH) is more important in the assimilation of ammonium in fungi (Genetet, Martin and Stewart, 1984), although it

is known that the GS/GOGAT pathway may be used under certain nutrient deficient conditions. In view of the changes in structure and function of host chloroplasts induced by many rust fungi (see Walters, 1985), it would be interesting to know something of the distribution of host GS activity between the cytosolic $GS_1$, and chloroplastic $GS_2$ isoforms. In wheat and barley, more than 80% of the GS present in leaves is of the $GS_2$ isoform, localized in the chloroplast (McNally, Hirel, Gadal, Mann and Stewart, 1983), and since there is a loss of chloroplast ribosomes in barley as early as one day after inoculation with powdery mildew (Dyer and Scott, 1972), it is difficult to reconcile these observations with the increased $GS_1$ activity demonstrated in mildewed barley (Sadler and Scott, 1974) and rusted wheat (Farkas and Kiraly, 1961). It is possible that either there is a much increased activity of the cytosolic GS, isoform in infected plants or infection in these particular systems has led to the appearance of new isoenzymes (Wheeler, 1975). Furthermore, given the accumulation of nutrients and assimilates at infection sites of rust fungi (Greenland and Lewis, 1983), and the effect of carbohydrates and ATP in enhancing GS activity (Lea and Fowden, 1975), it may be useful to examine the distribution of GS activity within discrete areas of infected leaves.

It has been shown that leaves of barley infected with powdery mildew liberated appreciable amounts of ammonia gas (Sadler and Scott, 1974). The results obtained for rusted leeks indicate that although healthy leeks exhibited slower rates of ammonia evolution, the rates were considerably higher in rust-infected leaves. Some of the ammonia may have been of fungal origin, but since separation of

the rust from the host plant is difficult, it is impossible to be more conclusive. However, it may be of importance to note that ammonia is evolved during the biosynthesis of polyamines from arginine (Adiga and Prasad, 1985) and that substantial increases in polyamine accumulation have been found in rusted and mildewed plants (Greenland and Lewis, 1984; Walters, Wilson and Shuttleton, 1985). The possibility that the increased ammonia evolution by rusted leek leaves stems from polyamine accumulation should not be ruled out.

SECTION 8

# A Look to the Future...

The research presented in this thesis has attempted to improve our understanding of the interaction between leeks and the leek rust fungus. As with other pathosystems, it seems clear that the changes elicited in the infected host's metabolism are far from straightforward, and although different areas of metabolism were examined, they should not be considered as being mutually exclusive. A lot of research still has to be done on this host/parasite interaction before we can claim to fully understand the leek/rust system. The following section describes avenues of research that are potentially important, and may yield interesting and valuable results.

In view of the results obtained from studies on discrete regions of infected leaves, and the value of such studies in determining pathogen-induced alterations **in situ**, more research could be carried out on localized areas of the infected leek leaf. Measurements of photosynthesis and growth regulators in different areas of rusted leaves provided useful information (Section 5), but, if time had permitted, there were several other parameters worthy of investigation. Due to limited availability of material, photosynthetic measurements were made at 14 and 21 days after inoculation, i.e. prior to green-island formation. The whole question of green-island physiology in rusted leek leaves has yet to be examined. Earlier investigations, particularly those of Thrower (1965) and Wang (1961), showed by use of $^{14}CO_2$, that green islands induced by rust in clover and in bean leaves were photosynthetically active. More recently, Scholes and Farrar (1985) showed that green islands surrounding pustules of **Uromyces muscari** on bluebell leaves were also photosynthetically active. They showed that the rate of

oxygen evolution and the fluorescence kinetics of green-island tissue were similar to those for presporulation pustules rather than to those of control leaves. However, they went on to suggest that this may not have been a direct consequence of fungal colonization, since hyphae were not present throughout all the green-island tissue (Scholes and Farrar, unpublished). Our results show that photosynthesis is affected by rust infection, being reduced in pustule regions.

Since discrete regions of infected leek leaves appear to be physiologically different, it may be useful to examine other areas of host metabolism in these areas, particularly carbohydrate and nitrogen metabolism, coupled with an examination of fungal nutrition. The pustules of rust fungi act as foci for the accumulation of many metabolites. Not only does the fungus derive nutrients by alteration of direction of transport in the phloem, nutrients are also made available by enhanced transpiration following rupture of host epidermis during sporulation (Lewis, 1976). Although some workers have recorded decreases in the carbohydrate content at the sites of biotrophic infection, most studies show a marked initial accumulation (see Long, Fung, McGee, Cooke and Lewis, 1975). Experiments involving rust infections of **Tussilago farfara** and **Poa pratensis** (both hosts for **Puccinia poarum**) revealed that at infection sites, there was an accumulation of host sucrose, glucose, free fructose and fructose polymers (Holligan, Chen and Lewis, 1973). Concomitant with these alterations to the carbohydrate composition of host cells, the fungus accumulated mannitol, arabitol and glycogen (Holligan **et al**, 1973; Holligan, Chen, McGee and Lewis, 1974).

Such accumulation of nutrients and metabolites in and around pustules may help maintain cellular function and 'juvenility' in these pustules and so lead to the phenomenon of green-island formation. Metabolite accumulation associated with pustules may help to explain the recently reported increases in polyamine concentrations in infected leaves (e.g. Greenland and Lewis, 1984; Walters, Wilson and Shuttleton, 1985; Bailey, Bower and Lewis, 1987). Walters and Wylie (1986) have shown that in mildewed barley, these increased polyamines are localised in regions around mildew pustules. Recent work in this laboratory has also shown that massive accumulations of polyamines occur in green-islands on detached barley leaves and are associated with a substantial increase in the activity of arginine decarboxylase (ADC) (Coghlan and Walters, personal communication). ADC activity and polyamine concentrations are known to increase in response to osmotic and water stress (Galston, 1983) and it is possible that accumulation of nutrients and metabolites in and around fungal pustules might lead to osmotic or water stress in these regions, resulting in enhanced ADC activity and polyamine concentration. If increased polyamine concentrations were involved in retardation of senescence in these regions, then green-island formation would again appear to be an indirect consequence of nutrient accumulation at infection sites.

It is also interesting to note that the application of any stimulating plant growth regulator (cytokinin, auxin, etc.) to plant tissues results in a marked increase in polyamine biosynthesis and concentration (Galston, 1983; Coghlan and Walters, personal communication). Walters and Wylie (1986) have suggested that the increased polyamine concentration in and around mildew pustules

could be involved in the formation of green islands and the maintenance of 'juvenility' in these areas. In view of the alteration in growth substances in discrete regions of rusted leek leaves and the other changes described above, a detailed time-course study of green-island physiology in the leek/rust system may prove to be very rewarding.

As with many host/pathogen interactions, photosynthesis was reduced in rusted leek leaves long before sporulation occurred. The question why photosynthesis is affected so early in the infection process has not yet been answered. One of the earliest alterations in host metabolism induced by infection with obligate parasitic fungi is the reduction in chloroplast polysome content described in mildewed barley by Dyer and Scott (1972). Scott (1972) suggested that this reduction was undoubtedly related to the disease symptoms detectable at later stages of infection, such as reduced photosynthesis and losses of chlorophyll. More recently, Manners and Scott (1983) reported reduced amounts of **in vitro** protein synthesis by chloroplast polysomes isolated from mildewed barley leaves, as early as 24 hours after inoculation. There is evidence to suggest that polysome breakdown is mediated by alterations in ribonuclease (RNase) activity following infection (Chakravorty and Scott, 1979). More specific evidence to suggest that early reductions in photosynthesis are linked to alterations in RNA metabolism comes from Higgins, Manners and Scott (1985). These authors have shown that following infection of barley with powdery mildew, three messenger RNA species coding for both the large and small subunits of RuBPcase, and for the thylakoid protein 32k DP, were reduced compared to controls. These reductions were observed

as early as one day after inoculation. The powdery mildew fungus infects only epidermal cells which lack developed chloroplasts, yet the effects on host gene expression must occur in photosynthetic cells. The mechanism by which these effects are transmitted through the host tissue is not known. However, since rust fungi develop throughout the host plant by means of intercellular hyphae, as well as haustorial structures, it may be easier for them to effect changes in host RNA metabolism. This is certainly an area that is worthy of further research, since it may enable us to explain why carbon fixation is altered so early after inoculation, before any visible symptoms of disease are present.

Finally, recent research has indicated that uninfected tissues within an infected host may compensate for lowered photosynthetic activity in diseased plant parts (Ayres, 1981; Williams and Ayres, 1981; Walters and Ayres, 1983; Roberts and Walters, 1986). It would seem that leeks infected by rust provide an ideal system for the elucidation of the mechanism underlying these stimulations in photosynthesis, since in this pathosystem, the stimulations are very large and of a very long duration. It would be interesting to examine whether the photosynthetic capacity of the uninfected leaf is related to, for example, observed changes in growth regulators. Is gene expression altered in these leaves, or chloroplast integrity perhaps? Further research in these areas would be useful, not only in explaining how the pathogen mediates changes in plant parts that are a great distance from the initial infection site, but also in examining the significance of these enhanced photosynthetic rates in both host and pathogen survival. Further, if such stimulations of photosynthesis in uninfected leaves on infected plants represents a

more general plant response (e.g. it does occur in partially defoliated plants) then it may prove useful to examine the phenomenon in plants infected with a range of different pathogens (e.g. necrotrophs like net blotch, leaf scald, etc.). Certainly, future work must include field measurements since plant responses to infection in the field may be different to responses observed in laboratory conditions.

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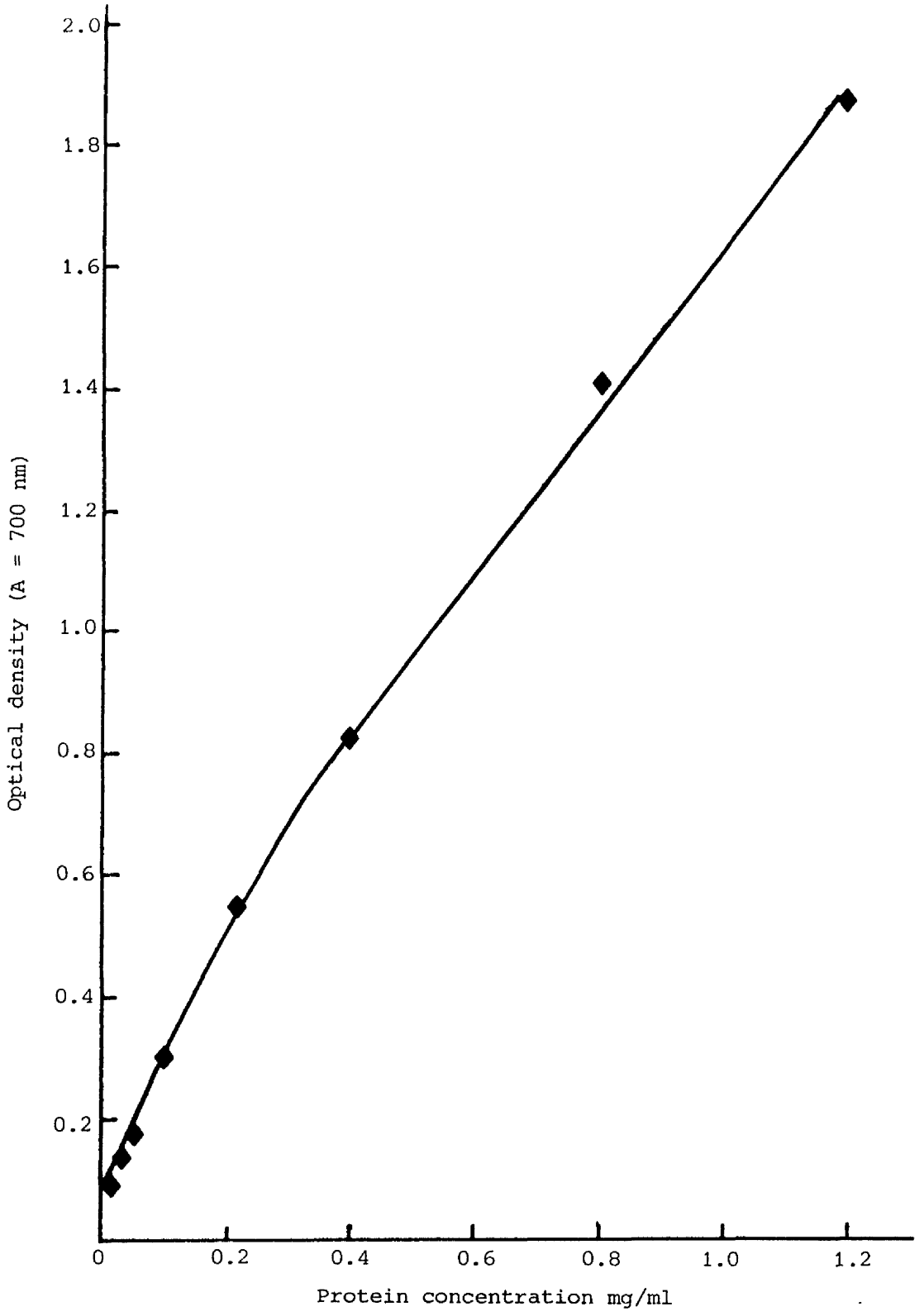
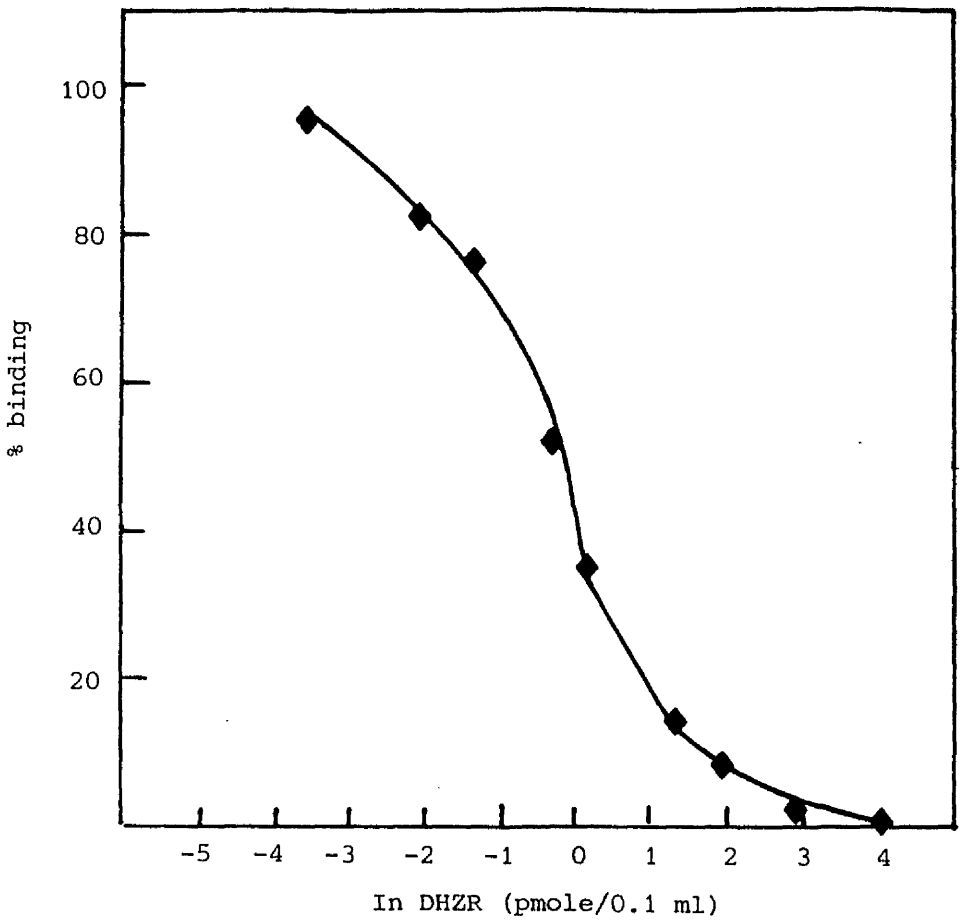
# APPENDIX I

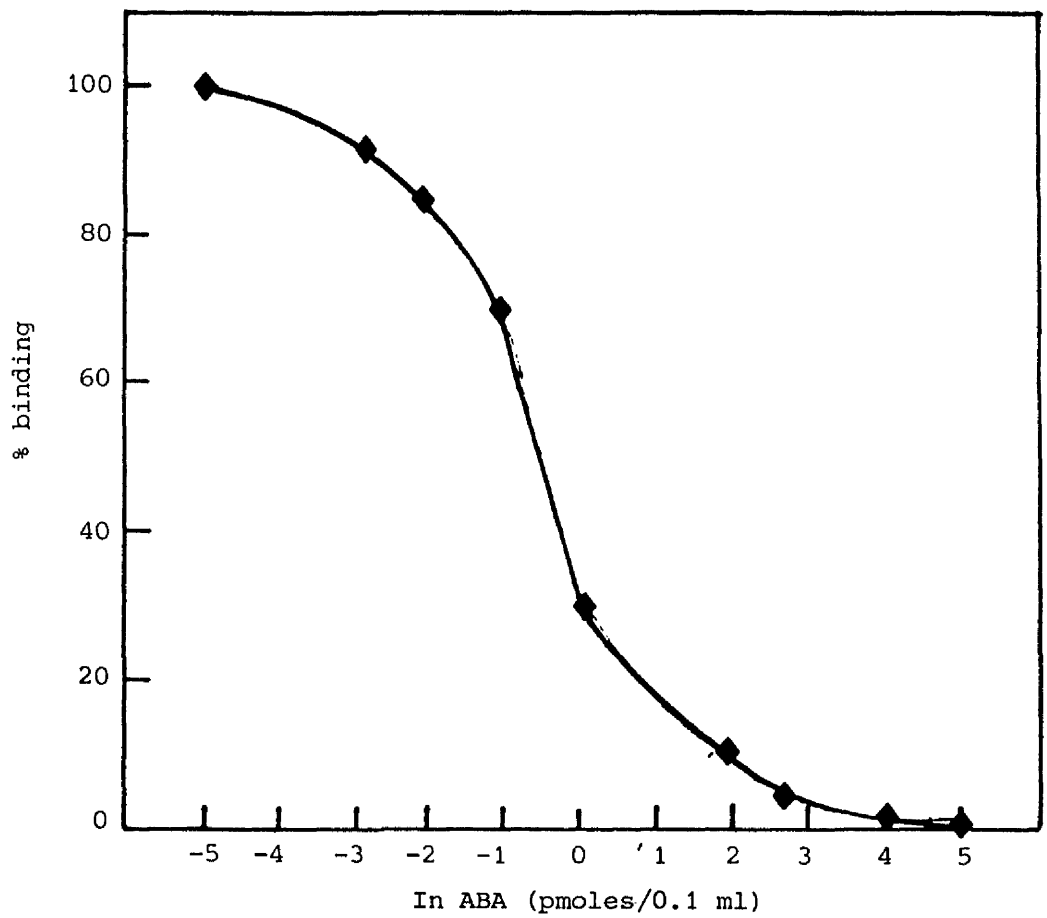
## Calibration Curves



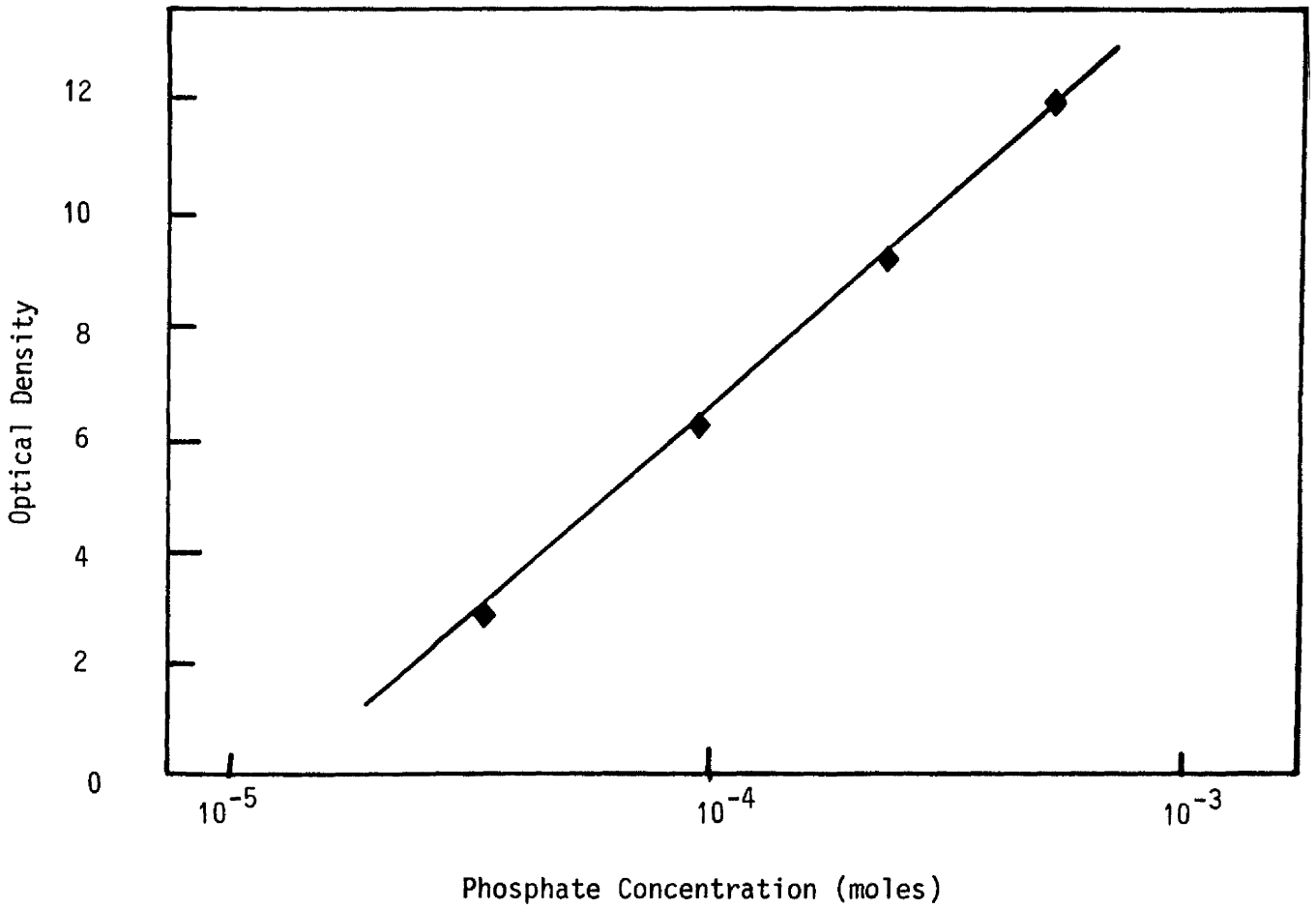

Protein Calibration Curve



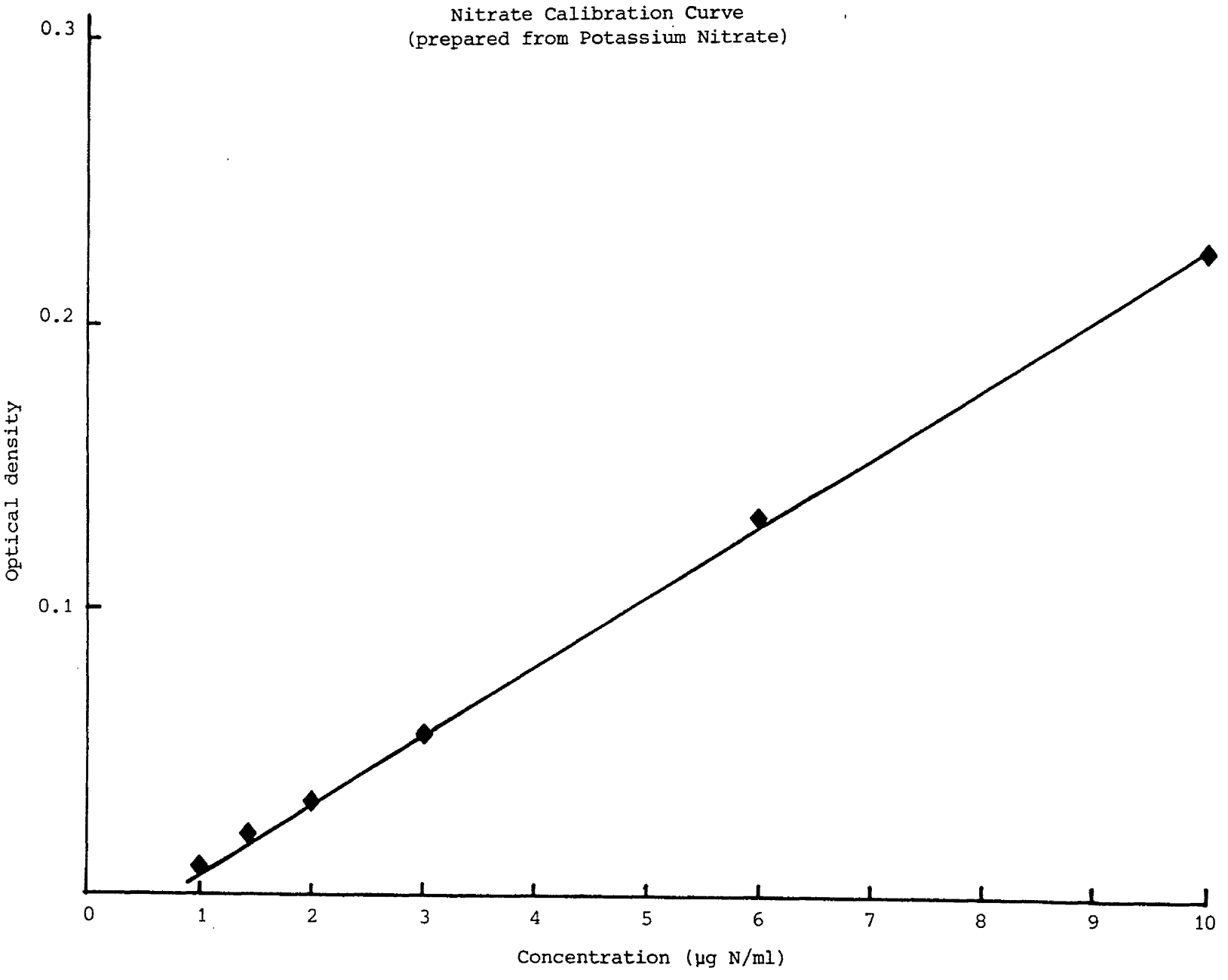

DHZR Standard Curve

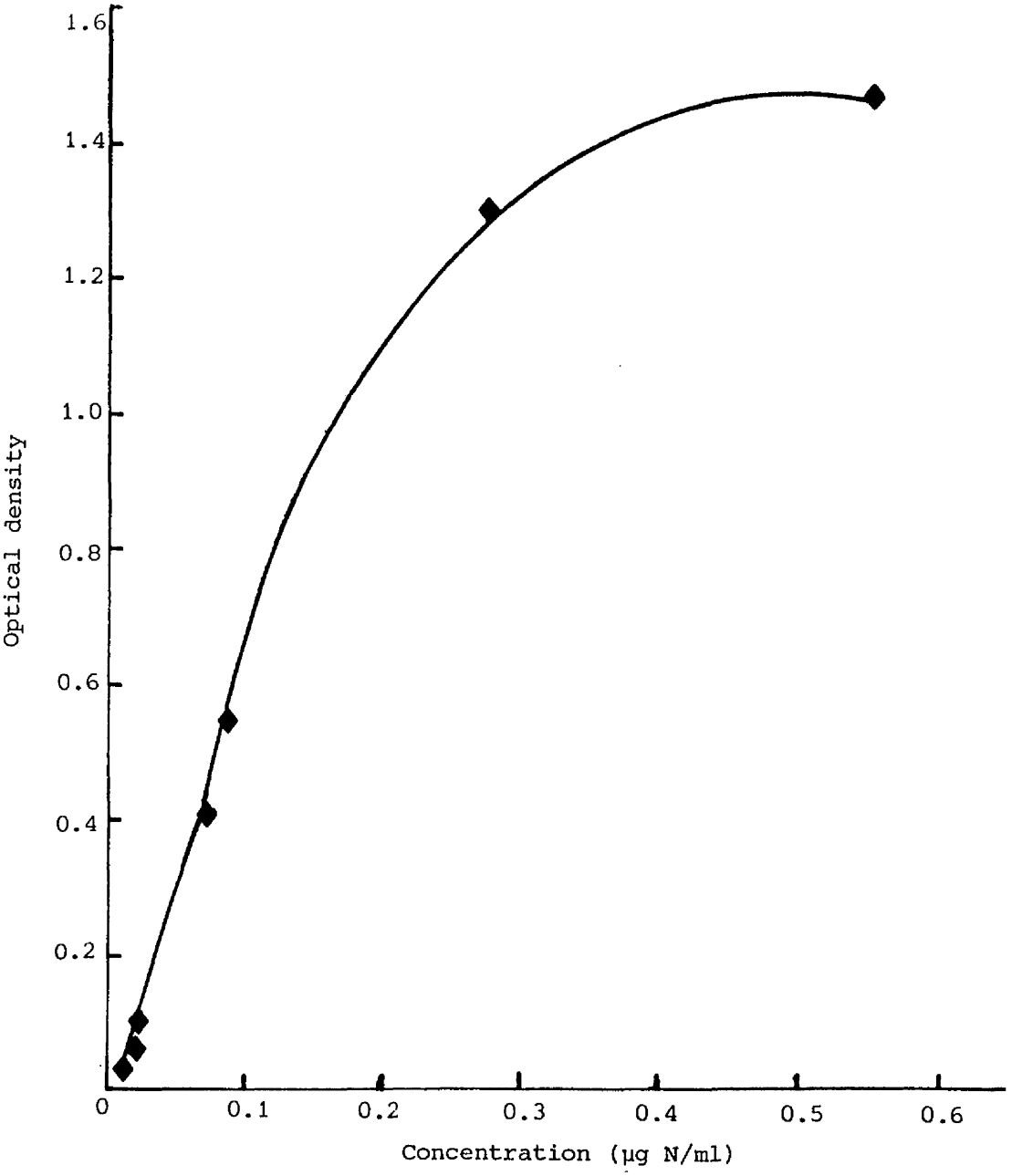



ABA Standard Curve

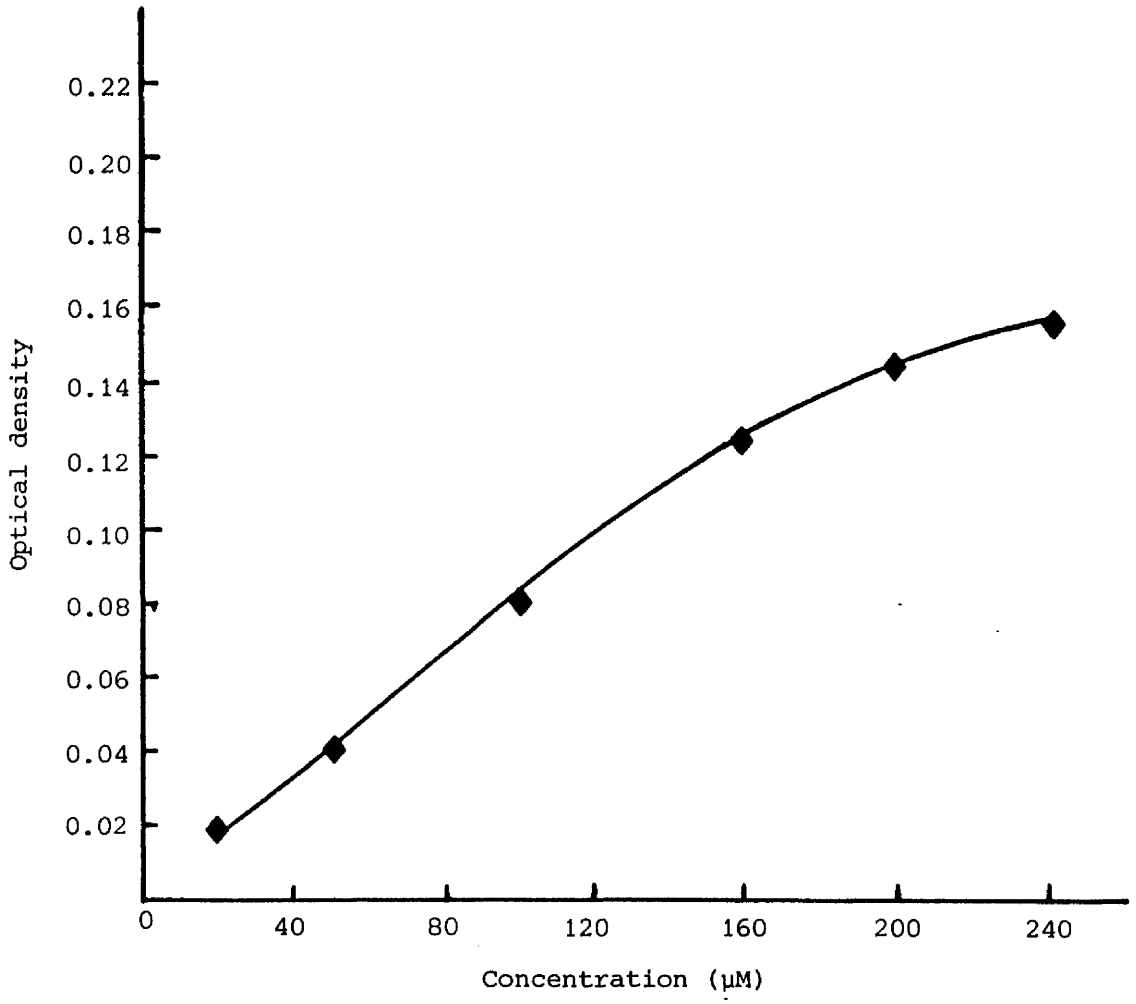



Calibration Curve for Phosphate ( using Potassium Phosphate )


Nitrate Calibration Curve
(prepared from Potassium Nitrate)
Optical density
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
Concentration (µg N/ml)




Nitrite Calibration Curve
(prepared from potassium nitrite)




Glutamine Synthetase Calibration Curve
(prepared from L-Glutamic Acid-8-hydroxymate)

APPENDIX II

**Content and Concentrations of Minerals in Roots and Shoots of Healthy and Rusted Leeks**

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 77.9 ± 6.23 | 110.7 ± 10.52 | 182.2 ± 10.93 | 230.1 ± 16.11 | 265.3 ± 18.83 |
| 10% rust | 88.5 ± 6.20 | 109.8 ± 5.27 | 148.9 ± 5.81 | 184.2 ± 7.18 | 380.4 ± 19.02 |
| 20% rust | 50.9 ± 5.12 | 91.8 ± 9.08 | 124.3 ± 7.45 | 150.4 ± 7.97 | 377.6 ± 15.10 |
| 30% rust | 56.4 ± 3.38 | 98.8 ± 6.52 | 124.7 ± 11.22 | 171.4 ± 12.00 | 268.4 ± 12.62 |
| Concentration (mg/g) | | | | | |
| Control | 42.1 ± 3.43 | 35.7 ± 3.79 | 36.0 ± 2.50 | 30.1 ± 2.11 | 18.2 ± 1.33 |
| 10% rust | 52.3 ± 3.56 | 41.4 ± 1.99 | 44.2 ± 1.73 | 45.3 ± 1.77 | 26.1 ± 1.32 |
| 20% rust | 52.3 ± 5.99 | 50.6 ± 5.01 | 47.7 ± 3.07 | 39.8 ± 2.11 | 37.9 ± 1.48 |
| 30% rust | 51.7 ± 2.98 | 47.4 ± 3.14 | 49.1 ± 4.43 | 38.6 ± 3.19 | 36.6 ± 1.73 |

Table 1. The amount and concentration of nitrogen in shoots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 19.0 ± 1.50 | 16.0 ± 1.70 | 27.8 ± 1.71 | 31.9 ± 2.04 | 68.9 ± 5.03 |
| 10% rust | 11.8 ± 0.80 | 23.8 ± 1.19 | 27.9 ± 1.11 | 30.5 ± 1.37 | 77.2 ± 3.90 |
| 20% rust | 6.4 ± 0.58 | 18.3 ± 1.46 | 24.2 ± 1.56 | 30.6 ± 1.53 | 63.1 ± 2.39 |
| 30% rust | 9.1 ± 0.55 | 13.3 ± 0.80 | 25.6 ± 2.84 | 29.4 ± 2.43 | 63.2 ± 3.16 |
| Concentration (mg/g) | | | | | |
| Control | 42.6 ± 3.46 | 29.3 ± 2.93 | 26.2 ± 1.61 | 20.6 ± 1.44 | 23.4 ± 1.68 |
| 10% rust | 31.7 ± 2.17 | 39.4 ± 1.89 | 35.8 ± 1.40 | 32.5 ± 1.30 | 34.2 ± 1.71 |
| 20% rust | 35.4 ± 4.06 | 42.4 ± 4.19 | 36.3 ± 2.36 | 32.2 ± 1.61 | 27.4 ± 1.09 |
| 30% rust | 37.3 ± 2.15 | 33.0 ± 2.04 | 40.8 ± 3.69 | 31.7 ± 2.63 | 28.3 ± 1.42 |

Table 2. The amount and concentration of nitrogen in roots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 10.3 ± 0.49 | 16.2 ± 1.22 | 25.9 ± 1.69 | 37.4 ± 4.23 | 73.5 ± 8.03 |
| 10% rust | 10.3 ± 1.28 | 15.9 ± 0.91 | 21.5 ± 1.49 | 24.6 ± 1.51 | 87.6 ± 7.59 |
| 20% rust | 5.8 ± 0.77 | 10.1 ± 0.86 | 15.8 ± 0.73 | 23.3 ± 1.64 | 61.8 ± 3.35 |
| 30% rust | 6.3 ± 0.53 | 12.1 ± 0.69 | 14.9 ± 2.36 | 27.6 ± 1.54 | 57.6 ± 3.50 |
| Concentration (mg/g) | | | | | |
| Control | 57.7 ± 1.49 | 55.1 ± 1.46 | 53.7 ± 2.93 | 51.2 ± 4.88 | 52.4 ± 1.46 |
| 10% rust | 62.8 ± 1.47 | 62.4 ± 0.73 | 66.6 ± 1.22 | 63.7 ± 1.22 | 61.4 ± 0.73 |
| 20% rust | 63.2 ± 2.75 | 59.0 ± 1.22 | 63.4 ± 1.22 | 64.4 ± 2.19 | 64.6 ± 0.73 |
| 30% rust | 60.0 ± 0.72 | 60.7 ± 0.97 | 60.5 ± 1.95 | 65.1 ± 2.44 | 63.2 ± 0.48 |

Table 3. The amount and concentration of phosphate in shoots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 2.0 ± 0.16 | 2.4 ± 0.38 | 4.7 ± 0.18 | 6.3 ± 0.29 | 12.3 ± 1.21 |
| 10% rust | 1.7 ± 0.14 | 2.9 ± 0.21 | 3.8 ± 0.21 | 4.6 ± 0.46 | 11.1 ± 0.92 |
| 20% rust | 0.8 ± 0.19 | 2.0 ± 0.14 | 3.2 ± 0.13 | 4.5 ± 0.37 | 10.9 ± 0.47 |
| 30% rust | 1.1 ± 0.17 | 1.9 ± 0.16 | 2.9 ± 0.31 | 4.4 ± 0.40 | 10.7 ± 0.63 |
| Concentration (mg/g) | | | | | |
| Control | 46.4 ± 0.38 | 45.6 ± 0.72 | 46.3 ± 0.24 | 42.4 ± 0.24 | 43.7 ± 0.24 |
| 10% rust | 49.5 ± 0.94 | 51.5 ± 0.24 | 51.5 ± 0.22 | 51.5 ± 0.24 | 51.5 ± 0.19 |
| 20% rust | 50.7 ± 0.89 | 49.3 ± 0.97 | 50.0 ± 0.24 | 49.8 ± 0.24 | 49.8 ± 0.24 |
| 30% rust | 48.8 ± 0.57 | 48.0 ± 0.17 | 48.5 ± 0.24 | 49.5 ± 0.24 | 50.2 ± 0.73 |

Table 4. The amount and concentration of phosphate in roots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 132.7 ± 10.61 | 172.5 ± 17.24 | 284.5 ± 17.49 | 383.7 ± 26.86 | 626.7 ± 45.75 |
| 10% rust | 136.4 ± 9.55 | 190.6 ± 7.62 | 249.4 ± 7.48 | 272.5 ± 10.62 | 762.3 ± 38.11 |
| 20% rust | 72.4 ± 7.96 | 149.9 ± 13.49 | 206.9 ± 13.34 | 236.5 ± 11.82 | 565.9 ± 22.07 |
| 30% rust | 77.9 ± 4.67 | 155.8 ± 9.34 | 176.7 ± 16.08 | 278.9 ± 23.09 | 338.1 ± 13.52 |
| Concentration (mg/g) | | | | | |
| Control | 71.7 ± 5.87 | 55.6 ± 5.89 | 56.2 ± 3.37 | 50.2 ± 3.56 | 43.0 ± 3.14 |
| 10% rust | 80.6 ± 5.64 | 71.9 ± 3.52 | 74.0 ± 2.89 | 67.0 ± 2.61 | 52.3 ± 2.65 |
| 20% rust | 74.4 ± 7.45 | 82.6 ± 8.17 | 79.4 ± 5.16 | 62.6 ± 2.32 | 56.8 ± 2.21 |
| 30% rust | 71.4 ± 4.07 | 74.7 ± 4.93 | 69.6 ± 6.26 | 62.8 ± 5.15 | 46.1 ± 2.30 |

Table 5. The amount and concentration of potassium in shoots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 43.4 ± 3.04 | 37.2 ± 3.45 | 84.2 ± 4.21 | 58.8 ± 4.41 | 154.4 ± 11.42 |
| 10% rust | 32.9 ± 1.97 | 52.5 ± 2.52 | 61.3 ± 2.39 | 72.4 ± 2.17 | 158.4 ± 8.01 |
| 20% rust | 16.1 ± 1.61 | 37.8 ± 3.40 | 60.6 ± 3.64 | 73.7 ± 3.32 | 125.9 ± 5.04 |
| 30% rust | 21.9 ± 1.26 | 27.5 ± 1.78 | 57.6 ± 5.22 | 68.4 ± 4.10 | 125.9 ± 7.55 |
| Concentration (mg/g) | | | | | |
| Control | 97.1 ± 7.77 | 68.1 ± 6.81 | 79.3 ± 4.92 | 38.0 ± 2.69 | 52.4 ± 3.82 |
| 10% rust | 88.2 ± 5.29 | 87.0 ± 3.48 | 78.7 ± 3.07 | 77.2 ± 3.09 | 70.2 ± 3.55 |
| 20% rust | 88.9 ± 9.78 | 87.7 ± 8.59 | 90.8 ± 5.81 | 77.7 ± 4.12 | 54.7 ± 2.13 |
| 30% rust | 89.9 ± 5.39 | 68.0 ± 4.08 | 91.9 ± 8.27 | 73.8 ± 6.12 | 56.4 ± 2.65 |

Table 6. The amount and concentration of potassium in roots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 8 |
| Amount (mg) | | | | | |
| Control | 12.9 ± 1.03 | 22.3 ± 2.23 | 36.4 ± 2.18 | 51.2 ± 3.58 | 104.9 ± 7.66 |
| 10% rust | 12.7 ± 0.76 | 20.4 ± 2.04 | 21.2 ± 0.64 | 37.0 ± 1.11 | 155.9 ± 7.79 |
| 20% rust | 7.3 ± 0.73 | 15.8 ± 1.54 | 19.0 ± 1.14 | 33.2 ± 1.76 | 93.6 ± 2.81 |
| 30% rust | 7.2 ± 0.43 | 16.3 ± 0.97 | 17.8 ± 1.60 | 38.2 ± 3.13 | 122.5 ± 6.12 |
| Concentration (mg/g) | | | | | |
| Control | 7.0 ± 0.56 | 7.2 ± 0.70 | 7.2 ± 0.43 | 6.7 ± 0.47 | 7.2 ± 0.53 |
| 10% rust | 7.5 ± 0.50 | 7.7 ± 0.40 | 6.3 ± 0.25 | 9.1 ± 0.36 | 10.7 ± 0.54 |
| 20% rust | 7.5 ± 0.82 | 8.7 ± 0.86 | 7.3 ± 0.44 | 8.8 ± 0.47 | 9.4 ± 0.38 |
| 30% rust | 6.6 ± 0.33 | 7.8 ± 0.32 | 7.0 ± 0.56 | 8.6 ± 0.71 | 16.7 ± 0.78 |

Table 7. The amount and concentration of calcium in shoots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

| | Weeks after inoculation | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 |
| Amount (mg) | | | | | |
| Control | 3.0 ± 0.24 | 3.7 ± 0.35 | 4.9 ± 0.31 | 6.6 ± 0.43 | 13.2 ± 0.93 |
| 10% rust | 2.1 ± 0.10 | 4.8 ± 0.23 | 7.8 ± 0.23 | 5.9 ± 0.24 | 16.2 ± 0.81 |
| 20% rust | 1.0 ± 0.12 | 3.2 ± 0.25 | 4.3 ± 0.27 | 5.8 ± 0.29 | 15.9 ± 0.63 |
| 30% rust | 1.3 ± 0.07 | 3.6 ± 0.24 | 3.3 ± 0.28 | 6.9 ± 0.48 | 13.4 ± 0.63 |
| Concentration (mg/g) | | | | | |
| Control | 8.5 ± 0.69 | 6.8 ± 0.66 | 4.7 ± 0.35 | 4.3 ± 0.29 | 4.5 ± 0.31 |
| 10% rust | 5.6 ± 0.37 | 8.0 ± 0.38 | 10.0 ± 0.25 | 6.3 ± 0.25 | 7.2 ± 0.36 |
| 20% rust | 5.7 ± 0.56 | 7.5 ± 0.71 | 6.4 ± 0.41 | 6.1 ± 0.32 | 6.9 ± 0.28 |
| 30% rust | 5.2 ± 0.31 | 9.0 ± 0.54 | 5.2 ± 0.36 | 7.5 ± 0.60 | 6.0 ± 0.28 |

Table 8. The amount and concentration of calcium in roots of leek plants infected with different levels of rust and in healthy controls. All values are the mean of four replicates with standard error.

**Excision of Discrete Regions of Rusted Leaves**

The different regions of the rusted leaf under examination were pustule regions and inter-pustule regions. These were excised from whole leaves by means of a 5mm cork borer. Twenty leaf discs (approximately 500mg) were collected for each tissue type, and were subsequently placed in the oxygen electrode. Photosynthetic measurements were then conducted as described in Section 4, page 58.

## Explanation of Tables 7a and 7b.

Table 7a

Assimilate production by individual leaves of control and rusted leeks over a 16h photoperiod was calculated by assuming a constant rate of net leaf photosynthesis throughout the photoperiod (see Owera **et al**, 1981). Indeed, measurements of net photosynthesis of leek leaves at periods throughout the photoperiod showed little variation. Total production of assimilate plant$^{-1}$ day$^{-1}$ was calculated as the sum of assimilate production by all individual leaves.

Plant growth in 1 16h photoperiod was calculated from the relative growth rates of healthy and rusted leaves. Relative growth rates were calculated using data from Table 5.

Table 7b

Assimilate translocation out of individual leaves is based on data from Table 8 and explanations of its calculation have already been given (see overleaf).

Total translocation plant$^{-1}$ day$^{-1}$ is the sum of assimilate translocation out of individual leaves.

**Explanation of Components of Carbon Budget (Table 8).**

NET PHOTOSYNTHESIS

Based on data from Table 4.

LEAF RESPIRATION

Based on data from Table 4: for leaves 1,2 and 3 (infected), this is the control value only. For leaves 4 and 5 (uninfected), the values are for control (healthy) and uninfected leaf on the infected plant (rusted).

C STORAGE IN LEAF

'C storage in leaf' was calculated by Owera **et al**(1981) from the difference in leaf weight in healthy and rusted leaves. This was not possible for the leek/rust system since rusted leaves weighed less than healthy controls. Therefore, it has been assumed that C storage in rusted leaves will be similar in proportion to that calculated for rusted barley (Owera **et al**,1981). A value for carbon storage in the uninfected fifth leaf on infected plants was estimated because these leaves were still rapidly expanding at the time of the experiment and would probably have been net importers of assimilate.

FUNGAL RESPIRATION

Based on rusted dark respiration-healthy dark respiration (from Table 4).

GROSS FUNGAL C UPTAKE

'C storage' + fungal respiration

TRANSLOCATION

Net photosynthesis - 'C storage'