Relative tolerances of wild and cultivated barleys to infection by *Blumeria graminis* f.sp. *hordei* (Syn. *Erysiphe graminis* f.sp. *hordei*).

By

ABDELLAH AKHKHA

A thesis presented for the Degree of

Doctor of Philosophy

In

The Institute of Biomedical and Life Science

Division of Environmental and Evolutionary Biology

At the University of Glasgow

August 1999

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

<u>In the Name of God, the Most Beneficent, the Most Merciful</u>

<< It is He Who sends down rain from the sky, and with it We bring forth vegetation of all kinds, and out of it We bring forth green stalks, from which We bring forth thick clustered grain, and out of the date-palm and its spathe come forth clusters of dates hanging low and near, and gardens of grapes, olives and pomegranates, each similar (in kind) yet different (in variety and taste). Look at their fruits when they begin to bear, and the ripeness thereof. Verily! In these things there are signs for people who believe >>.

Qur'an, Chapter 6: Verse 99.

Declaration

I hereby declare that the work submitted in this thesis is the result of my own investigations except where references are mentioned and assistance is acknowledged. Therefore no part of this thesis has been previously presented for any degree.

Abdellah Akhkha

August 1999

Dedication

To my mum and late father, wife and my brothers

and sisters.

Acknowledgements

I wish to express my thanks and appreciation to, my supervisor Dr. D. D. Clarke for his guidance and stimulating discussions of many aspects of this thesis, Dr. Peter Dominy for his help and advice with the physiological aspects of this work and the construction of O_2 electrode system and Dr. Kevin Murphy for his help and advice throughout. I would like also to thank Dr. Chris Wheeler for his encouragement.

I wish also to acknowledge the help of Aileen Adam, the technician and all members of staff in the Brian Laboratory and in the Arnott Laboratory for their help and interest regarding my research.

Finally, a warm thanks to my mother, and my late father (who died before the completion of this thesis) who dedicated their lives for my education, to my dear wife for her support and patience, to my mother in law for her encouragement and to many friends who have made my stay in Scotland so enjoyable.

Table of contents

List of Tables

List of Figures

List of plates

List of abbreviations

ADP : Adenosine diphosphate

ANOVA : Analysis of variance

ATP : Adenosine triphosphate

Chl : Chlorophyll

Cv. : Cultivar

G.S : Growth stage

GAPDH : Glyceraldehyde phosphate dehydrogenase

GLA : Green leaf area

HCL : Hydrochloric acid

HI : Harvest index

KCL : Potassium chloride

L_A : Leaf area

LAR : Leaf area ratio

L_W : Leaf dry weight

LWR : Leaf weight ratio

MBC : Carbendazim

Mv : Millivolts

N_2 : Nitrogen

NAD : Nicotinamide adenine dinucleotide

NADP : Nicotinamide adenine dinucleotide phosphate (oxidised)

NADPH : Nicotinamide adenine dinucleotide phosphate (reduced)

nm : Nanometre, 10^{-9} metres

PAR : Photosynthetic active radiation

PEP : Phosphoenol pyruvate

PePcase : Phosphoenol pyruvate carboxylase

Pg : Gross photosynthesis

Pg_{max} : Maximum gross photosynthesis

Pi : Orthophosphate, inorganic phosphate

PLRC : Photosynthetic light response curve

Pn : Net photosynthesis

Pn_{max} : Maximum net photosynthesis

PPFD : Photosynthetic photon flux density

PSI : Photosystem I

PSII : Photosystem II

R_A : Root surface area

RAR : Root area ratio

Rd : Dark respiration

R_{dw} : Root dry weight

RGR : Relative growth rate

RH : Relative humidity

R_L : Root length

RLR : Root length ratio

r_p : Physical resistance

RPPC : Reductive pentose phosphate cycle

RSR : Root : shoot ratio

RuBPcase : Ribulose-1,5-bisphosphate carboxylase

RuBPoxygenase : Ribulose-1,5-bisphosphate oxygenase

RWR : Root weight ratio

r_x : Carboxylation resistance

S_{dw} : Shoot dry weight

SLA : Specific leaf area

SRA : Specific root area

SRL : Specific root length

S_W : Shoot dry weight

SWR : Shoot weight ratio

TIFF : Tagged image files

ULR : Unit leaf rate

W : Total plant dry weight

α : Quantum efficiency of photosynthesis

μm : Micrometre, 10^{-6} metres

θ : The ratio of physical to total resistance of CO_2 diffusion into the leaf.

SUMMARY

The relative tolerances of two lines of wild barley (*Hordeum spontaneum*), B19909 and I-17-40 and one cultivated barley (*Hordeum vulgare*), cv. Prisma infected by *Blumeria graminis* f.sp. *hordei* (syn. *Erysiphe graminis* f.sp. *hordei*) were investigated by growth analysis.

Mildew development was assessed as percentage leaf area colonised, and by numbers of conidia produced. At all stages of plant growth, the percentage leaf area colonised by the mildew was slightly higher on the wild line B19909 than on cv. Prisma and much more than on the wild line I-17-40. When mildew was measured as conidial production, line B19909 was found to support more fungal biomass than cv. Prisma and cv. Prisma much more than line I-17-40.

Although line B19909 supported the development of more fungal biomass than cv. Prisma and line I-17-40, its total dry weight was reduced less by infection than either of the latter lines, even cv. Prisma. The growth components of line I-17-40 were reduced to the same level as line B19909 or less presumably because it supported the lowest levels of infection and lowest levels of conidial production. The reduction in total plant dry weight was reflected in reduced primary shoot height, fewer tillers, and reductions in leaf area. All these reductions occurred to a lesser extent in line B19909 than in cv. Prisma and sometimes to a greater extent than in line I-17-40. Root dry weight was also reduced less in line B19909 than in cv. Prisma, but least in line I-17-40. Root development was inhibited more than shoot development in all the lines but the least difference occurred in line B19909. Total length

and surface area of the seminal roots as well as the number of laterals on the seminal roots were reduced in line B19909 to about the same level as in line I-17-40 but much less than in cv. Prisma. Number, total length, diameter and surface area of nodal roots were also reduced much less in line B19909 than in the other two lines.

The reductions in the vegetative parts were not reflected in reductions in yield components such as number and dry weight of grains on the primary shoot, total number and total dry weight of grains on tillers and the proportion of total biomass converted to grain (harvest index). Although reductions in all these components occurred in cv. Prisma they were not affected in the wild lines.

The reductions in dry matter production were partly due to reductions in leaf development and partly to reductions in the rate of photosynthesis per unit leaf area. The effects of infection on the rates of photosynthesis were investigated in three cultivated barleys, Golden Promise, Prisma and Triumph, and in three wild barleys, line B19909, I-17-40 and B8893. Photosynthesis was reduced more in cvs Prisma, Golden Promise and Triumph and in the moderately resistant line I-17-40 than in line B19909, although the latter supported the highest levels of infection. Although cv. Golden Promise supported the production of a similar number of conidia as line B19909, its rate of photosynthesis was more reduced. In contrast, the most resistant line B8893 that supported the production of very few conidia was not affected.

High levels of infection increased the rate of dark respiration less in line B19909 than low levels of infection in cv. Prisma. In contrast, the low levels

of infection on line I-17-40 increased dark respiration more than did higher levels of infection on cvs Golden Promise and Triumph. Dark respiration was not affected in the wild line B8893.

Infection increased stomatal resistance in the light in all three lines indicating that mildew induced stomatal closure and consequently limiting CO_2 diffusion to the carboxylation sites and causing a decline in the rates of photosynthesis. In contrast, stomata failed to close completely in the dark in all three lines.

The compensatory photosynthesis in uninfected fourth leaf of infected plants was also investigated in cv. Prisma and the two wild lines B19909 and I-17-40. Infection of the three lower leaves increased the rate of photosynthesis as well as the quantum efficiency in the more tolerant line B19909 only. This indicates that compensatory photosynthesis may play an important role in tolerance of the parasite.

Thus both from growth analysis and from measurements of photosynthesis and respiration it appears that line B19909 was more tolerant of mildew infection than the other lines.

Chapter 1

INTRODUCTION

1.1. The importance of barley as a crop and the economic significance of barley mildew (*Blumeria graminis* f.sp. *hordei*)

Barley (*Hordeum vulgare*), a small-grain cereal, belongs to the tribe Hordeae of the family Gramineae. It is a major world crop and ranks as the most important cereal after rice, wheat and maize (Bengtsson, 1992). Barley is widely cultivated, being grown extensively in Europe, around the Mediterranean rim, and in Ethiopia, Russia, China, India and North America (Harlan, 1995). In Britain, barley has been the crop with the largest land acreage for a considerable period of time and still represents today, together with wheat, one of the major crops.

It has been suggested that cultivated barley originated from the wild barley, *Hordeum spontaneum* C. Koch, which has its centre of origin in the Fertile Crescent of the Middle East (Zohary, 1969), with scattered stands over a much wider area from Tunisia to Afghanistan and with doubtful occurrence in Morocco and Abyssinia (Clarke, 1967; Harlan & Zohary, 1966).

The fungus *Blumeria graminis* (DC.) Speer f.sp. *hordei* Marchal (Syn. *Erysiphe graminis* DC. f.sp. *hordei* Marchal) (Braun, 1995) causes powdery mildew, the most important disease of barley throughout the world where the crop is grown (Brooks, 1970).

The importance of powdery mildew on barley was recognised at the beginning of this century when the disease was observed to cause economic

losses (Wolfe & Schwarzbach, 1978). Since then barley mildew has remained a constant problem in many parts of the world, including Europe. For example, annual losses of about 9% are reported in England and Wales (King, 1972; 1977), 10% in Denmark (Slootmaker & Essen, 1969), 25% in USA (Schaller, 1951) and 30% in North Africa (Yahyaoui *et al.*, 1997). Even greater yield reductions have been found in experimental studies and losses in grain yield in excess of 50% have been reported (Rea & Scott, 1973).

1.2. Interactions between barley and *B. graminis* f.sp. *hordei*

The responses of barley to infection by *B. graminis* have been found to be extremely varied. Some cultivars may be highly susceptible and support high levels of fungal development resulting in the death of the host, while others may be immune. In between the two extremes there is a continuous range of cultivars which support all levels of fungal development (Jones & Clifford, 1983a).

1.2.1. Host resistance

Resistance of a host to a parasite is defined by Agrios (1997) to be the ability of the host to prevent, completely or in some degree, the growth and development of that parasite. Different types of resistance in barley to infection by *B. graminis* f.sp. *hordei* have been noted from complete resistance to varying levels of partial resistance.

1.2.1.1. Complete resistance

In many instances, complete or near complete resistance has been found to be controlled by one or at most two or three genes with major effect. This type of resistance is often called race-specific resistance or major gene resistance and is often expressed as a hypersensitive reaction (Jones, 1987).

The specificity of most types of major gene resistance suggests that there is some relationship between specific avirulence genes in the different physiologic races of the pathogen and the different resistance genes in the host cultivars incorporating them.

Many *B. graminis* f.sp. *hordei* avirulence alleles and barley resistance genes were found to follow a gene-for-gene relationship, a relationship which was first demonstrated by Flor in 1956 between flax and flax rust (Moseman, 1957, 1959).

The application of Flor's gene-for-gene hypothesis has facilitated the identification of specific resistance genes in barley and their corresponding pathogenicity genes in *B. graminis* f.sp. *hordei*. In this way, a large number of resistance genes in cultivated barley and wild species of barley have been identified and mapped (Giese, 1981; Giese *et al.*, 1981; Wolfe, 1972; Søgård & Jørgensen, 1987).

Resistance to at least some variants of *B. graminis* f.sp. *hordei* is determined by alleles located in at least seven loci. At least five of these loci appear to be located on the long arm of chromosome 5, whereas the other two loci, *Ml-g* and *Ml-o*, are located on chromosome 4 (Wolf, 1972). The resistance alleles are designated after the cultivar or line in which they were

first identified; eg. *Ml-a* (cv. Algerian), *Ml-at* (cv. Atlas), *Ml-g* (cv. Goldfoil), *Ml-h* (cv. Hanna), *Ml-k* (cv. Kwan), *Ml-p* (cv. Psaknon) and *Ml-o* (McIntosh, 1978).

Plant breeders produce new resistant cultivars by incorporating single major genes derived from *Hordeum spontaneum*. These resistant cultivars are extensively used, particularly in Europe and North America, because resistance provides the cheapest and the most effective means of controlling pathogens, particularly powdery mildews of cereals. However, the cultivation of resistant cultivars on a large scale imposes a strong selection pressure on the pathogen population for virulent races that can overcome the resistance. In general, major gene resistance remains effective for only a few years before a virulent race of the mildew pathogen arises which can overcome the resistance. This was first observed in the case of the major resistance gene *Ml-g*, which was introduced into European barley varieties in the 1930s (Wolfe & Schwarzbach, 1978). For example in Germany, when the area under cultivation with cultivars with *Ml-g* gene was still small during the 1930s and 1940s, it remained effective. However, when areas under cultivation in the late 1940s started to increase rapidly, this resistance gene was defeated (Wolfe & Schwarzbach, 1978; Wolfe, 1984). The instability of major gene resistance has caused plant breeders to look for ways to use it, which might make it more durable.

Three methods have been used to improve the durability of major gene resistance, pyramiding resistance genes, multiline varieties and variety mixtures. Pyramiding resistance genes consists of breeding as many of the

genes as possible into a cultivar before releasing it into commercial production. This means that the parasite must overcome all the resistance genes before it becomes virulent.

Multiline varieties are formed as combinations of isogenic lines, identical in all agronomic characters but differing in the race-specific resistance gene they contain (Jones & Clifford, 1983b and Manners, 1993). In order to break down the resistance of the multiline, the pathogen must acquire enough different virulence alleles to overcome all the resistance genes present.

Variety mixtures consist of several varieties, which are similar to each other in agronomic characters, but which have different resistance genes. Mixed varieties have been shown to have reduced levels of infection and consequently reduced yield loss of the individual components of the mixture, when compared to the mean yield from pure stands (Wolf, 1985). However, mixed varieties, like multiline varieties and pyramiding, could lead to the development of new and more virulent races (super-races) that are virulent on all of the varieties in the mixture (Groth, 1976). This possibility was supported experimentally by the work of Huang *et al.* (1994) who suggested that super-races would dominate a mildew population when the same cultivar mixtures or multilines were used continuously over long periods and large areas. The search for more durable forms of resistance should continue.

1.2.1.2. Partial resistance

Many barley varieties have been found to be partially resistant *to B. graminis* pathotypes. Such varieties support the growth of the fungus but the

growth is limited. Partial resistance tends to be more durable than race specific resistance (Roberts & Caldwell 1970). It is in fact not specific and affects several of the pathogen infection processes, pathogenicity and sporulation. It is believed to be controlled by a number of genes each with small effect (Parlevliet, 1981) and it is sometimes referred to as polygenic resistance (Asher & Thomas, 1987). Partial resistance is more durable because the pathogen has to undergo several genetic changes to overcome the resistance (Jørgensen, 1994).

Partial resistance has been transmitted to new varieties in breeding programs (Roberts & Caldwell, 1970). Unfortunately, this type of resistance rarely provides an adequate level of resistance. It is also difficult to evaluate its level in the field without growing the plants to maturity and it is more difficult than major gene resistance to manipulate in breeding programs (Jones, 1987). However, if supported by other control measures, such as the use of fungicides, it can give useful mildew control.

Since partially resistant cultivars are susceptible in wild populations, genotypes which are least affected by that level of infection are likely to have the higher reproductive output and thus have a selective advantage over genotypes which are affected more. Genotypes which are least affected are the most tolerant genotypes and thus tolerance is likely to play a significant role in a host's survival strategy and could be used as a crop protection measure.

1.2.2. Tolerance of the parasite

Tolerance of the parasite in plants is defined as the ability of a plant to endure the effects of levels of parasitic development, which if they occur at equivalent levels in other plants of the same or of similar species would cause greater impairment of growth or yield (Clarke, 1986).

1.2.2.1. Evidence for tolerance in crop plants

That some cultivars of some crops may vary in their tolerance of infection has been suggested for many years. One of the first reports of tolerant cultivars in cereals is that of Salmon & Laude (1932). They claimed that Fulhard wheat was more tolerant of leaf rust (*P. recondita*) than were other cultivars used in a trial. These results were verified and supported by Caldwell *et al.* (1934).

Newton *et al.* (1945) compared the reactions of six barley cultivars to leaf rust (*P. hordei*) and concluded that two cultivars (Mensury and O.A.C.21) were more tolerant than the other four cultivars. Similarly, Kramer *et al.* (1980) compared the reactions of fifteen spring barley cultivars to leaf rust (*P. hordei)* and observed that some cultivars appeared to have stable tolerance which was expressed in each season, while others expressed unstable tolerance which was expressed in one season but not in another.

Simons (1966) examined 24 oat cultivars for their reactions to crown rust (*P. coronata*) and concluded that cv. Cherokee and several other cultivars with susceptible reactions were significantly more tolerant of given levels of infection, as measured by kernel weight ratio, than cvs Clinton and Benton.

However, in none of these cases, were the rates of development of parasite biomass and or disease examined. Clarke (1986) concluded that none of the studies clearly established that the named cultivars were really more tolerant of infection than some of the other cultivars with which they were compared. In contrast, the experiments carried out on oat plants by Sabri *et al.* (1993, 1995 and 1997) in which reductions in host plant growth and changes in photosynthesis and respiration were related to parasite biomass development, gave results which clearly showed that one cultivar cv. Lustre was more tolerant of given levels of mildew infection than another, cv. Peniarth.

1.2.2.2. Evidence for tolerance in native plants

It has been commonly observed (Tarr, 1972) that some wild plants can be very susceptible to a parasite yet appear to be little affected by it. This general view that wild plants may be more tolerant of parasitic infection than cultivated plants gained some support experimentally from the work of Ben-Kalio & Clarke (1979) on the effects of the powdery mildew fungus *Erysiphe fischeri* on the growth and development of *Senecio vulgaris* (groundsel). They observed that up to 30% mildew cover had no effect on plant growth. Even heavy levels of infection, when up to 75% of total leaf area were colonised, did not effect chlorophyll levels in the leaves nor the rate of dry-matter production per unit area of leaf, and nor did they affect photoassimilate distribution between the different parts of the plant. However, leaf expansion was reduced and so total plant growth and number of flowers and fruits were

reduced.

Similarly, comparative studies of the effects of powdery mildews on willow herb (*Epilobium montanum*) and couch grass (*Elymus repens*) indicated that infection did not reduce growth until more than 30% of the aerial surfaces were colonised (Clarke, 1988). Levels of infection above 30% progressively reduced growth but to a lesser extent compared to crop plants such as cereals, where growth is generally depressed substantially even by low levels of infection (Ayres, 1984).

More recently, Sabri (1993), (Sabri *et al.*, 1995) compared the effects of *B. graminis* f.sp. *avenae* on the growth of one wild line of oat (*Avena fatua*) and two cultivated oats Lustre and Peniarth (*A. sativa*) and found that although the wild oat supported the highest levels of mildew development, its growth and yield were reduced less than those of the two cultivated oats particularly cv. Peniarth. Thus the wild line appeared to be much more tolerant of given levels of mildew infection than either of the two cultivated oats particularly cv. Peniarth.

Since reductions in growth were due to the effects of mildew infection on leaf development and function, Sabri (1993), (Sabri *et al.*, 1997) investigated the effects of infection on photosynthesis and respiration in the three oat lines. The results indicated that gross and net photosynthesis and chlorophyll levels were reduced by infection in all three lines, but to the greatest extent in cv. Peniarth, to a lesser extent in cv. Lustre and to the least extent in the wild oat despite the latter supporting the development of the highest level of mildew biomass. This study supported the results of the growth analysis that wild oat

possessed more tolerance of infection than either of the two cultivated oats. It was also found that for given levels of mildew biomass development, cv. Lustre was less affected than cv. Peniarth indicating that cultivars may differ in their tolerance. From these studies on tolerance of wild plants, it was concluded that wild plants might possess higher levels of tolerance of parasites than crop plants.

1.3. How pathogens affect the growth and yield of susceptible hosts

1.3.1. Effects on the shoot growth and yield

Detailed growth analysis on some important crop species has been carried out to investigate just how pathogens affect the growth and development of the plant.

In glasshouse experiments, Last (1962) studied the effects of *B. graminis* f.sp. *hordei* on the growth and development of spring barley plants. He showed that although total plant dry weight of infected plants continued to increase, final dry weights were reduced by about 59% compared to the uninfected controls by 11 weeks after inoculation. At this stage the mean level of infection, measured as percentage mildew cover, was about 30%. Infected plants also produced shorter primary shoots, fewer shoots per plant and developed a smaller leaf area per shoot than the uninfected controls. The reduced dry weight of the shoot system of infected plants was found to be closely paralleled by a reduced leaf area. During the early stages of infection, reduced growth was attributed mainly to fewer shoots per plant, but later,

also to reductions in total leaf area as well as to fewer shoots. The mean unit leaf rate or net assimilation rate was also reduced by infection, by about 27% compared to the uninfected plants. Unexpectedly, infection reduced root growth more than shoot growth. The reductions in leaf area, net assimilation rate and root growth were reflected in smaller grain yields, due to the production of smaller and fewer ears. The average decreases in dry weight per ear and in number of ears per infected plant were about 21% and 12% respectively.

Although Paulech (1969) obtained similar results to Last, the results of both workers were open to criticism for three reasons. Firstly, the experiments were carried out in glasshouses where mildew developed more severely than in the field. Secondly, plants were grown in pots and so may be qualitatively and quantitatively different from those grown in the field. Thirdly, the effects on growth depend on the growth stage at which infection occurs (Brooks, 1972).

Working with spring barley in field trials, Brooks (1972) found that the growth and yield of winter barley, which can be subject to severe mildew attack in both autumn and in spring, was significantly reduced. He observed that when an early and severe attack was contained for the whole season, there was about 26% increase in the yield because of the increased numbers of fertile tillers produced and the increased ear weight. When mildew infection occurred late in plant development, reductions in grain size were the only effect. In general, Brooks (1972) confirmed the observations on pot grown plants made by Last (1962) and Paulech (1969).

Griffiths *et al.* (1975), in pot experiments, investigated the effects of mildew epidemics of different duration and with varying times of inoculum arrival on grain production. They showed that an early mildew attack not only reduced tiller number but also grain size and number of grains per tiller, but late mildew attack reduced only the number of fertile tillers with no significant effect on grain size or number. The change in the plant response to mildew infection was found to occur about G.S. 5.0, i.e. at the end of tillering, but after this stage, the effect of mildew epidemics on tillering was much reduced. This was due to the fact that tillering in barley is completed at G.S. 3.0 (Zadoks *et al.*, 1974).

These studies were confirmed and investigated in more details by Scott *et al.* (1980) who found that significant reductions in grain number and size could occur even with late mildew attack because of the effects of infection on photoassimilate production during the period of grain filling.

The greatest effect of mildew is to accelerate leaf senescence so that green leaf area (GLA) is reduced (Last, 1962; Brooks, 1972). Many studies have revealed close relationships between GLA or GLA duration and crop yield losses (e.g. Rea & Scott, 1973; Jenkyn, 1976; Carver *et al.*, 1981, 1982; Lim & Gaunt, 1986; Waggoner & Berger, 1987). For example, Carver *et al.* (1981) examined the relationships, using greenhouse grown barley plants, between the severity of powdery mildew, green leaf area (GLA) and grain yield. The results showed that mildew reduced GLA in proportion to its severity and there was an almost complete correlation ($r = 0.99$) between GLA and grain yield in both primary shoots and tillers. There was also a good

correlation between disease progress and total yield of primary shoots (r = 0.95). Detailed analyses of the data indicated a dominant role for GLA pre-anthesis on grain yield associated with mildew epidemics. GLA before anthesis determined the amount of stored photosynthate generated before anthesis and available for retranslocation to the developing grain.

However, field-grown plants generally differ from those grown in the glasshouse in the development of smaller leaves due to the effects of temperature and light intensity (Carver *et al.*, 1982) and so it is important to establish whether the responses of field grown plants to mildew attack are similar to those of glasshouse grown plants. For this reason, spring barley plants, cv. Julia, were grown in micro-plots in the field (Carver *et al.*, 1982), but the observations from these field experiments confirmed almost all the details gained from greenhouse experiments (Carver *et al.*, 1981).

In contrast to the findings of Carver and Griffiths (1981, 1982), that late mildew epidemics had only small effects on grain yield, Wanzhoug (1988) using cv. Triumph, observed that although early mildew attacks were more damaging to plant growth and yields than late attacks, late epidemics also caused significant losses in most yield components. The difference in yield responses to late mildew epidemics found between Carver *et al.*'s (1981) and Wanzhoug's (1988) experiments may be explained by different degrees of tolerance between the cvs Julia and Triumph or by differences in environmental conditions.

1.3.2. Effects on root growth and physiology

The effects of powdery mildews and other foliar pathogens on plant growth were usually considered in relation to the leaf environment and little thought was given to possible effects on root growth. Roots take up water and nutrients and transport these to the shoots together with certain plant growth regulators that they synthesise (Ayres, 1984). The growth and physiological efficiency of cereal roots can be disrupted directly by root parasites (Asher, 1972; Clarkson *et al.*, 1975; Fitt *et al.*, 1978) or indirectly by foliar parasites such as powdery mildew (Last, 1962; Paulech, 1969; Brooks, 1972; Walters & Ayres, 1981a).

Last (1962) was the first to note that mildew infection of the leaves reduced root growth relatively more than shoot growth. He found that root dry weight per unit leaf area was decreased in infected plants by up to about 32% of the level in uninfected plants. He suggested that some of the efficiency of the assimilatory apparatus could be affected by the large reductions in the absorbing systems (roots). Paulech (1969) and Brooks (1972) confirmed that roots were affected more than shoots by mildew infection in barley plants.

The branching pattern of roots was also found to be affected by mildew infection. Vizárová & Minarčic (1974) observed that in barley plants, four days after inoculation with *B. graminis* f.sp. *hordei*, the rate of elongation of the seminal roots was reduced as also was the growth and formation of lateral roots. They also noticed that the diameters of the roots were smaller and thus that the roots had a much smaller stele than the roots of healthy plants. Minarčic & Paulech (1975) also observed this reduction in the stele size.

More detailed investigations of the effects of mildew on the growth of barley roots were carried out by Walters (1981) and Walters & Ayres (1981a). They observed that total root dry weight, total root length, as well as the length of individual roots (seminals, nodals and laterals) were significantly decreased by mildew infection. The numbers of seminal and nodal roots were not affected by infection but there was a significant reduction in the number of primary and secondary laterals formed by both types of roots. Reduced stele size was also reported which was in agreement with the results of Vizárová & Minarčic (1974) and Minarčic & Paulech (1975). The results presented by Walters (1981) showed that mildew infection lead to a reduction in the number and size of the inner metaxylem vessels and in the size of the endodermis.

The effects of mildew infection on mitotic cell division in the apical meristems of the roots of barley plants were first investigated by Minarčic & Paulech (1975). They observed that infection reduced mitotic cell division in the apical root meristems of a highly susceptible barley cultivar in response to mildew infection and Walters (1981) obtained similar results. Lewis & Deacon (1982) investigated the effects of mildew infection on the senescence of the root cortex of barley seedlings, but found little difference from uninfected plants. Last (1962) suggested that the reductions in the root systems in barley caused by mildew infection was a secondary effect of the lower unit leaf rates, but a stage may, however, be reached when the smaller root system itself affects leaf efficiency. Fric (1975), attributed effects on root growth to the reduction in the quantity of photoassimilates reaching the root,

but he also suggested that other factors might be responsible such as disturbances to the hormonal balance of the roots. Vizárová & Minarčic (1974) in fact found that treatment of healthy barley with cytokinin did result in alterations in the root system which were similar to those observed in mildewed plants. Thus, the increase in cytokinin levels detected around four days after inoculation in infected plants may be associated with the morphological changes observed in the roots. However, the level of cytokinins was observed to decrease later and thus effects on root growth must be due to other factors. The reduction in the mitotic cell division of the root apices may also be attributable to a decreased photoassimilate supply from the shoots to the roots (Minarčic & Paulech, 1975) and such a reduction was later reported by Walters (1981) who observed a reduced supply of ^{14}C photoassimilates to the root tips of infected plants.

Undoubtedly, the changes in root anatomy and growth of mildewed barley plants would have an effect on root physiology. In the work carried out by Walters (1981), it was shown that roots of mildewed barley were taking up more ^{32}P-labelled phosphate than uninfected plants by 24 hours after inoculation. He suggested that this increase was due primarily to the creation of a sink in the shoot system created by the mildew infection and secondly to ammonium ions, which were found, to accumulate in roots of infected plants. It was also noticed that mildewed plants absorbed more potassium and chloride from the growth medium than healthy plants, and consequently, the ionic content of the tissues of infected plants was greater than those of uninfected plants (Walters, 1981). In contrast, infection by powdery mildew

was found to decrease nitrate uptake thereby lowering the nitrate content of roots and this was explained by the lack of photassimilates received by the roots from infected leaves (Walters & Ayres, 1980). However, sodium uptake and content was unaffected by infection (Walters, 1981). Walters (1981) did suggest that the increased levels of indole-acetic acid (Shaw *et al*, 1958) and cytokinin (Vizárová & Minarčic, 1974) found in infected plants could cause increases in the movement of inorganic and organic nutrients to sites of mildew infection.

Other physiological processes in the roots were found to be altered by infection, e.g. respiration which increased in the roots of barley plants whose shoots were infected by mildew (Fric, 1975; Walters, 1981).

From all the studies presented here, it is clear that the roots of barley are significantly altered morphologically, anatomically and physiologically by infection with powdery mildew fungi. These changes must have a detrimental effect on the growth of the plants and thus on yield.

1.3.3. Functional equilibrium between roots and shoots of infected plants

Shoots, together with the roots, constitute the entire plant structure and the root : shoot ratio can provide an index of the performance of each organ in a given growth environment.

Based on the work of Davidson (1969) and Thornley (1972), several workers have demonstrated a functional equilibrium between root and shoot growth and the development of uninfected plants (e.g. Richards, 1977, 1978). Ayres (1984) suggested that the equilibrium between root and shoot growth is

mediated by water and nutrients moving from root to shoot, by photoassimilates moving from shoot to root and by growth regulators moving in both directions. However, it was mentioned earlier that the dry weights of the roots of barley plants infected with *B. graminis* f.sp. *hordei* were reduced more than shoot dry weights and consequently, the root : shoot ratio had been decreased by infection. It has already been noted that nutrient uptake, photoassimilate distribution and the balance between growth regulators were all altered following infection.

Walters (1985) suggested that, powdery mildew infected barley plants might be able to maintain a functional equilibrium between root and shoot growth during the early stages of infection, but that the equilibrium may become increasingly unstable as the pathogen colonises the majority of the plant's leaf area and the host's physiology becomes increasingly altered.

In contrast to the findings on the effects of powdery mildew infection in cereals, some wild plants have been observed to have their roots and shoots more or less equally affected by infection e.g. infection by *B. graminis* f.sp. *avenae* had no effect on the root to shoot ratio in a wild oat line compared to the cultivated oats (Sabri et al., 1995). *Erysiphe fischeri* had also no effect on the root to shoot ratio in *Senecio vulgaris* (groundsel) (Ben-Kalio *et al.*, 1979). A similar result was reported for willow-herb infected with the powdery mildew, *Sphaerotheca epilobii* (Clarke, 1988). The failure of powdery mildew infection to alter root to shoot ratios in wild oat, groundsel and willow-herb was explained by the ability of these hosts to tolerate infection.

1.4. The effects of infection on host metabolism

Invasion of plants by parasitic micro-organisms alters the metabolism of the host in various ways. Infections by necrotrophic fungi are generally associated with extensive damage and the rapid death of affected tissues, but relatively simple changes in metabolism (Manners, 1993). In contrast, biotrophic fungi obtain their nutrients from living cells and appear to be able to manipulate their host's metabolism to a significant extent in order to ensure a continued supply of carbohydrates and nutrients (Ayres *et al.*, 1996).

Biotrophic fungi certainly alter most of the physiological and biochemical processes of their host, including photosynthesis (Ahmad *et al.*, 1983; Buchanan *et al.*, 1981; Ayres *et al.*, 1996; Scholes *et al.*, 1985; Scholes, 1992), respiration (Last, 1963; Daly, 1976; Kosuge *et al.*, 1981; Raggi, 1980), carbohydrate metabolism (Farrar, 1985; Whipps *et al.*, 1981; Scholes, 1992; Ayres *et al.*, 1996), transport systems (Farrar, 1984), water relations (Ayres 1981a; Duniway *et al.*, 1971b), nucleic acid metabolism (Chakravorty and Scott, 1982; Higgins *et al.*, 1985) and protein synthesis (Manners and Scott, 1984). Since this project deals with the effects of *B. graminis* f.sp. *hordei* infection on photosynthesis and respiration in barley, this review will concentrate mainly on these two processes and only refer to effects on other systems where they impinge on these processes. Furthermore, because of the extensive literature on biotrophic parasites, only the effects of powdery mildews and rusts will be considered in detail in this introduction.

1.4.1. Effects on carbon gain through photosynthesis

1.4.1.1. Effects on photosynthesis in infected leaves

Since photosynthesis is the process by which green plants obtain their energy, any pathogen interference with this process will clearly have adverse effects on the plant, leading to decreased growth and yield. Powdery mildew and rust infections have generally been found to reduce the rates of photosynthesis in their host (Allen, 1942; Gordon *et al.*, 1982a and 1982b; Owera *et al.*, 1981; Daly, 1976; Magyarosy *et al.*, 1976; Habeshaw, 1979, 1984; Mitchell, 1979; Ellis *et al.*, 1981; Ahmad *et al.* 1983).

In the case of powdery mildews, Allen (1942) reported that the rate of photosynthesis declined rapidly in wheat leaves heavily infected with *B. graminis* f.sp. *tritici*, but in lightly infected leaves the rate of photosynthesis still declined but more slowly. Similarly, when barley leaves were infected with *B. graminis* f.sp. *hordei*, the rate of photosynthesis began to decline progressively from two days after inoculation according to Scott & Smillie (1966), but not until four days after inoculation according to Last (1963) and Hibberd *et al.* (1996). Habeshaw (1979) examining the effects of *B. graminis* f.sp. *hordei* on the susceptible cultivar Golden Promise also found that infection decreased the rate of photosynthesis from a very early stage of infection. A biphasic inhibition of photosynthesis was observed by Edwards (1970) in barley leaves infected with *B. graminis* f.sp. *hordei*. The first phase occurred within 24 hours after inoculation and the second phase occurred six days after inoculation when fungus sporulation on the leaf had reached its maximum.

In the case of oat plants, the effect of infection was delayed in comparison with barley. Haigh *et al.* (1991) reported that photosynthesis did not decline in leaves of oat plants infected with *B. graminis* f.sp. *avenae* until five days after inoculation. Similarly, Sabri *et al.,* (1997) observed that the rates of maximum gross and net photosynthesis following infection by *B. graminis* f.sp. *avenae* decreased eight days after inoculation in one cultivar, but not until ten days after inoculation in another (Sabri *et al.,* 1997).

Working with pea (*Pisum sativum*) plants infected with *Erysiphe pisi*, Ayres (1976) showed that the rate of photosynthesis reduced rapidly from 24 hours of inoculation and had decreased to less than one third of that in uninfected plants by the seventh day after inoculation. Similar reductions have been observed in powdery mildew-infected (*Erysiphe polygoni DC*) sugar beet leaves (*Beta vulgaris* L) (Magyarosy *et al.*, 1976).

Rust fungi have been reported to have the same effects on photosynthesis as powdery mildew fungi. Wheat leaves infected with *Puccinia graminis* f.sp. *tritici* were shown to have a reduced rate of photosynthesis together with reduced chlorophyll content from the third day after inoculation (Mitchell, 1979). Barley plants infected with the brown rust fungus, *P. hordei*, at the first leaf stage showed no reduction in photosynthesis until nine days after inoculation after which the rate of photosynthesis declined to about half that of the uninfected leaf (Owera *et al.*, 1981). Scholes *et al.* (1985) reported that the reduction in the rate of photosynthesis, both per unit leaf area and per unit of chlorophyll, and the changes *in vivo* chlorophyll fluorescence kinetics clearly indicated that photosynthesis was being progressively inhibited within

developing pustules of *Uromyces muscari* on bluebell leaves [*Hyacinthoides non-scripta* (L.) Chouard ex Rothm.].

It appears that results even for the same species are contradictory both concerning the time and the rate at which changes in photosynthesis occur following infection. Some of these differences could be due to the use of different cultivars with varying degrees of susceptibility and which differed in their level of tolerance of infection.

The reductions in photosynthesis, caused by biotrophic fungi, are sometimes preceded by an increased rate during the very early stages of infection particularly when high concentrations of CO_2 were used (Scott & Smillie, 1966). This effect of high CO_2 concentration was shown for bean leaves infected by *Uromyces phaseoli* (Livne, 1964), wheat leaves infected by *Puccinia striiformis* (Doodson *et al.* 1965), wheat leaves infected by *B. graminis* f.sp. *tritici* (Allen, 1942) and barley leaves infected by *B. gramins* f.sp. *hordei* (Scott & Smillie, 1966). Studies on barley leaves infected with *B. graminis* f.sp. *hordei* by Edwards (1970), using ambient CO_2 concentration, observed no initial stimulation but instead a biphasic inhibition. However, when a high concentration of CO_2 (1%) was used stimulation was observed. This stimulation in photosynthesis was attributed to an impairment in glycollate metabolism in infected leaves especially at high CO_2 concentrations.

1.4.1.2. Causes of changes in photosynthesis

A variety of mechanisms have been considered to be responsible for the decrease observed in photosynthesis following infection. Photosynthesis can

be described as a diffusion process where the flux of CO_2 into leaf is driven by the concentration gradient between the atmosphere and the carboxylation sites. Models of this diffusion pathway have been applied to the analysis of photosynthesis in several host-pathogen systems (Duniway & Slater, 1971; Hall & Loomis, 1972; Gordon & Duniway, 1982a). For example, in powdery mildew infection, stomatal resistance was increased by infection, but this change was not apparent until three days after inoculation in barley (Ayres, 1979), four days after inoculation in pea (Ayres, 1976) and six days after inoculation in oak (Hewitt & Ayres, 1975). In powdery mildew-infected sugar beet leaves, the decline in net photosynthesis was attributed not to increased stomatal resistance but mainly to increases in mesophyll resistance to CO_2 (Gordon & Dunway, 1982a). Similarly, in barley infected with *Puccinia hordei*, diffusion of CO_2 into the leaf was found not to be an important limiting factor in photosynthesis. The main effect of infection, in this case, was to increase CO_2 concentrations in the intercellular spaces and to double mesophyll resistance (Owera, Farrar & Whitbread, 1981).

In certain infections, particularly those caused by powdery mildew, the decrease observed in the rate of photosynthesis has been partly attributed to a reduction in the amount of irradiance reaching the chloroplasts due to the shading effect of the fungal mycelium present over the surface of the leaf (Misaghi, 1982). However, in powdery mildew of apples, removal of the mycelium did not lead to any increase in the rate of photosynthesis and so a reduction in light reaching the chloroplasts was not a factor in this case.

It is widely recognised that infection by fungal biotrophs causes a

reduction in chlorophyll content although some evidence is contradictory. The rate of photosynthesis expressed per unit of chlorophyll was reported to decrease in barley leaves infected with *B. graminis* f.sp. *hordei* (Allen, 1942). Scott & Smillie (1963) reported a similar effect in barley, but after recalculation of their data, Waygood *et al.* (1974) found that net photosynthesis per milligram of chlorophyll was actually 50% higher in infected than in uninfected leaves.

Other researchers (Paulech & Haspelová - Harvatoviăová, 1970) found that total chlorophyll was reduced by *B. graminis* f.sp. *hordei* in barley leaves before photosynthesis had begun to decline. In contrast, in oak (*Quercus robur* L.) leaves infected with the powdery mildew (*Microsphaera alphitoides*), total chlorophyll content only began to reduce after photosynthesis had begun to decline and changes in the chlorophyll a : b ratio were not found until six days after inoculation (Hewitt, 1976).

Even more contradictory results have been reported in the case of rust fungal infections. For example, in *Vigna sesquipedalis* infected by *Uromyces appendiculatus*, the reduction in photosynthesis was significantly correlated with the reduction in chlorophyll content between 0 and 14 days after inoculation (So & Thrower, 1976). Similarly, in wheat infected with stem rust (*P. graminis* f.sp. *tritici*), the decline in the rate of photosynthesis per unit of chlorophyll was directly correlated with chlorophyll loss, suggesting that loss of chlorophyll was a major contributory factor to the reduction in photosynthesis (Mitchell, 1979).

In contrast, no correlation was observed between the reduction in

photosynthesis and the reduction in chlorophyll levels in wheat infected by *P. striiformis* (Doodson, Manners & Myers, 1964). Owera *et al.* (1981) also observed that although chlorophyll content was depressed in barley leaves infected with *P. hordei*, both net and gross photosynthesis, when expressed per unit green leaf area, or per chlorophyll content, increased slightly. This is in agreement with the findings by Last (1963) on the effects of *B. graminis* f.sp. *hordei* on photosynthesis in barley leaves.

Results from studies on the effects of downy mildews on photosynthesis in their hosts are also contradictory. In lettuce infected by *Bremia lactucae*, chlorophyll levels were found to be significantly reduced by six days after inoculation (Mason, 1973), but in cabbage infected with *Peronospora parasitica*, no chlorophyll loss was observed up to seven days following inoculation although chlorophyll content may have been altered in the later stages of infection.

However, although reducing chlorophyll content is one of the features of powdery mildew infections of crop plants, it was not shown by some wild plants in response to infection; eg. no loss of chlorophyll following infection was found in the case of willow-herb, couch grass or groundsel (Clarke, 1988).

Other evidence suggests that changes in the enzyme activities of the chloroplasts and of alterations to chloroplast structure may play a role in the decline of photosynthesis. Powdery mildew infection of sugar beet was found to result in a substantial reduction in the activity of ribulose-1,5-bisphosphate carboxylase (RuBPcase), a key enzyme in the reductive pentose phosphate

pathway (Gordon & Dunway, 1982b). This decrease was attributed to a reduction in the concentration of RuBPcase as there was no apparent change in the specific activity of this enzyme in infected tissue. Similar effects were observed in barley leaves infected with powdery mildew (Walters & Ayres, 1984). Following infection, there was a progressive reduction in host mRNAs encoding both large and small subunits of RuBPcase. Stem rusts have also been reported to reduce the activity of RuBPcase in wheat (Wrigley & Webster, 1966).

Reductions in the amount of RuBPcase were reported to be caused by reductions in plant nitrogen in barley infected with *B. graminis* f.sp. *hordei* (Walters & Ayres, 1980) and in barley infected with *P. hordei* (Ahmad *et al.*, 1982). The pathogen may also affect the retranslocation of nitrogen out of infected leaves (e.g. Ahmad *et al.*, 1983).

Gordon & Duniway (1982b) have suggested that changes in RuBPcase activity may not be entirely responsible for limiting carbon flux through the reductive pentose phosphate cycle (RPPC) in mildewed barley, since the activities of RPPC enzymes may also be reduced by infection. Walters & Ayres (1984) in fact noted that the activities of some enzymes of the pathway were reduced by mildew infection of barley leaves e.g. 3-phosphoglycerate kinase and NADP – glyceraldehyde phosphate dehydrogenase (GAPDH).

A further explanation for the decline in photosynthesis could be fungal sequestration of inorganic phosphate (Pi) from the host tissues. Whipps & Lewis (1981) suggested that fungal infection of the leaf induces Pi-deficiency since biotrophic pathogens act as a sink for Pi. Thus the fungus could reduce

the concentration of host cytosolic and thus chloroplastic Pi, causing a reduction in the rate of photosynthesis. On the other hand, other investigations do not support this work. For example, in powdery mildew-infected barley and wheat leaves the Pi content of the leaf was either unaffected or slightly increased by the time of fungal sporulation (Walters & Ayres, 1981b; Zulu *et al.*, 1991; Scholes *et al.*, 1992; Wright, 1992). Furthermore, in barley leaves infected with *P. hordei* (Ahmad *et al.*, 1982, 1984) and in wheat leaves infected with *P. graminis tritici* (Bennet & Scott, 1971), Pi was found to have at least doubled in concentration compared to uninfected leaves. Additionally, when Pi was fed to rusted leaves of barley (Scholes & Farrar, 1986) and to mildewed wheat (Zulu *et al.*, 1991), the rate of photosynthesis did not increase. Other examples have been discussed by Scholes (1992), who concluded that Pi is not the primary factor responsible for the decline in photosynthesis following infection.

Studies of the effects of biotrophic pathogens on the photochemical reactions of photosynthesis are limited and there is little agreement as to their effects. Montalbini *et al.* (1974) and Magyarosy *et al.* (1976) reported that infection of broad bean leaves by *Uromyces fabae* and sugar beet leaves infected by *Erysiphe polygoni* led to a preferential inhibition of non-cyclic photophosphorylation (non-cyclic electron transport chain) as measured in isolated chloroplasts. Magyarosy *et al.* (1978) investigated the effects of *E. polygoni* infection on sugar beet further and found that the cytochrome content of the electron transport chain was reduced by 33% in comparison to the controls. This would suggest that infection by biotrophic pathogens

specifically alters the content of certain carriers involved in electron transport, and consequently reduces the rate of non-cyclic electron transport. This view however is not supported by the work of Wynn (1963) using chloroplasts isolated from rust-infected oat leaves, or of Ahmad *et al.* (1983) using barley leaves infected with brown rust, or of Holloway *et al.* (1992) using chloroplasts from mildewed-barley leaves. All these authors observed no reduction in non-cyclic electron transport.

Recently, Scholes (1992) reviewed possible mechanisms responsible for the reduced photosynthesis in infected plants and suggested that the enzyme invertase could play a central and linked role in both reducing photosynthesis and in retaining photoassimilates within infected tissues (see section 1.5). Increased invertase activity in powdery mildew infected barley leaves resulted in the accumulation of sucrose, glucose and fructose, causing down-regulation of the Calvin cycle by end-product inhibition and by a direct effect on genes encoding photosynthetic enzymes (Scholes, 1992).

These contradictory results concerning the effect of infection on photosynthesis and the mechanisms responsible could well be explained by the fact that the different cultivars were used of the species investigated.

1.4.2. Compensatory photosynthesis in uninfected parts of infected plants

The reductions in the rates of photosynthesis described above have been measured in the infected leaves. It is always possible that uninfected tissues of that leaf or the uninfected leaves may develop increased rates of

photosynthesis to compensate for the losses from the infected tissues. In this way infected plants may provide sufficient photosynthates to satisfy all or most sinks at least for some time following infection.

For example, Williams & Ayres (1981) demonstrated that net photosynthesis was stimulated in the uninfected third leaf of barley plants whose lower two leaves were heavily infected by *B. graminis* f.sp. *hordei*. This stimulation was greater in water-stressed than in well-watered plants. Similarly, infections by *Erysiphe pisi* on the lower three leaves of pea (*Pisum sativum* L.) stimulated photosynthesis in the uninfected fourth leaf (Ayres, 1981b).

Different mechanisms have been suggested to explain stimulated photosynthesis in the uninfected leaves of infected plants. Walters & Ayres (1983) suggested that this stimulation in the uninfected upper leaves of mildewed barley plants could be due, in part, to a transient increase in the content and activity of RuBPcase in these leaves. Increases in the activities of phosphoenol pyruvate (PEP) carboxylase and NADP malic enzyme were also observed in this study.

Walters (1985) suggested that changes in the nitrate : ammonium balance in infected shoots may also affect the activity of RuBPcase. Furthermore, an increased uptake of ^{32}P-labelled phosphate in barley could stimulate net photosynthesis, either by increasing RuBPcase activity or by affecting the ratio of ATP : ADP.

Williams & Ayres (1981) suggested that this stimulated photosynthetic activity in uninfected leaves may allow the plant to compensate for the

reductions in photosynthesis in infected tissues and for the loss of photoassimilates due to the pathogen acting as a sink. Such compensatory activity could well serve to protect the yield of lightly infected plants (Ayres & Zadoks, 1979).

1.4.3. Effects on carbon loss through dark respiration

1.4.3.1. Changes in dark respiration

Another universal effect of biotrophic fungi is an increase in the rate of dark respiration (Daly, 1976; Farrar & Lewis, 1987). Increased dark respiration in diseased plants means that as infection progresses, an increasing proportion of newly fixed assimilates is lost via respiratory processes (Walters, 1985).

An increase in dark respiration in infected plants may be expected, because in addition to the host, the fungus will have an energy demand for growth. Furthermore, the host, in addition to normal activities, will have a demand to support defence reactions. Such increases in dark respiration would provide both energy [NAD(P)H and ATP] and the carbon skeletons needed for the necessary biosynthesis (Farrar & Rayns,1987).

One of the earliest reports of increased dark respiration in response to infection is that of Yarwood (1934) who showed that infection of clover by powdery mildew (*Erysiphe polygoni*) increased dark respiration up to 50% above the levels of the controls. Working with wheat infected with *B. graminis* f.sp. *tritici*, Allen & Goddard (1938) showed that infected leaves respired four-to five-fold more than equivalent uninfected leaves. Other

authors have confirmed these results for barley infected with *B. graminis* f.sp. *hordei* (Millerd & Scott, 1956; Scott, 1965; Bushnell & Allen, 1962). Increased respiration has also been reported in oak leaves infected with *Microsphaera alphitoides* (Hewitt & Ayres, 1975).

With powdery mildew infections, it is possible to wipe the surface fungal mycelium from the surface of the leaf leaving only the haustoria in the epidermal cells. The leaf, plus haustoria have been found to respire at a rate little short of that occurring before removing the superficial mycelium, and so most of the increased respiration can be attributed to the host (Daly, 1976). Furthermore, protoplasts isolated from barley leaves infected with *B. graminis* f.sp. *hordei* were found to respire faster than those from uninfected leaves (McAinsh *et al.*, 1989).

Dark respiration was also found to increase in hosts infected with rusts. The rate of dark respiration in whole barley leaves infected with *P. hordei* at the time of sporulation was found to be at least twice that of uninfected tissues (Scholes, 1985). Similar values were reported for wheat leaves infected with *P. graminis tritici* (Mitchell, 1979; Shaw & Samborski, 1957), for wheat leaves infected with *P. recondita tritici* (Staples, 1957) and for barley leaves infected with *P. hordei* (Owera *et al.*, 1981).

The rate of dark respiration is also substantially higher within individual pustules of rusts on many hosts than in surrounding uninfected tissues. For example, within pustules of *P. hordei* on leaves of barley (Scholes, 1985; Scholes & Farrar, 1986), of *Uromyces muscari* on leaves of bluebell (Scholes & Farrar, 1985) and of *Puccinia allii* on leaves of leek (Roberts & Walters,

1988). These findings lead researchers to hypothesise that in contrast to mildew infections, most of the increase in respiratory activity in rusted tissues is contributed by the fungus (Owera *et al.* 1981; Raggi, 1980). However, it is not possible to test this hypothesis, since separating rust fungal tissue from host tissues is not yet possible. However, since the uninfected regions in wheat around *P. graminis tritici* pustules (Bushnell, 1970) and in barley around *P. hordei* pustules (Scholes, 1985; Scholes & Farrar, 1986) also show increased respiration, the host is clearly contributing to the overall increase in respiration. Respiration was found to increase in the regions between pustules at both flecking and sporulation stages, but was negligible in these regions at the green island stage (Scholes, 1985).

1.4.3. 2. Causes of changes in respiratory rates

Different mechanisms have been proposed to explain the rise in dark respiration following infection. Allen & Goddard (1938) suggested that the increased dark respiration in wheat leaves infected with *B. graminis* f.sp. *tritici* was due to substances produced by the fungus, which diffused into the mesophyll. Similarly, the accumulation of metabolites, often in mobile form, in uninfected cells adjacent to the mildew colonies and in the tissues immediately below, led Bushnell & Allen (1962) to suggest that the fungus produced diffusible toxic substances that caused the rise in the rate of respiration. Later, Allen (1953) suggested that the toxin increased dark respiration by uncoupling respiration from energy-requiring processes through activities on oxidative phosphorylation. These activities prevented ATP synthesis and lead

to ADP accumulation and a higher rate of respiration. A high ADP : ATP ratio was in fact found by Poszar & Király (1958) in wheat leaves infected with *P. graminis* f.sp. *tritici*.

On the other hand, Scott (1972) suggested that the increase could be due to quantitative changes in the existing pathways or, alternatively, to qualitative changes in respiratory pathways. Daly (1976) in fact suggested that the most likely cause of the rise in dark respiration is a shift from the glycolytic pathway to the pentose phosphate pathway with increased activity of the latter pathway. The involvement of the pentose phosphate pathway in the rise in rates of respiration is supported by the finding that the activities of the enzymes of this pathway increased after infection. Indeed, Scott (1965) reported a two to three fold increase in the activities of glucose-6-P dehydrogenase and 6-phosphogluconate dehydrogenase.

The pentose phosphate pathway seems to be located in the cytosol and is limited by the availability of $NADP^+$. The rise in the respiratory activity observed in mildewed barley leaves may be a direct response to the change in the $NADP^+$: NADPH balance (Scott & Smillie, 1966; Dyer & Scott, 1972). Ryrie & Scott (1968) suggested that the enhanced activity of the pentose phosphate pathway observed in rust and mildew infections could be due to the release of $NADP^+$ into the cytosol following chloroplast breakdown, the latter was observed by Dyer & Scott (1972).

Chakravorty & Scott (1982) suggested that the decline in photosynthesis in rusted and mildewed leaves could also lead to increased respiration, since it could lead to the release of control mechanisms on glucose-6-P

dehydrogenase and 6-phosphogluconate dehydrogenase and consequently an increased activity of the pathway. However, as well as increases in the pentose phosphate pathway, Daly (1976) suggested that in the later stages of infection there may be some uncoupling of oxidative phosphorylation.

In the investigation carried out by Farrar & Rayns (1987) on barley infected with powdery mildew, dark respiration was increased by about 80% during fungus sporulation. About half of the increase was due to increased electron flow through the cytochrome chain and about half through the alternative pathway. The latter showed increased engagement following infection but no increase in capacity. The authors suggested that the increase in activity of the cytochrome path was due to adenylate regulation, but that of the alternative pathway was not understood.

In conclusion, whatever the mechanism or mechanisms behind the increased respiratory activity following infection, it results in the loss of photosynthate that would otherwise be utilised for plant growth.

1.4.4. Effects on carbon loss through photorespiration

1.4.4.1. Changes in photorespiration

The effects of plant infections on photorespiration have not been widely investigated and the existing reports are contradictory. Most reports in fact indicate that leaves infected with biotrophic fungi have lower rates of photorespiration than equivalent uninfected leaves (Daly, 1976; Farrar & Lewis, 1987). For example, reductions were found in barley leaves infected with *B. graminis* f.sp. *hordei* (Ayres, 1979; Walters & Ayres, 1984) and also

in oak leaves infected with *M. alphitoides* (Hewitt & Ayres, 1975). Similarly, some rust fungi e.g. *Melampsora lini* in flax were found to decrease the rate of photoresoiration (Kakkar, 1966).

However, in a few cases increases in the rate of photorespiration following infection have been reported. Thus Ayres (1976) observed an increase in pea leaves infected with the powdery mildew *Erysiphe pisi*, while a similar increase was observed in barley leaves infected with *P. hordei* (Owera *et al.*, 1981). To add to the confusion, Mitchell (1979) found no differences in rates of photorespiration between healthy wheat leaves and leaves infected by *P. graminis* f.sp. *tritici*.

1.4.4.2. Causes of changes in photorespiration

Walters (1985) reported that reductions in photorespiration in infected plants could be due to reductions in the activities of associated enzymes. For example, in barley leaves infected with *B. graminis* f.sp. *hordei* (Walters & Ayres, 1984) and in oak leaves infected with *M. alphitoides* (Hewitt & Ayres, 1977), the activity of the enzyme glycolate oxidase was found to be lower than in uninfected tissues. The activity of this enzyme was reported to have decreased in wheat leaves infected with *P. graminis tritici* (Kiraly & Farkas, 1957). In addition, Walters & Ayres (1984) found that the activities of glyoxylate reductase and RuBPoxygenase also decreased following infection, however, the mechanism responsible for the stimulation of photorespiration in barley leaves infected with *P. hordei* reported by (Owera *et al.*, 1981) has not been not understood.

1.5. Effects of infection on translocation

The effects of biotrophic pathogens on the translocation and distribution patterns of assimilates throughout the plant have been investigated by many workers (eg. Crowdy & Manners, 1971; Manners & Myers, 1973; Whipps *et al.*, 1981; Farrar, 1984; Farrar, 1992). Because an infected leaf typically has a lowered rate of photosynthesis, an additional sink for assimilates in the form of the pathogen, and an increased rate of dark respiration, less translocation from it would be expected. In general, infection by biotrophic fungi results not only in a reduction in the export of assimilates from infected leaves (Doodson *et al.*, 1965), but it also promotes imports into those tissues (Livne *et al.*, 1966; Shaw *et al.*, 1956).

In the case of powdery mildew infections, Fríc (1975) observed that export from the first leaves of barley plants infected with *B. graminis* f.sp. *hordei* was less, although export from uninfected second leaves was greater, five days after inoculation.

Rust fungi were found to behave in the same way as powdery mildews. Thus Doodson *et al.* (1965) found that when a single leaf of a wheat plant was infected with *Puccinia striiformis*, the proportion of the assimilates exported was only 0.4% compared with 20% from a corresponding healthy leaf on an uninfected plant. It has also been shown that uninfected primary leaves of *Phaseolus vulgaris* fed with ^{14}C-labelled CO_2 exported 50% of the label in a five-hour period, whereas primary leaves infected with *Uromyces appendiculatus* exported less than 2% in a similar time interval (Livne *et al.*, 1966).

In some cases infection may not only reduce export of carbohydrates from an organ, but also promote import into that organ. For example, in oak plants infected with *M. alphitoides*, Hewitt & Ayres (1976) observed that assimilates continued to be imported from uninfected leaves into infected leaves. Similarly, Livne *et al.* (1966) observed that when ^{14}C-labelled CO_2 was fed to uninfected trifoliate leaves of bean plants the label accumulated in primary leaves infected with *U. appendiculatus*. Siddiqui & Manners (1971) also showed that infection of wheat leaves by *Puccinia striiformis* increased the amount of ^{14}C-labelled assimilates moving to infected leaves. However, when single leaves of wheat were infected with *P. striiformis* they did not attract assimilate movement from other leaves (Doodson *et al.*, 1965).

Inorganic ions have also been shown to preferentially move to infected parts of the plant (Gerwitz *et al.*, 1965; Yarwood *et al.*, 1955). For example, Ahmad *et al.* (1982) demonstrated that not only carbohydrates but also nitrogen, phosphorus and potassium were retained to a greater extent in barley leaves infected with *P. hordei* than in uninfected leaves as the leaves age.

Conversely, other workers demonstrated a decrease in sugar content of leaves infected by rusts. Thus Murphy (1936) observed a decrease in soluble sugars in oat plants infected with *Puccinia coronata*. Similarly, the total sugar content and especially the sucrose fraction, decreased only slightly in leaves of a resistant wheat cultivar infected with *P. graminis* but to a significant extent in a susceptible one (Krog *et al.*, 1961).

The reduction of assimilate export from infected organs and the increased

import of assimilates to that organ would deprive other organs, such as roots, of required assimilates. In the case of powdery mildew infections, reductions in the percentage of assimilates translocated to roots were observed in barley plants by Edwards (1971) and Fríc (1975) and in wheat by Lupton *et al.* (1973). Edwards (1971) attempted an analysis of the fate of imported assimilates within roots of barley plants infected by *B. graminis* f.sp. *hordei*. He found that when $^{14}CO_2$ was fed to the tips of infected leaves, labelling of ethanol-soluble compounds was reduced much more than that of ethanol-insoluble compounds when import was reduced. Fric (1975) observed that when barley leaves were infected with *B. graminis* f.sp. *hordei*, the absolute amount of labelled assimilate reaching the roots from the infected leaves in the 24 hours after feeding was reduced by 27% following infection, while the amount remaining in the shoots was reduced by 20%. Walters & Ayres (1982) concluded that reduction in the growth of primary roots of barley infected with *B. graminis* f.sp. *hordei* was due to a reduction in the specific activity of different assimilates fractions within the roots (soluble, storage and structural). Similarly, infection of wheat leaves with *B. graminis* f.sp. *tritici*, also reduced the percentage of labelled assimilates exported to the roots in three susceptible cultivars fed $^{14}CO_2$ at the third, fifth or flag leaf stages (Lupton *et al.*, 1973).

Rust fungi were also reported to reduce the amount of assimilates translocated to roots. For example, Siddiqui & Manners (1971) showed that infection of wheat leaves by *Puccinia striiformis* decreased the amount of ^{14}C-labelled assimilates moving to the roots.

The magnitude of changes in assimilate translocation in response to biotrophic pathogen attack may depend on many factors, including environmental conditions, level of infection, level of host resistance and level of host tolerance of the parasite.

From the considerable experimental evidence currently available, several mechanisms have been proposed to be responsible for the disruption of assimilates translocation in infected plants. Firstly, Thrower (1965) attributed the reduced translocation from infected leaves to the rest of the plant simply to the direct effect of the pathogen providing an active sink for nutrient substances. This was experimentally demonstrated by the work of Edwards *et al.* (1966) who found that carbon transfer from infected barley leaves *to B. graminis* f.sp. *hordei* was very rapid.

In contrast, Farrar (1984) and Whipps & Lewis (1981) proposed a number of combinations of mechanisms, which may be responsible e.g. changes in growth regulator concentrations, altered permeability of infected cells, increased activity of invertase and amylase and changes in the concentration of orthophosphate (Pi).

1.6. Effects of infection on water relations

Water plays a very important role in all physiological processes in plants including photosynthesis, respiration, translocation, partitioning of metabolites, stomatal behaviour, protein synthesis, cell division, cell elongation and cell wall synthesis. Water stress will lead to the perturbation of all or some of these physiological processes and consequently will lead to

reductions in plant growth and yield (Kramer, 1983).

Healthy plants can protect themselves against the development of water stress by regulating stomatal aperture. The stomata are sensitive structures that represent the greatest variable resistance in the pathway of water movement through the plant (Ayres, 1981a) and any biotic or abiotic factor causing changes in the pattern of stomatal behaviour will affect plant water relations and consequently perturb growth and development. Many investigations have been carried out to determine the effects of obligate biotrophs on stomatal behaviour. These effects have been found to differ from one pathogen to another and from one host to another.

1.6.1. Effects on stomatal opening and closure

Infections of barley leaves by *Rhynchosporium secalis* (Ayres *et al.*, 1975) and of potato by the blight fungus, *Phytophthora infestans* (Farrell *et al.*, 1969) caused an increase in the rate of transpiration from the infected area of the leaf both in the light and in the dark. This increase was attributed to an increase in the mean stomatal aperture in the infected area in the light and the failure of the stomata to close in the dark. The downy mildew fungus *Peronospora tabacina* has also been found to affect stomatal opening in the leaves of its host tobacco in a similar manner (Cruickshank *et al.*, 1961).

In contrast, stomatal opening in the light has been reported to be inhibited by rust and powdery mildew infections as well as by some viruses such as sugar beet yellows virus (Hall *et al.*, 1972).

Transpiration in rust and powdery mildew infected tissues usually follows

the pattern of stomatal behaviour, decreasing in the light and increasing in the dark (Walters, 1985). Rust fungi enter their hosts through stomatal pores, develop mainly in the intercellular spaces of the leaf and inhibit stomatal movements progressively until eventually the stomata became fixed in an almost closed position (Duniway *et al.*, 1971a). However, with rust fungi, once fungal sporulation has ruptured the cuticle, non-stomatal transpiration increases and becomes the significant factor (Johnson *et al.*, 1934, 1940 and Murphy, 1935).

Paul *et al.* (1984) showed that after sporulation, groundsel (*Senecio vulgaris*) leaves infected with *Puccinia lagenophorae* transpired much more rapidly than did healthy controls. The same results were shown by Duniway *et al.* (1971a) in bean (*Phaseolus vulgaris*) leaves infected with *Uromyces phaseolus.*

Powdery mildew infections result generally in a failure of stomata to open fully in the light and to close fully in the dark (Majernick, 1971; Ayres, 1976). Wheat leaves infected with *B. graminis* f.sp. *tritici* were shown to have a significantly reduced stomatal opening within three to six hours after inoculation (Martin *et al.*, 1975). Majernick (1965) working with barley leaves infected with *B. graminis* f.sp. *hordei* reported that stomatal transpiration had reduced within one day after inoculation. Ayres (1979) also, using barley leaves infected with *B. graminis* f.sp. *hordei*, observed that reduced stomatal opening was not apparent until three days after inoculation. Other plant species other than cereals showed similar responses to mildew infection. For example, although garden pea (*Pisum sativum*) leaves infected

with *E. pisi* showed an initial increase in stomatal opening within the first 48 hours of inoculation, the stomatal opening became progressively reduced in the light and stomata failed to close completely in the dark (Ayres, 1976). Thomas *et al.* (1982) observed a 50% reduction in stomatal aperture five days after inoculation in sugar beet (*Beta vulgaris*) leaves infected with *E. polygoni*. Similar responses were observed in leaves of oak plants infected with *Microsphaera alphitoides*, but not until six days after inoculation, although, transpiration rates increased within two to three days after inoculation (Hewitt *et al.*, 1975).

1.6.2. Causes of changes in stomatal function

Ayres (1972 and 1975) investigated stomatal functioning in barley leaves infected with *Rhynchosporium secalis* and suggested that in the early stages of infection the increase in stomatal aperture was a result of the loss of osmotically active solutes from the epidermal cells of diseased leaves which consequently altered the turgor relations between guard cells and their surrounding epidermal cells. The increase in transpiration at later stages of infection was attributed to water loss through the ruptured cuticle (Ayres, 1975).

In the case of barley infected with *B. graminis* f.sp. *hordei*, Majernick (1965) suggested that a volatile product was involved in the inhibition of stomatal opening in the light. A similar suggestion was made by Martin *et al.* (1975) for wheat leaves infected with *B. graminis* f.sp. *tritici*.

The increased stomatal opening in the light that occurs in pea leaves

infected with *E. pisi* 48 hours after inoculation contrasts with the reduced stomatal opening in wheat within 6 hours of inoculation (Martin *et al.*, 1975) and in barley within 24 hours after inoculation (Majernick, 1965) with the cereal powdery mildew. The difference between peas and cereals (barley and wheat) was attributed to the lack of production of a volatile substances in peas infected with *Erysiphe pisi* or to the differences in the turgor pressures of guard cells and epidermal cells (Ayres, 1976). Furthermore, Ayres (1980) suggested that stomatal opening could be inhibited by substances synthesised by the host such as pisatin (a pterocarpan) which accumulates in pea leaves infected with *E. pisi.*

The increased rate of transpiration observed in barley leaves infected with *B. graminis* f.sp. *hordei* when 50% of the leaf was covered by mildew, was attributed to cuticular injuries caused by the infection (Paulech *et al.*, 1970; Majernick, 1965). In contrast, the increase in the rate of transpiration observed in oak leaves infected with *Microsphaera alphitoides* was attributed mainly to the fungal mycelium itself (Hewitt *et al.*, 1975).

Many of the differences in host response to different pathogens are most likely to be mainly due to the different ways the pathogens grow and develop on or within their host's tissues. However, the experimental differences in host response reported for particular pathogens are also likely to be due to an extent to the experimental procedures used, but are also likely to be due to the fact that different cultivars were used.

One significant factor missing from most of the studies was any measure of the way in which or the rate at which parasite biomass accumulated during

the course of the experiments. Even when parasite biomass accumulated to similar extents in the different cultivars used, reactions may be different due to different tolerances of the parasite in the tissues.

1.7. Aim of the project

The aim of this study was to investigate the levels of tolerance of *B. graminis* f.sp. *hordei* in wild barley lines compared with cultivated barley lines. Wild and cultivated barley lines are very close relatives, crossable, morphologically similar and infection by *B. graminis* f.sp. *hordei* progresses in the same manner on both taxons.

Studying tolerance in such systems would increase our understanding of tolerance and help us to identify tolerance characteristics, which could be useful in breeding programs. All components of vegetative growth of both shoots and roots were investigated as well as yield.

The photosynthesis and respiration rates in both wild and cultivated barley lines were determined as well as stomatal resistance to CO_2 diffusion through the leaf, which is an important photosynthesis-limiting factor. The effect of infection on cell division and elongation in leaves was checked by counting the number of stomata and epidermal cells other than stomata per leaf area. The compensation of uninfected leaves of infected plants was investigated by measuring photosynthesis rate in the fourth uninfected leaf of infected plants.

Chapter 2

GENERAL MATERIALS AND METHODS

2.1. Plant material

The lines of wild barley (*Hordeum spontaneum)* and of cultivated barley (*Hordeum vulgare*) used in this study were obtained either from the John Innes Centre, Norwich Research Park or from the Scottish Crop Research Institute, Invergowrie, Dundee. Before use, the wild lines were inbred for two generations to ensure that, as far as possible, genetically uniform populations of plants were used in the experiments.

2.2. Growth media

2.2.1. Solid media

Peat based potting compost (Levington Horticulture Ltd.) was used for all experiments except where it was necessary to harvest the root systems. Several rooting media were investigated for the ease with which the root systems could be harvested for analysis.

Horticultural grade sand (Silvaperl) alone was a very poor medium in which to grow plants because of the difficulty of maintaining an adequate water supply. In sand, the nutrient solution drained rapidly; growth was very slow, and the plants were clearly stressed because necrotic spots developed on the leaves. In contrast, a medium consisting of a 50:50 mixture of equal parts of horticultural grade sand and horticultural grade perlite (Celite) was found to allow good water retention and growth. The

nutrient solution used for this rooting medium was that recommended by the Agricultural Research Council, Letcombe Laboratory (Table 1). It was added 3 times a week, up to 8 weeks after planting and then once a week until the end of the experiment. The rooting medium was kept moist by adding water twice a day. The medium surface was covered with circular sheets of black plastic to prevent evaporation as well as algal growth.

2.2.2. Hydroponic solution

For the study of the mitotic index of root tips, plants were grown hydroponically in the Letcombe nutrient solution. Pregerminated grains were placed on a platform, which was then submerged in a tank containing 33 litres of the nutrient solution. The medium was aerated through a plastic tube fixed in the bottom of the tank, which was connected to an air pump.

The pH was maintained between 5.3 and 5.5 by the addition of concentrated H_2SO_4 as required, and the solution was changed weekly.

2.3. Plant growth

2.3.1. Grain germination and plant growth

Cultivated barley: Grains were germinated on damp filter paper in trays and incubated in the growth cabinet for four days before transplanting into the required growth medium.

Wild barley: Initial treatments were required to break the dormancy of the grain. Grains were placed on filter paper moistened with distilled water in

Petri dishes and allowed to imbibe for half an hour. The dishes were then wrapped in plastic film to conserve moisture, and placed in a refrigerator at 4°C for 7 days. After chilling, the vernalised grains were germinated in the same way as the cultivated barley grains. This method gave high percentage germination.

After germination, both cultivated and wild barley seedlings were transplanted into pots as required.

For growth analysis experiments, plants were grown singly in 12.7-cm plastic pots containing sand and perlite mixture (section 2.2.1).

For measurements of photosynthesis and stomatal resistance, germinated seedlings of both wild and cultivated lines were planted in 12.7 or 15-cm plastic pots containing Levington potting compost. Two seedlings were planted per pot. A week later, the seedlings were thinned to one per pot all of equal size or left as two per pot as required and any non growing seedlings were replaced. No supplementary feeding was carried out as most of the experiments lasted five to six weeks only.

2.3.2. Growth conditions

All the experiments were carried out in the same growth cabinet at a temperature of 20°C ± 2°C. The cabinet was illuminated during a 16h photoperiod by Kolorarc high-pressure mercury vapour lamps giving 130 μmol quanta m^{-2} s^{-1} of PAR at plant level. The relative humidity within the cabinet ranged between 65% and 80%.

Table 1: Composition of Letcombe Laboratory nutrient solution:

Compound	Formula	mmoles l^{-1}
Macronutrients		
Calcium nitrate	$Ca(NO_3)_2$ $4H_2O$	1.5
Potassium nitrate	KNO_3	5.0
Potassium di-hydrogen ortho-phosphate	KH_2PO_4	1.0
Magnesium sulphate	$MgSO_4$ $7H_2O$	1.5
Sodium nitrate	$NaNO_3$	2.0
Micronutrients		**µmoles l^{-1}**
Ferric EDTA	$C_{16}H_{12}O_8N_2FeNaH_2O$	9.220
Boric acid	H_3BO_3	9.220
Cupric sulphate	$CuSO_4$ $5H_2O$	0.160
Potassium chloride	KCl	14.100
Manganese sulphate	$MnSO_4$ $4H_2O$	3.600
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}$ H_2O	0.016
Zinc sulphate	$ZnSO_4$ $7H_2O$	0.770

2.4. Experimental design

The pots in the growth cabinet were arranged in a randomised design with three or four replicates and re-randomised at weekly intervals to ensure even growth.

2.5. Growth analysis

After each harvest, the plants were dissected and the following measurements were made:

2.5.1. Shoot and reproductive structures

1. Primary shoot height: The height of the primary shoot was measured from the rooting medium to the base of the youngest leaf or to the base of the flag leaf after ear emergence.
2. Number of leaves: Number of leaves was determined only for the primary shoots.
3. Leaf blade area: Leaf blade area of individual leaves on the primary shoot as well as total leaf blade area on all tillers was determined. Either a Delta-T flat bed scanner or a Delta-T photoelectric leaf area meter (Delta-T Devices Ltd. 128 Low Road, Burwell, Cambridge CB5 0EJ, England) was used. Leaf images were saved as tagged image files (TIFF) and Delta-T scan image software was used to calculate leaf area. Total green leaf blade area, including that affected by mildew, of each leaf on the primary shoot and total green leaf area of the tillers was measured. Prior to green leaf

blade area measurements, yellow and brown areas were excised from the leaves.

4. Number of tillers: Number of tillers per plant was recorded visually.
5. Dry weight: Plant parts were dried at 70°C in an oven for 48 hours, after which the dry weights were obtained.
6. Grain yield components: Counts and measurements were made when the grains were fully ripened in the ears of cv. Prisma. Because of possible grain loss through shattering from the wild lines, the grains were collected just before they were fully dried. For both cultivated and wild lines, ten plants of each treatment and each line were sampled and the following measurements made:
   - Number of fertile ears.
   - Number of grains per primary shoot ear.
   - Dry grain weight of primary shoot.
   - Thousand-grain dry weight on primary shoot:

     [(grain weight/number of grains) x 1000]
   - Number of fertile and unfertile tillers.
   - Total number of grains produced on all tillers.
   - Total grain dry weight on all tillers.
   - Thousand-grain dry weights on all tillers:

     [(grain weight/number of grains) x 1000]
   - Total dry weight of grains per plant.
   - The harvest index (HI), calculated from the equation:

$$HI = \frac{Y_{grain}}{Y_{total}}$$

Where: Y_{grain} : Total grain dry weight.

Y_{total} : Total plant dry weight.

2.5.2. Measurements of Root production:

The roots were recovered from the sand and perlite mixture by washing using a Delta-T root washer (Fig. 1). The root washer consisted of 4 buckets, each with a water supply and a central overflow pipe. Two jets in the base of the bucket created an upward turbulent flow that separated the roots from the sand and perlite. The lighter roots floated into the central overflow pipe, and were caught on a filter with a 550 μm mesh in a funnel under the table. The root washer was provided with an electric water pump to ensure adequate water pressure and a recirculation tank for conserving the water. After collection, the seminal and nodal roots were separated and counted manually. The nodal and seminal roots were stained with methylene blue to facilitate measurements using the Delta-T splash proof flatbed scanner. Root images were saved in the same way as the leaves and Delta-T scan image software was used to calculate the various parameters: root length, root surface area, root diameter or thickness and the number of lateral roots of both seminal and nodal roots.

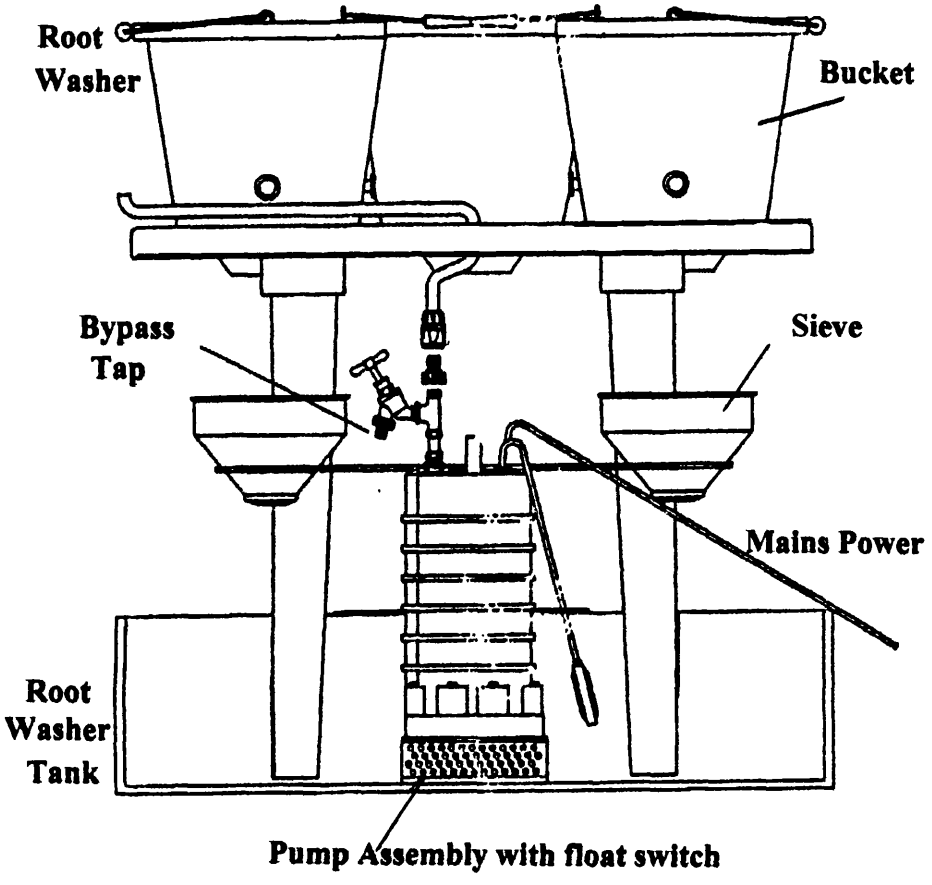


Fig. 1: Delta-T Root Washer

1. Root length: Root lengths were determined according to the procedure of Newman *et al.* (1966) with the correction introduced by Harris & Campbelle (1989), which takes account of roots that overlap.

2. Root Surface area: Root surface area was calculated from the formula: $S = \pi.T.L_s$

   Where T is the thickness and L_s is the total length of the sample.

3. Average root diameter: Average root diameters were determined separately for nodal and seminal roots including their laterals.

4. Number of lateral roots: Numbers of lateral roots on the intact nodal and seminal roots were determined using the Delta-T scan from root tip counts. The numbers of nodal and seminal root tips were subtracted from the total values given by the scanning image analysis to give an estimate of the number of lateral roots alone.

2.6. Growth indices

2.6.1. Relative growth rate (RGR)

The most important parameter in classical growth analysis is the RGR, first introduced by Blackman (1919) as an efficiency index. It is defined as the increase in plant dry weight relative to the total dry weight of that plant at a single time point (Fisher, 1921). In practice, RGR is estimated as a mean RGR between two time points. RGR is calculated

using the following equation:

$$RGR = \frac{1}{t_2 - t_1} \int d(\log_e W) = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where: W_1 = total plant dry weight at the first harvest.

W_2 = total plant dry weight at the second harvest.

t_2-t_1 = the time interval between the two harvests.

$\log_e$ = natural logarithm, with base e.

2.6.2. Unit leaf rate (ULR)

The ULR is the rate of plant dry matter production per unit of assimilatory area (leaf) and is often called net assimilatory rate. Williams (1946) provided a formula to estimate the ULR from measurements at two harvests:

$$ULR = \frac{(W_2 - W_1)(\log_e L_{A2} - \log_e L_{A1})}{(L_{A2} - L_{A1})(t_2 - t_1)}$$

Where: L_{A1} = total leaf area at the first harvest.

L_{A2} = total leaf area at the second harvest.

Radford (1967) has pointed out, that this relationship can not be integrated unless total leaf area and total plant weight are linearly related over the time between measurements. In this study, since W and LA of barley were linearly related over the short period of 7 days (Appendix Fig.1), the equation was applied. Radford (1967), Evans (1972), Coombe (1960) and Hughes *et al.* (1962) have listed other formulae for the

calculation of ULR where the relationship between W and LA is not linear.

2.6.3. Simple ratios

Leaf area ratio (LAR):

LAR is the ratio between the assimilatory area (leaf area) and total dry matter. It is a morphological index of the leafiness of the plant (Hunt, 1990). It describes the relative size of the assimilatory apparatus (Briggs *et al.* 1920b) and is calculated by the equation:

$$LAR = \frac{L_A}{W}$$

Where: L_A = total leaf area.

W = total plant dry weight.

Specific leaf area (SLA):

SLA is the ratio between total leaf area per plant and total leaf dry weight per plant (Hunt, 1990). It is an index of leaf thickness and is derived from the equation:

$$SLA = \frac{L_A}{L_W}$$

Where: L_A = total leaf area.

L_W = leaf dry weight.

Leaf weight ratio (LWR):

LWR is the ratio between total leaf dry weight per plant and total dry weight per plant (Hunt, 1990). It represents the mean fraction of the

plant's total dry matter distributed between the photosynthetic apparatus and the rest of the plant. LWR is calculated from the equation:

$$LWR = \frac{L_W}{W}$$

Where: L_W = total plant dry weight.

W = leaf dry weight.

The growth indices described above are interrelated as follows:

$$LAR = SLA \times LWR$$

$$RGR = ULR \times LAR$$

Root : Shoot ratio (RSR):

RSR is one of several ratios, which give estimates of the distribution of dry matter between the different plant organs. It is a measure of the distribution of dry matter between the root and the shoot systems and it is calculated by the equation:

$$RSR = \frac{R_{dw}}{S_{dw}}$$

Where: R_{dw} = root dry weight

S_{dw} = shoot dry weight

Shoot weight ratio (SWR):

SWR is the ratio between total shoot dry weight per plant and total dry weight per plant. It is an index of shootiness and is calculated by the equation:

$$SWR = \frac{S_W}{W}$$

Where: S_W = total shoot dry weight.

W = total plant dry weight.

Root weight ratio (RWR):

RWR is the ratio between total root dry weight per plant and total dry weight per plant. It is an index of rootiness and is calculated by the equation:

$$RWR = \frac{R_W}{W}$$

Where: R_W = total root dry weight.

W = total plant dry weight

Root area ratio (RAR):

RAR is a further morphological index of the rootiness of the plant being defined as the ratio between total root area per plant and total dry weight per plant (Evans 1972). It is calculated from the formula:

$$RAR = \frac{R_A}{W}$$

Where: R_A = total root area.

W = total plant dry weight.

Specific root area (SRA):

SRA is defined as the ratio of total root area to total root dry weight

(Evans 1972) and is calculated from the formula:

$$SRA = \frac{R_A}{R_W}$$

Where: R_A = total root area.

R_W = total root dry weight.

Specific root length (SRL):

SRL is the ratio of total root length to total dry weight of the roots and is calculated from the formula:

$$SRL = \frac{R_L}{R_W}$$

Where: R_L = total root length.

R_W = total root dry weight.

Root length ratio (RLR):

RLR is the ratio between total root length per plant to total plant dry weight per plant and is calculated from the formula:

$$RLR = \frac{R_L}{W}$$

Where: R_L = total root length.

W = total dry weight of the plant.

2.7. Determination of the mitotic index of root tips

The mitotic index of the root tips was examined in root preparations

made by a rapid squash technique using lacto-propionic orcein as a chromosome stain (Prakash 1986). Root tips, 2 mm in length, were detached and immersed in 0.2% colchicine for 2 hours and then fixed in Farmer's fluid (3 parts absolute alcohol :1 part glacial acetic acid) overnight. After removing from the fixing solution, the root tips were rinsed in distilled water and softened in 1N HCL at 60°C for 5 minutes. Finally, the tissues were mounted on a clean glass slide and a drop of lacto-propionic orcein was added. After 2 minutes a cover glass was applied and the root tips were squashed and heated gently. The numbers of cells undergoing cell division (metaphase, anaphase or telophase) were counted at a magnification of x 312.5.

2.8. Measurement of photosynthetic oxygen evolution

2.8.1. The principle of O_2 evolution measurements

Photosynthetic rates were determined polarographically using a Hansatech LD2 leaf disc O_2 electrode (Hansatech Ltd., Paxman Road, Hardwick Industrial Estate, King's Lynn, Nofolk, UK) designed by Delieu and Walker (1981, 1983).

The O_2 electrode:

The O_2 electrode (Fig. 2) is a conventional Clark-type $Pt/Ag/AgCl_2$ electrode (Delieu and Walker, 1972). It comprises a relatively large (2 mm) platinum cathode and a silver anode immersed in, and linked by, a KCl bridge. Both electrodes are set in a plastic (epoxy resin) disc; the cathode at the centre of a dome and the silver anode in a circular groove (electrolyte reservoir). The two electrodes are separated from the reaction medium by a Teflon membrane, which is permeable to oxygen. A cigarette paper "spacer" is usually placed beneath the membrane in order to provide a uniform layer of electrolyte between the electrodes. When a small voltage of 600-700 mV is applied, oxygen is reduced at the platinum surface, initially to hydrogen peroxide so that the polarity tends to discharge as electrons are donated to oxygen, which acts as an electron acceptor. The current which then flows is stoichiometrically related to the oxygen consumed at the cathode (Walker, 1987; Delieu & Walker, 1972).

The leaf-disc electrode assembly

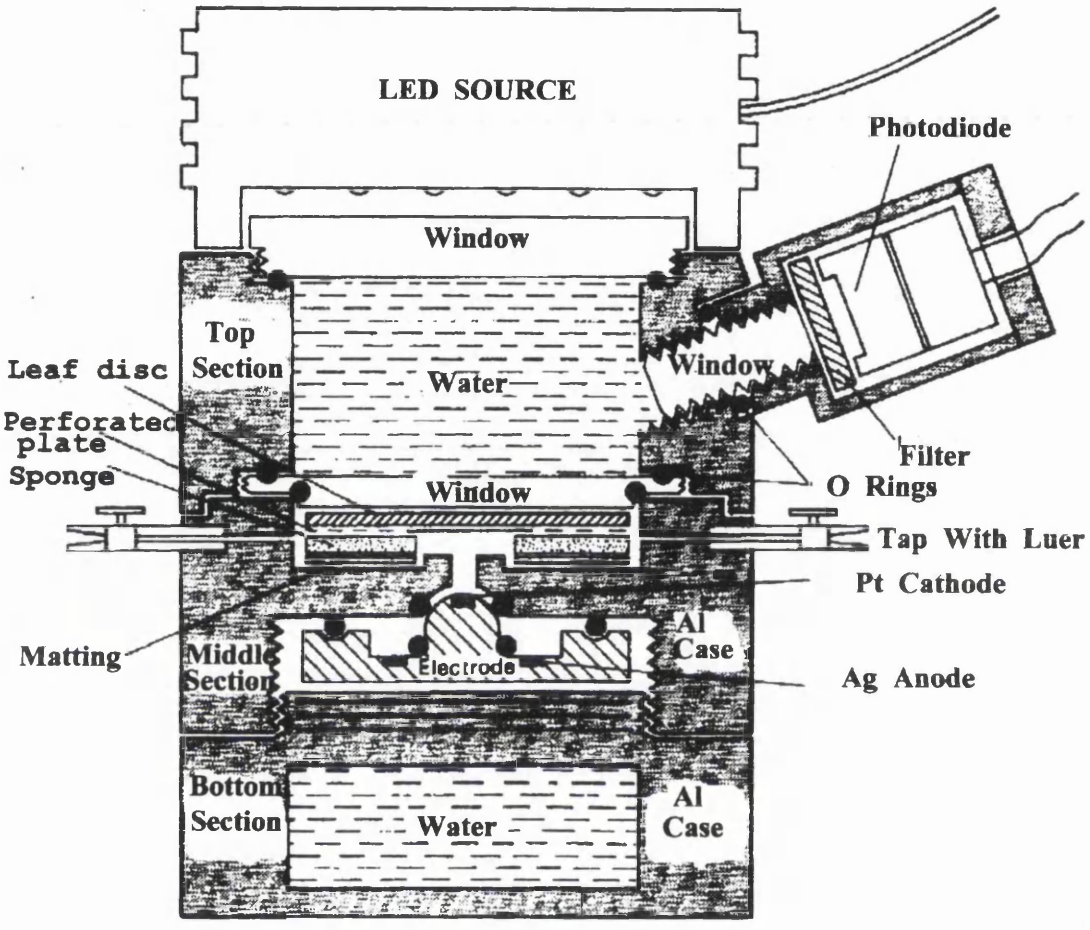


Fig. 2: The apparatus is made of anodised aluminium. Above and below the leaf chamber, circulating water maintains a constant temperature. A disc or piece of leaf is accommodated immediately below a window in the top section and immediately above the Clark-type O_2 electrode. The O_2 electrode measures the partial pressure of O_2 within the closed chamber (Walker, D. A. 1993).

The leaf chamber:

The leaf chamber is made of anodised aluminium, and good temperature control is provided by circulating thermostatically controlled water through it (25 ± 0.01°C). The electrode lies beneath the leaf chamber with its Pt cathode exposed to the atmosphere. An effective air-tight seal is produced by pressing the electrode against an O-ring. The leaf chamber is cylindrical and accommodates a leaf disc of 10 cm^2 area, or smaller pieces of leaf supported on a stainless steel grid and capillary matting, which presses the leaf against the temperature controlled water jacketed roof of the chamber. During use, the water-jacketed roof, through which the leaf is illuminated, is held down on the walls of the chamber by two clips, thereby compressing an O-ring in order to form a seal. Carbon dioxide can be generated within the chamber from a bicarbonate buffer or supplied in the gas-phase or both (Walker, 1987).

Calibration procedure:

The leaf chamber is fitted with two taps that communicate with the external atmosphere. These allow the chamber to be flushed with N_2, air or a CO_2 / air mixture. The nitrogen line and the air line on the chart recorder were obtained by flushing N_2 and air respectively. The difference between the electrical output of the electrode in air and N_2 is a measure of the partial pressure of O_2 in the internal atmosphere (Delieu and Walker, 1981). The deflection recorded in millivolts on the chart, caused by flushing N_2 (zero oxygen) and air corresponds to the number of moles of

O_2 at a particular temperature (T°C).

Calculations relating to volume and calibration:

The O_2 electrode measures concentration but it is also necessary to know the effective volume of the chamber. For this purpose, successive 200-µl volumes of air were introduced into the chamber using a 1-ml gas-tight syringe. This causes a deflection of the electrode trace that is proportional to the number of µmoles of O_2 present in the added air. The effective volume of the chamber is calculated using the general equation given by Walker (1987, 1993):

$$v = \frac{R_1 - [R_2 \times (1-V)]}{R_2 - R_1}$$

Where: R_1 = the initial electrode output.

R_2 = the electrode output when the plunger is depressed.

v = the effective volume of the chamber.

V = the volume of air injected by the syringe.

At the standard temperature and pressure the volume of O_2 in 1 ml of atmospheric air (21%, by volume) is 210 µl. This volume is equivalent to 9.37 µmol of O_2. Therefore, the amount of O_2 can be determined at any temperature (T) from the equation 9.37 x (273/273+T). If the temperature inside the chamber is 25°C and the volume is 5ml, the amount of O_2 is given by:

5x[9.37x(273/273+25)]= 42.920 µmoles of O_2

2.8.2. Photosynthesis measurements

All measurements were made at a constant temperature (25 ±0.01°C). Illumination was provided by 24° 50 Watt dichroic quartz halogen lamps (Wotan) and different light intensities, ranging from 1 to 1363 μmol quanta m^{-2} s^{-1}, were obtained using neutral light filters (Balzar, Lichtenstein).

The CO_2 concentration in the chamber was kept constant by mixing CO_2-free air with 10% CO_2 in air to give the desired CO_2 partial pressures (5 kPa).

After determining the chamber volume by the air injection method (see Hansatech LD2 instruction manual), the chamber containing the leaf disc was purged with air containing the desired CO_2 partial pressure (50 ml min^{-1}) for 5 minutes. The chamber was then closed and the leaf disc illuminated with 150 μmol quanta m^{-2} s^{-1} until a steady rate of O_2 evolution was obtained. The leaf disc was then darkened for 15 minutes to obtain a steady rate of O_2 consumption in order to determine dark respiration rate, and the light response of O_2 evolution was measured during a sequence of PPFD light intensities up to 1363 μmol quanta m^{-2} s^{-1}.

After measurement at each light level, the leaf chamber was opened to relieve any build up of pressure and purged again for 1 minute with the air at the desired CO_2 partial pressure. Eight light levels between 1 and 180 μmol quanta m^{-2} s^{-1} were used to obtain a reliable estimate of the quantum efficiency α.

2.8.3. Modelling photosynthesis

Different models have been proposed to describe the photosynthesis light response (PLR) curve. Blackman (1905) derived one of the earliest models which describes a response of photosynthesis which increases linearly with irradiance (light-limited) until the CO_2 supply becomes limiting (CO_2-limited). This model is inadequate, because the PLR curve shows no sharp discontinuity between the light-limited and CO_2-limited regions. For these reasons, other models were proposed:

2.8.3.1. The linear or rectangular hyperbola model

A later model proposed by Rabinowich (1951) describes the relationship between photosynthesis and irradiance in terms of a rectangular hyperbola (Fig. 3). This model, known as the linear model, was derived from the Michaelis-Menten relation between the rate of an enzyme-catalysed reaction and the concentration of its substrate. It defines two parameters, P_{max} the maximum (gross or net) photosynthesis rate and the quantum efficiency:

$$Pn = \frac{Pn\_ \times \alpha \times I}{Pn\_ + \alpha \times I} \quad (1)$$

Where Pn is net photosynthesis, Pn_{max} is the maximum rate of net photosynthesis, α is the quantum efficiency at low irradiance and I is the irradiance.

Gross photosynthesis (Pg) is defined by the equation:

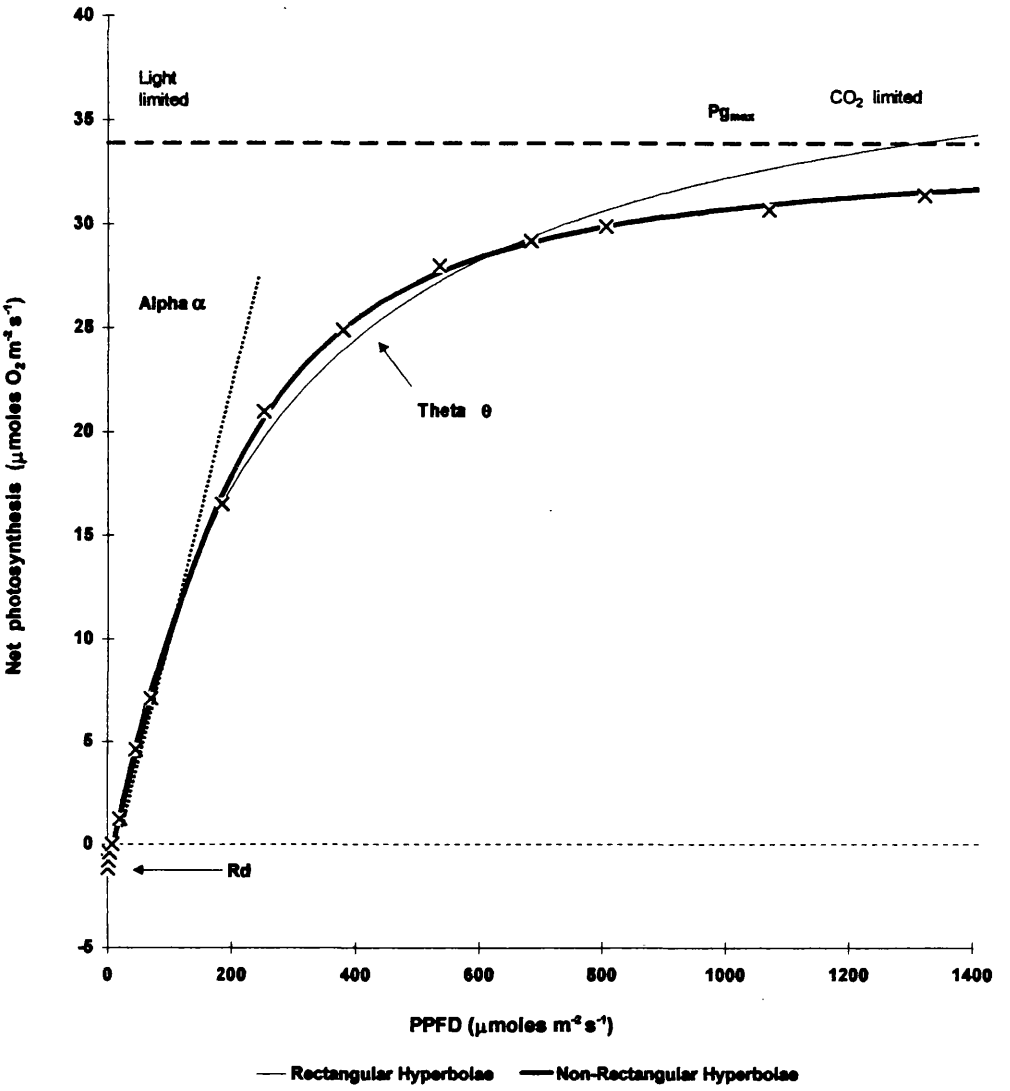


Fig. 3: The light response curve of the net photosynthesis in The cultivated barley cv. Prisma. (x) is the experimental data, Pg_{max} is the maximum rate of gross photosynthesis, α is the quantum efficiency calculated from the inintial slope of the PLR curve, Rd is the dark respiration and θ is the convexity of the PLR curve (rp / rp + rx).

$$Pg = Pn + Rd \quad (2)$$

Where Rd is the rate of dark respiration.

Equations (1) and (2) combine to become:

$$Pg = \frac{Pn_{-} \times \alpha \times I}{Pn_{-} + \alpha \times I} + Rd \quad (3)$$

By using equation (3), the linear model can be used to estimate a third parameter, dark respiration (Rd). However, this model has been shown to be a poor description of the PLR curve at certain saturating levels of CO_2 by some workers (Thornley, 1976; Chartier, 1970). Working with the flag leaf of winter wheat, Marshall and Biscoe (1980) demonstrated that the linear model over estimates the quantum efficiency (α), the maximum rate of photosynthesis (P_{max}) and dark respiration (Rd), and underestimates the rate of photosynthesis at the intermediate light-levels (100-500 μmol m^{-2} s^{-1} PPFD) range. This is because the model describes only the biochemistry of photosynthesis, taking no account of CO_2 transfer from the atmosphere to the chloroplasts. Other attempts have been made to find adequate mathematical models to describe the PLR curve. Using a general asymptote, Peat (1970) was able to obtain a more accurate description of the PLR curve of tomatoes, as did Biscoe *et al.* (1975) using barley.

2.8.3.2. Non linear model or non-rectangular hyperbola

This model was derived by Thornley (1976b) who realised that the actual PLR curve was better described by a quadratic model with three parameters, Pg_{max}, α and θ, where θ is a term that governs the 'convexity' of the PLR curve:

$$P'\theta - (\alpha . I + P_{max})P + \alpha . I . P_{max} = 0 \qquad (4)$$

When set at the limit where $\theta = 0$, the response degenerates into the rectangular hyperbola response described above, but at the other limit, where $\theta = 1$, the response becomes a Blackman-type curve (Blackman, 1905). This model combines a simplified description of the biochemical reactions occurring within the chloroplasts with the physical diffusion of CO_2 from the stomata to the chloroplasts. Marshall and Biscoe (1980) later extended this model to include estimates of dark respiration rate and therefore Pn. A derivation of the non-linear model is described here:

The model of Rabinowich was modified by Thornley (1976a,b) to give:

$$Pg = \frac{\alpha \times I \times Pg_{max}}{\alpha \times I + Pg_{max}} = \frac{\alpha \times I \times \left(C_f / r_x \right)}{\alpha \times I + \left(C_f / r_x \right)}$$

Where: C_f = is the CO_2 concentration at the site of fixation.

r_x = is the chemical or carboxylation resistance.

If: $Pg = Pn + Rd$

Then:
$$Pn + Rd = \frac{\alpha \times I \times \left(C_f / r_x \right)}{\alpha \times I + \left(C_f / r_x \right)} \qquad (5)$$

The net flux of CO_2 from the atmosphere to the site of fixation is:

$$Pn = \frac{C_a - C_f}{r_x}$$

and
$$C_f = C_a - Pn \times r_p$$

If C_f is eliminated from the equation (5), then:

$$Pn + Rd = \frac{\alpha \times I \times \left(C_a / r_x - Pn \times r_p / r_x \right)}{\alpha \times I + \left(C_a / r_x - Pn \times r_p / r_x \right)} \qquad (6)$$

To remove uncertainties created by r_x the numerator and denominator are multiplied by $r_x / (r_p + r_x)$ to give:

$$Pn + Rd = \frac{\alpha \times I \times \left(Pg_{max} - \theta \times Pn \right)}{\alpha \times I \times \left(1 - \theta \right) + \left(Pg_{max} - \theta \times Pn \right)} \qquad (7)$$

Where: $Pg_{max} = C_a / \left(r_p + r_x \right)$ and $\theta = r_p / \left(r_p + r_x \right)$

If equation (7) is expanded, equation (8) is obtained:

$$\theta.P^2n-(Pg_{\max}+\alpha.I-\theta.Rd)Pn+\alpha.I.Pg_{\max}-(1-\theta)Rd.Pg_{\max}=0 \quad (8)$$

In equation (8), α, the initial slope, is the quantum efficiency at zero irradiance, θ is the ratio of physical-to-total resistance (also called the convexity or rate of bending of the PLR curve) where, Pg_{max} is the maximum rate of net photosynthesis calculated from the equation of the asymptote:

$$Pn_{\max}=Pg_{\max}-(1-\theta)\times Rd \quad (9)$$

When θ is zero, the carboxylation resistance r_x is greater than the physical resistance r_p and equation (8) reduces to a rectangular hyperbola, but when θ is unity, r_p is greater than r_x and equation (8) reduces to a Blackman type response. The model is in a quadratic form:

$$Y=a.Pn^2+b.Pn+c=0 \quad (10)$$

Where: $a=\theta$,

$$b=-(Pg_{max}+\alpha.I-\theta.Rd)$$

$$c=\alpha\cdot I\cdot Pg_{\max}-(1-\theta)\cdot Rd\cdot Pg_{\max}$$

Equation (10) can then be solved for P_n as:

$$x=\frac{-b\pm\sqrt{b^2-4ac}}{2a} \quad (11)$$

2.9. Chlorophyll analysis

Chlorophyll concentration was determined in extracts made using hot methanol as described by Hipkins and Baker (1986), since hot methanol was found to result in rapid chlorophyll extraction.

Healthy and infected leaf samples used for photosynthesis measurements were cut into small pieces before placing in 10-ml methanol in 15 ml centrifuge tubes to allow the solvent to penetrate the tissues. The tubes were wrapped in aluminium foil to avoid light-induced breakdown of chlorophyll, and placed in a water bath heated to 60°C for 40 minutes. The chlorophyll/methanol solution was allowed to cool to room temperature and then centrifuged at 1500 rpm for 5 minutes in order to remove the leaf tissues. The supernatant was poured into a 15-ml volumetric flask and made up to a known volume. Chlorophyll content was determined spectrophotometrically using methanol as a standard at 650 and 665 nm.

From the specific absorption coefficients given by Mackinney (1941) for chlorophyll a and b in methanol, equations similar to those used for 80% acetone solutions (Arnon, 1949) have been derived (Holden, 1965):

$$Chlorophyll\ a\ (mg\ l^{-1}) = 16.5 \times D_{665} - 8.3 \times D_{650}$$

$$Chlorophyll\ b\ (mg\ l^{-1}) = 33.8 \times D_{650} - 12.5 \times D_{665}$$

$$Total\ Chlorophyll\ (mg\ l^{-1}) = 25.5 \times D_{650} - 4.0 \times D_{665}$$

2.10. Stomatal measurements

2.10.1. Epidermal cell size

The numbers of epidermal cells and stomata per unit area of the lower epidermis of the third leaf of uninfected and infected barley plants were determined microscopically. Clear nail varnish was applied to the lower epidermis and left for two hours to dry. The epidermal imprints were then peeled off with fine forceps and placed in distilled water on a microscope slide. A cover slip was then placed over the imprints to prevent curling or blowing away. Numbers of stomata and epidermal cells other than stomata were counted per field of view (0.623 mm^2) at x 126 magnification.

2.10.2. Stomatal resistance

An automatic diffusion porometer MK3 Delta-T Devices (128, Low Road, Burwell, Cambridge CB5 OEJ, UK) was used to measure stomatal diffusive resistance.

2.10.2.1. Principle of the measurements

The diffusion porometer measures the approximate rate of diffusion of water vapour through the stomata. Its operation assumes that water vapour diffusion out of a leaf into dry air is regulated by the degree of opening of the stomata (neglecting cuticular transpiration). A small chamber containing a relative humidity sensor is clamped to the leaf. Prior to reading, a small electric diaphragm pump blows a stream of air, dried

by passing through silica gel, into the chamber. Water vapour emitted by the transpiring leaf surface causes the relative humidity (RH) within the chamber to rise and the sensor becomes moist. As the sensor becomes moist its conductivity increases and the rate of increase in conductivity to a set value is directly proportional to the rate of outward diffusion of water vapour through the stomata. The difference in temperature between the leaf and the cup is measured by two thermistors, which are built into the leaf clamp.

2.10.2.2. Porometer calibration

The porometer is supplied with a moulded polypropylene calibration plate with six groups of holes each of known diffusion resistance. A source of water vapour is provided by backing the plate with damp filter paper, which is sealed to the plate with waterproof tape. The sensor head is clipped onto the calibration plate and readings are taken from each set of holes. A calibration graph of plate resistances is plotted against the corresponding counts (Automatic porometer MK3 operating manual,) and this graph is used to convert the counts obtained from the leaf measurements into diffusion resistance values.

2.10.3.Experimental procedure

Fifty seedlings were raised as described in section 2.3. When two weeks old, the fully expanded third leaves on 25 plants of each line were inoculated in the middle region of the adaxial surface, using a camel hair

brush (section 2.11.3.2). The tip and the base of the leaf blades were kept free of mildew. The other 25 plants of each line were kept free of mildew by adding 0.05% Benlate solution to the pots at weekly intervals. The inoculated and uninoculated plants were then placed randomly in the growth cabinet.

The stomatal resistance measurements were taken from four plants per treatment per line. The first measurements in the light were made 24 hours after inoculation, and then the same plants were placed in a dark room for 24 hours after which porometer measurements were made under green light. Subsequent measurements were made at two-day intervals until 5 sets of measurements had been made.

Stomatal resistances were measured in the middle and tip of both adaxial and abaxial surfaces of infected and uninfected third leaves on each plant line.

2.11. Mildew cultures

2.11.1. Single-spore isolation

The initial mildew inoculum was obtained from naturally infected barley plants growing in the greenhouse. The inoculum used in all the experiments was derived from a single conidial chain in an attempt to ensure genetic purity. Each single conidial chain isolate was first cultured on a detached leaf maintained on benzimidazole agar in a Petri dish (See below). The conidia produced from that generation were then used to inoculate either, other detached leaves, or whole plants grown in an isolation plant propagator. In this way, the large amounts of inoculum required for the inoculation of plants in all the experiments were produced.

2.11.2. Maintenance of mildew isolates

2.11.2.1. On whole plants using a Burkard isolation plant propagator

An isolation plant propagator (Burkard Manufacturing Co Ltd.) of a type devised by Jenkyn *et al.* (1973) was used to maintain mildew on whole plants. Seedlings of the susceptible barley cv. Golden Promise were grown in the propagator pots in a growth cabinet free from mildew. When 12 days old, the seedlings were inoculated, covered with a transparent polystyrene cover and placed on the propagator. The multiple units of plant pots were provided with filtered moistened air under positive pressure thus keeping the plants within the units free from contamination. The plants were watered from below using wicks of absorbent cotton.

Mildewed plants were replaced every four to five weeks with a set of newly inoculated young seedlings.

2.11.2.2. On detached leaf pieces

Detached leaves were cut into pieces of approximately 2-cm length and placed, 4 per Petri dish, on 0.5% water agar containing 100 mg l^{-1} benzimidazole. The benzimidazole was included in the agar in order to delay the senescence of the leaf segments (Person *et al.*, 1957) and thus, to allow appropriate development of the mildew. The agar medium was prepared by boiling distilled water containing 0.5%-powdered agar while stirring until the agar was completely dissolved. After cooling to 50°C, benzimidazole was added to give a concentration of 100 mg l^{-1}, and the medium was poured into Petri dishes. After inoculating the leaf segments, the dishes were incubated for 10 to 14 days in a growth room maintained at 20 ± 2°C with a 16 hours photoperiod providing a PPFD of 130 μmol quanta m^{-2} s^{-1}. Mildew colonies were apparent 5 days after inoculation and by the 10th day, leaf segments had developed large sporulating colonies.

2.11.3. Methods of inoculation

Three different inoculation procedures were used depending upon whether whole plants or parts of plants were being inoculated.

2.11.3.1. Whole plants

Whole plants for growth analysis experiments were inoculated by

shaking heavily infected young seedlings over them daily over a period of one week.

2.11.3.2. Individual leaves on whole plants

This method was used to inoculate the middle region of the third leaves for stomatal resistance measurements. Inoculations were made using a soft camel-hair brush to transfer conidia from recently produced colonies on whole plants or on detached leaves to the part of the leaf required. The brush after sterilising in 95% ethanol, was stroked over the surface of an infected leaf to pick up conidia, and then tapped lightly to dislodge the spores as it was passed above the adaxial surface of the leaf to be inoculated. This method produces a relatively uniform distribution of conidia (Russell *et al.* 1975).

2.11.3.3. Spore-settling tower

This method was used to inoculate the third leaves of plants used for photosynthesis and respiration measurements. The spore-settling tower produced relatively uniform distributions of conidia over individual leaves.

The leaves to be inoculated were aligned horizontally, with their adaxial side uppermost on the inoculation table of the tower. Newly produced conidia from two-week-old (from planting) heavily mildewed seedlings were used as inoculum; older conidia were removed by shaking the infected plants 24 hours prior to inoculation so that only conidia produced during the intervening 24 hours were used as inoculum. Conidia

were blown into the tower using a blowing brush and allowed to settle for three minutes. A coverslip covered in Vaseline grease was also placed in the tower and the amount of inoculum landing on the leaves was estimated by counting conidia that had settled on the coverslip. The density was generally 5-8 conidia per mm^2 in all the experiments. After inoculation, the plants were grown on in the growth room.

2.11.4. Mildew assessment

Two methods were used to estimate the amount of mildew growth on infected leaves: Visual assessment of mildew cover on the leaves and assessment of fungal biomass produced from counts of the number of conidia produced on the leaves.

2.11.4.1. Visual assessment of mildew cover

Visual assessment of the level of mildew present on the leaf is relatively rapid but is a subjective method and liable to error. The method involves comparing standard diagrams (Fig. 4) defining specific levels of mildew cover on leaves and stems, with infected leaves to determine percentage leaf blade area covered by mildew colonies. Pustules on the main stem were not included in the assessment since stem area contributes only a negligible portion of the total photosynthetic area of the plant. Leaf area of each leaf on the main axis was measured either using a photoelectric leaf area meter or the Delta-T scanner. Total percentage area of all leaf blades covered by mildew was calculated as follows:

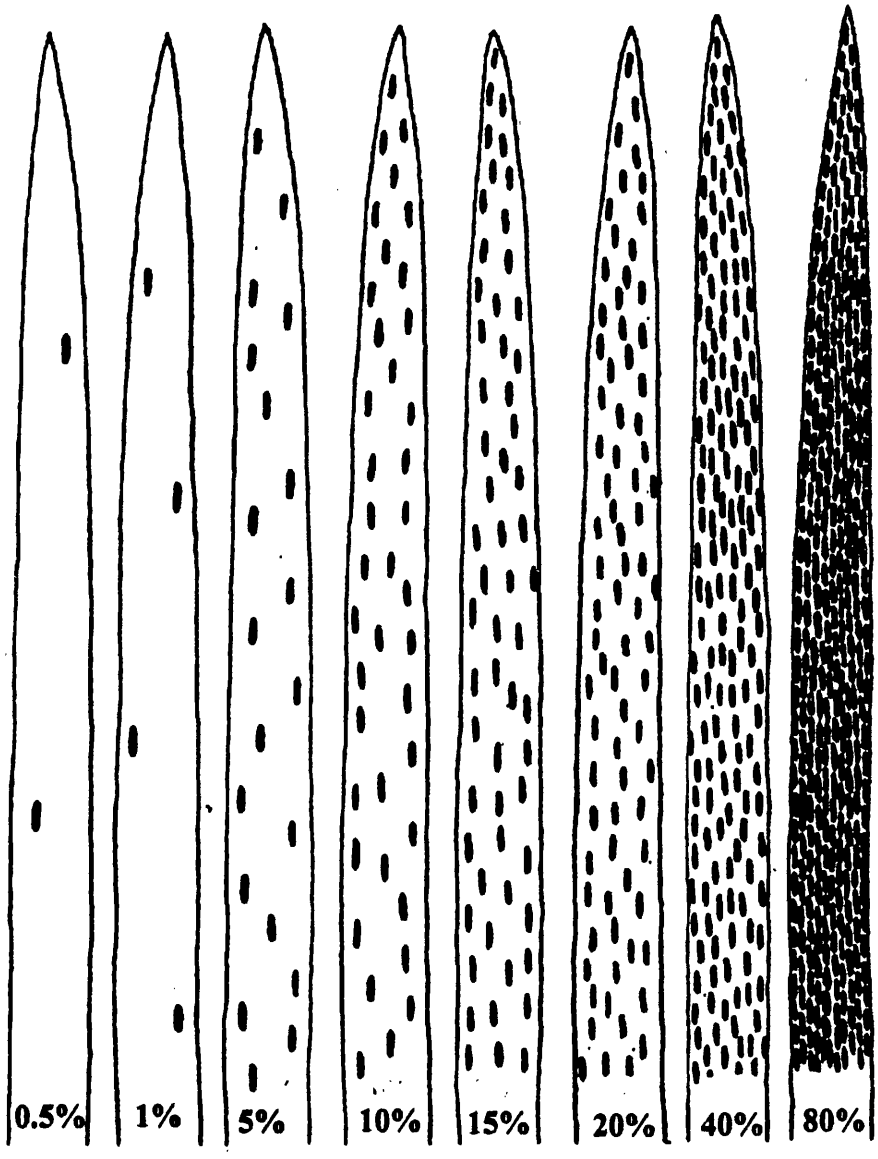


Fig. 4: The 'standard area' diagrams used for the determination of percentage leaf area covered by powdery mildew.

$$Z = \frac{y_1.a_1 + y_2.a_2 + y_3.a_3 + \ldots\ldots y_n.a_n}{a_1 + a_2 + a_3 + \ldots\ldots a_n}$$

Where y_1, y_2, y_3...y_n are the percentage areas of the leaf blade of leaves 1, 2, 3,...n (on the primary shoot) with leaf blade areas of a_1, a_2, a_3,...a_n covered by mildew colonies.

To estimate the percentage leaf segment covered by mildew in the photosynthesis experiment, the diagram in Fig. 5 was used.

2.11.4.2. Measuring mildew biomass production

Several methods of collecting conidia were tried. Initially a small cyclone collector was made and tested, but it proved difficult to collect conidia from one leaf without disturbing other leaves and dislodging their conidia. In the method finally adopted, infected leaves were inserted singly into glass tubes, which were long and wide enough to insert the leaves without loss of conidia. The leaves were then cut carefully from the plant using a new razor blade and the tubes sealed prior to transfer to the laboratory. Using a vortex mixer, conidia were detached from the leaves into distilled water containing the wetting agent Tween 80; the wetting agent facilitates conidia dislodgement. The conidial suspension was then centrifuged at 1000 rpm for 10 minutes, the supernatant discarded and the pellet of conidia resuspended in 5 ml distilled water and the number of conidia present in the suspension was determined using a hemocytometer. Ten counts were made per leaf sample and the mean number of conidia per unit leaf area was calculated. The washed leaves were blotted dry and

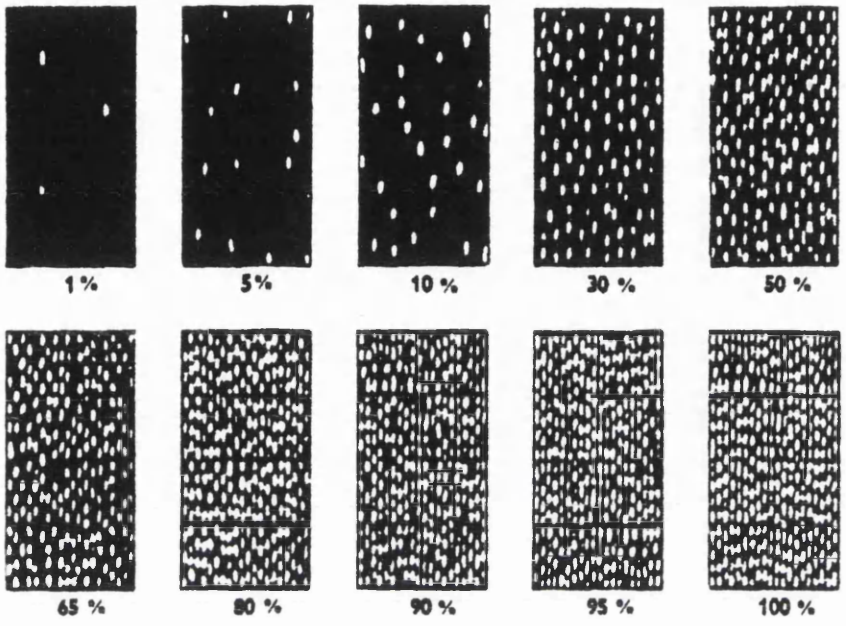


Fig. 5: Scale for estimating mildew infection on leaf segments.

the leaf area was determined using the Delta-T scanner.

2.11.5. Maintenance of mildew-free control plants

The control uninfected plants were maintained free of mildew using fungicides. The main fungicide used was Benlate (Benomyl; methyl-1-(butycarbamoyl)-2-benzimidazole-carbamate). It is a systemic fungicide, which is absorbed through the foliage or roots, converted to carbendazim (MBC) and translocated within the plant through the xylem. It inhibits the growth of sensitive fungi by blocking nuclear division. It is non-phytotoxic when applied at low but fungitoxic concentrations (Thanassoupoulos *et al.* 1970; Reyes, 1975; Ben-Kalio, 1976; Fraser, 1981 and Paul *et al.* 1989). Meek (1981) observed that when applied at low concentration (0.02%), it did not affect the growth of barley, while Cameron (1993) demonstrated that it could be used at even higher concentrations (0.05%) without growth being affected.

In this study, Benomyl was applied weekly as a soil drench at 0.05% concentration. The pots were placed on saucers to ensure that the fungicide did not transfer from treated to untreated pots. Sometimes Benomyl treatments did not control mildew completely over the whole experiment in growth analysis experiments, and an application of the systemic fungicide Patrol (Fenpropidin) was then applied. Fenpropidin is based on piperidine whose activity is directed at the disruption of the synthesis and function of fungal cell membranes by the inhibition of ergosterol biosynthesis. When applied at low concentrations (0.1%), Fenpropidin has been found to be non-

phytotoxic to oats (Sabri 1993). Prior to spraying, the plants requiring treatment were moved to another growth cabinet, sprayed and then returned.

To investigate compensatory photosynthesis in the fourth leaves of plants whose three lower leaves were inoculated, applications of yellow sulphur were applied weekly to the fourth leaf as finely ground dust. When the fourth leaves were fully expanded, the lower three leaves of one set of plants were inoculated (section 2.11.3.2). The fourth leaves were covered with a plastic bag to protect them from the inoculum during inoculation. Tillers appearing in addition to the main shoot were removed to maintain a constant ratio of healthy to infected tissues.

Chapter 3

REACTIONS OF BARLEY LINES TO MILDEW INFECTION

3.1. Introduction

A number of wild and cultivated spring barley lines were tested for their reactions to powdery mildew in order to select lines expressing a range of different reactions for study (Table 2).

Three seedlings of each line were grown in the greenhouse and inoculated by shaking heavily infected seedlings over them (see Materials and Methods).

3.2. Results

3.2.1. Cultivated barley

Four cultivars were tested for their reaction to mildew infection:

1) Golden Promise: A highly susceptible cultivar (plate 1) with no known resistance genes. It is a mutant of the cultivar Maythorpe being produced using gamma rays.

2) Prisma: A very susceptible cultivar (Plate 2), with at least one defeated resistance gene. It was derived from a hybrid between cv. Triumph x cv. Cambrinus crossed with cv. Piccolo.

3) Triumph: A moderately susceptible cultivar (Plate 3), with at least one defeated resistance gene. It develops considerable necrosis in response to mildew infection. It was derived from cv. Diamant x St. 14029/64/9.

Plate 1: Infected leaf of cv. Golden Promise

Plate 2: Infected leaf of cv. Prisma

Plate 3: Infected leaf of cv. Triumph

Plate 4: Infected leaf of the wild barley line B19909

4) Camargue: A highly resistant cultivar (Immune) to powdery mildew infection. When inoculated with mildew conidia, it shows no sign of necrosis or mycelium development. It was derived from a hybrid between cv. Diamant x14029/64/6 crossed with Km 1192.

Only the first three cultivars were used in this investigation.

3.2.2. Wild lines

Twenty five wild barley lines were tested for their reaction to powdery mildew infection. The reactions of all the wild and cultivated lines were summarised in Table 2.

The tests showed a range of reactions from highly susceptible through high levels of partial resistance to complete resistance. The two most susceptible wild lines B19909 (Plate 4) and I-17-40 and line B8893 (Plate 5), which reacts hypersensitively to *B. graminis* f.sp. *hordei* were selected for use.

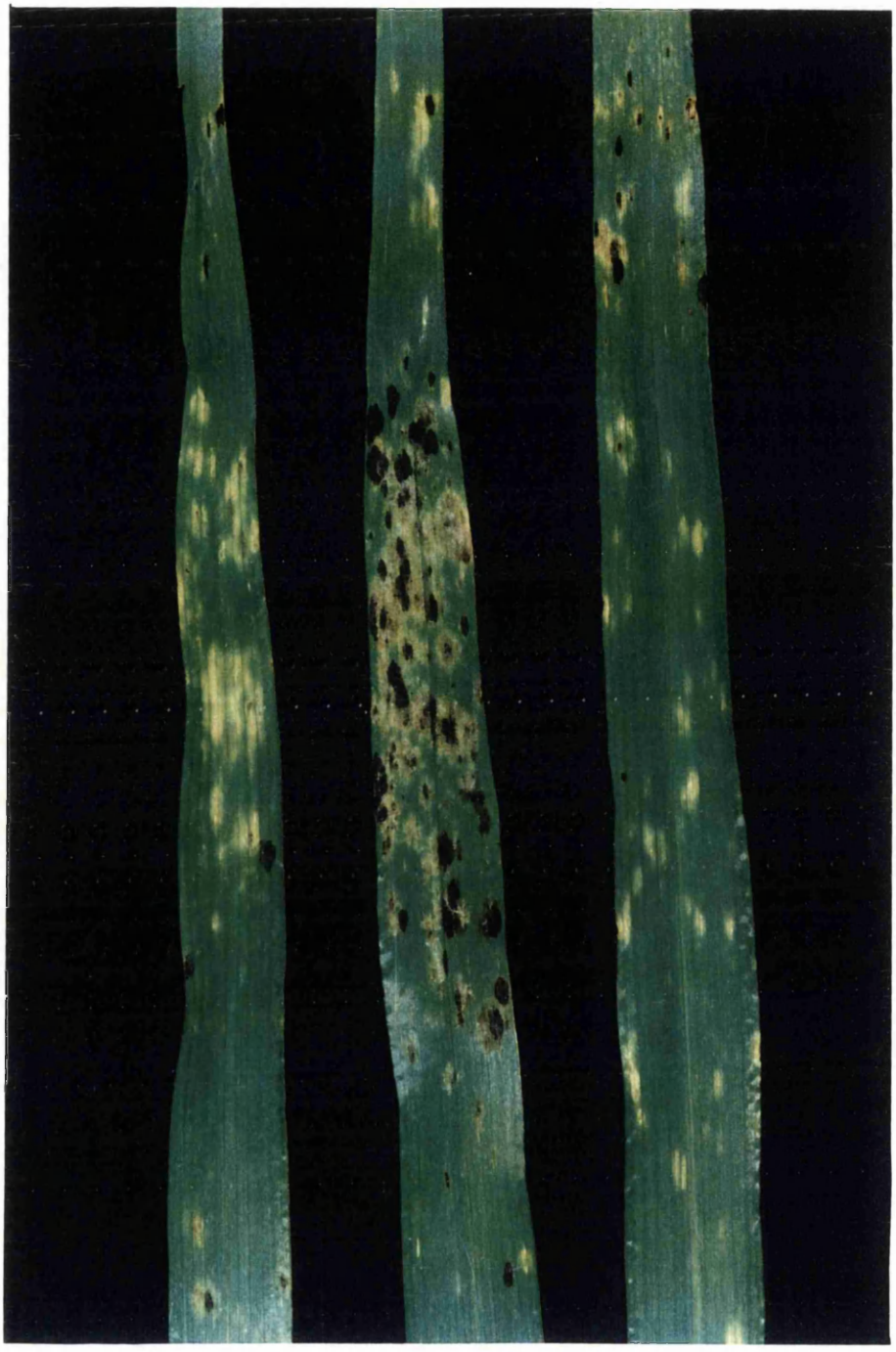


Plate 5: Infected leaves of the wild barley line B8893.

Table 2: Reactions of barley lines to powdery mildew.

Plant line	Reaction to mildew (scores)
Wild lines	
8876	0
D-49	0
8861	1
8878	1
8894	1
8895	1
8925	1
19917	1
8854	2
8858	2
8888	2
8893	**2**
8911	2
8919	2
8936	2
8937	2
8938	2
19926	2
8850	3
19905	3
19935	3
19937	3
I-16	3
19909	**4**
I-17-40	**4**
Cultivated cultivars	
Golden Promise	**4**
Prisma	**4**
Triumph	**3**
Camargue	0

0: Very resistant **1**: Resistant. **2**: Moderately resistant.

3: Moderately susceptible. **4**: very susceptible.

2 and **3** show hypersensitive necrosis to B. graminis mycelium.

The lines selected are shown in bold type.

Chapter 4

THE EFFECTS OF MILDEW INFECTION ON THE GROWTH AND DEVELOPMENT OF THREE BARLEY LINES.

4.1. Introduction

The effects of powdery mildew infection on host growth and development were studied in one cultivated barley, cv. Prisma and two lines of wild barley, I-17-40 and B19909. The experiment was carried out in a growth cabinet and the results are presented in Text Table 3 and Appendix Tables 1 to 10, and summarised in Appendix Tables 13A and 13B.

4.2. Mildew development

4.2.1. Levels of infection

Percentage mildew cover on each leaf surface at each harvest is presented in Fig. 6A. The levels of infection developing on the growth cabinet grown plants were much higher than those developing on plants grown in the greenhouse.

The plants were inoculated two weeks after planting when the third leaves were fully expanded. Within five days of inoculation small isolated mildew colonies were visible scattered over the whole plant. From four weeks after inoculation, about 27% of the leaf blades on the primary shoot of cv. Prisma and line I-17-40 was covered by mildew and about 34% on line B19909. The fungal colonies continued to increase rapidly on cv. Prisma and line B19909 until about six weeks after inoculation when the experiment was terminated, the levels of infection had reached about 40% on both.

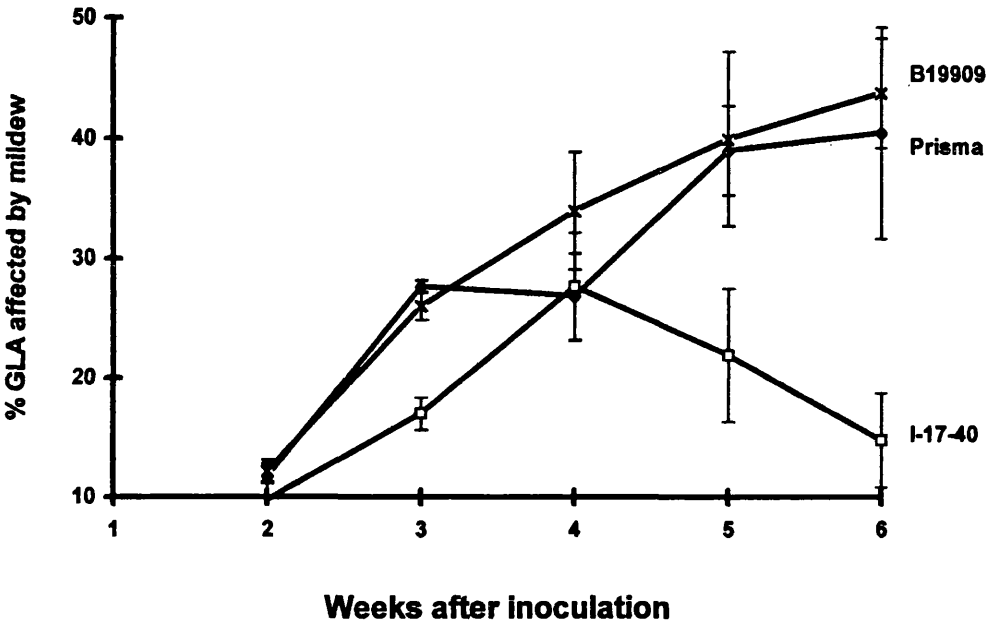


Fig. 6A: **The percentage green leaf area affected by mildew infection. Each datum point is the mean of three replicates. The vertical bars are the standard errors.**

In contrast, mildew cover developed differently on line I-17-40. Although it reached 27% by the fourth week after inoculation the same as cv. Prisma, it then decreased dramatically falling to 15% six weeks after inoculation, because of the high level of adult plant resistance expressed by the upper leaves.

4.2.2. The development of fungal biomass

4.2.2.1. On the primary shoot

Measurements of conidial production involved destructive sampling, and so a different set of plants from those used for the growth analysis was used for this study. Conidial production per unit area of the individual infected leaves on the primary shoot of the three lines is plotted in Fig. 6B while the cumulative number of conidia produced on the whole primary shoot of each line is plotted in Fig. 6C.

Fig. 6B shows that individual leaves of line B19909 generally produced more conidia than those of cv. Prisma, which in turn produced more conidia than those of line I-17-40. However, four weeks after inoculation, leaf five of cv. Prisma and line I-17-40 had produced about the same number of conidia, while that of line B19909 had produced a slightly higher number. As infection progressed, the older leaves supported the production of more conidia in line B19909 than in cv. Prisma or line I-17-40, the latter producing the lowest number of conidia.

The total number of conidia produced on the primary shoot of line B19909, by each sampling time, was significantly higher ($p < 0.001$) than on line I-17-40, and also higher than on cv. Prisma (Fig. 6C). By the end of the

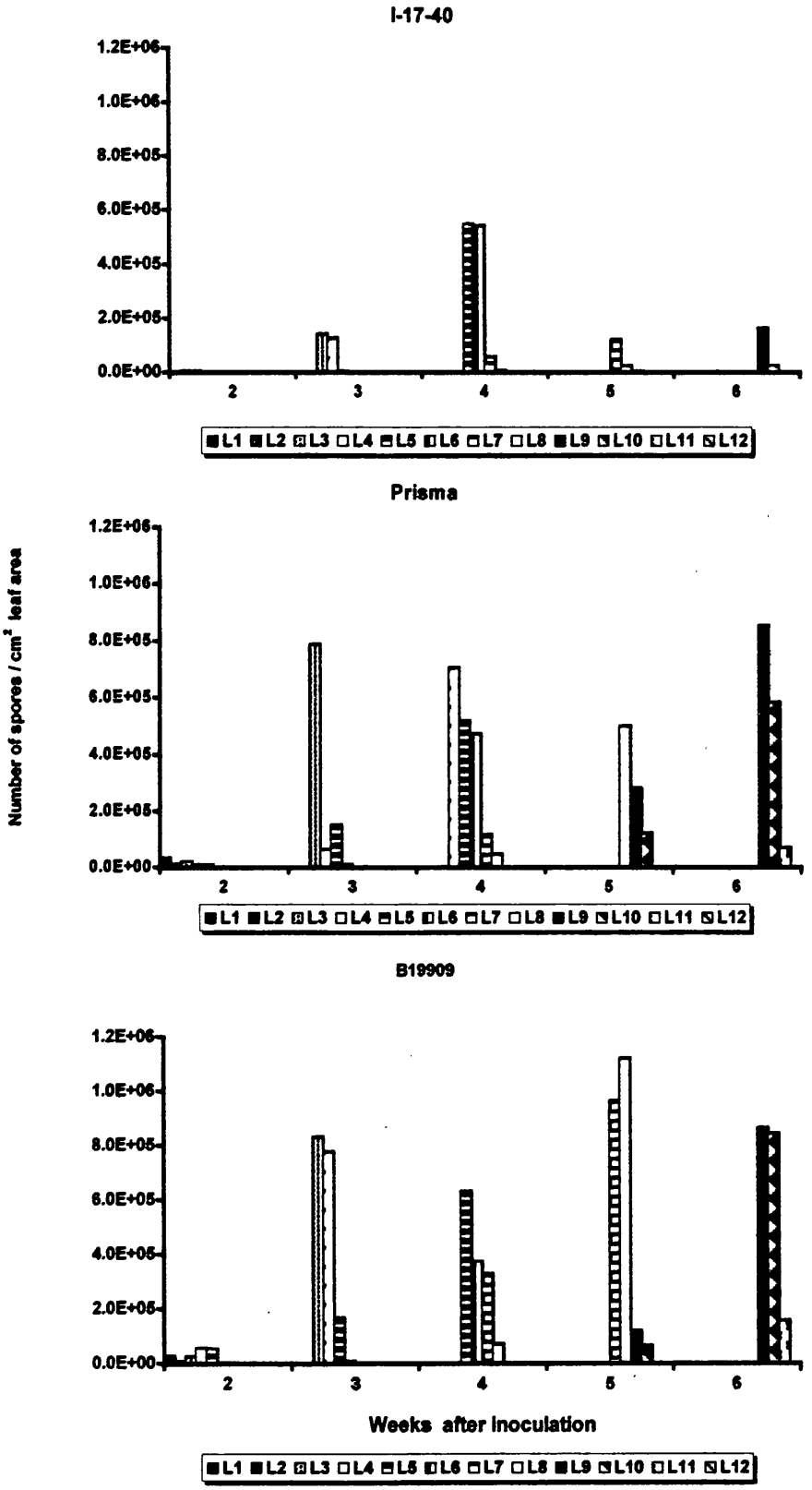


Fig. 6B: The number of spores produced by mildew on the individual leaves expanded on the primary shoot of the three barley lines.

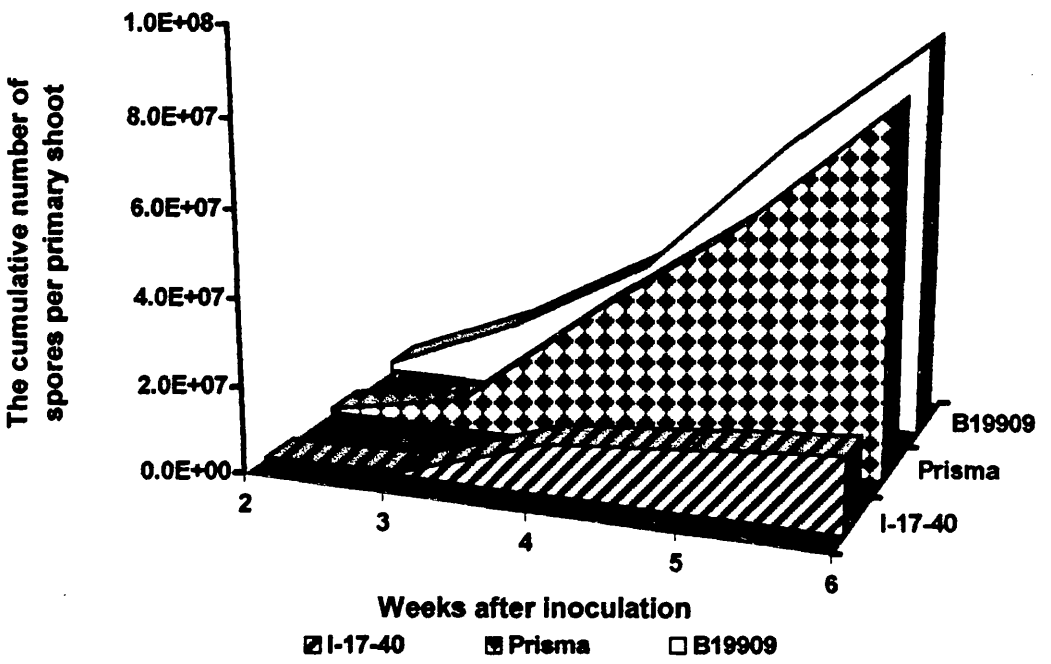


Fig. 6C: The cumulative number of spores produced by mildew on the leaves of the primary shoot of the three barley lines.

experiment (six weeks after inoculation), the total number of conidia produced by line B19909 was about $9.1x10^7$, by cv. Prisma about $8.3x10^7$ and by line I-17-40 about $1.7x10^7$.

4.2.2.2. On the whole plant

The cumulative number of conidia produced on each plant was calculated from total green leaf areas of tillers and primary shoots assuming that conidial production on the leaves of each tiller followed the same pattern as on the primary shoot.

The results plotted in Fig. 6D, show significantly higher ($p < 0.05$) conidial production on line B19909 compared to the other two lines, with line I-17-40 supporting the production of the lowest number of conidia. At the final harvest, the total number of conidia produced was about $1.4x10^8$ on line I-17-40, about $3.1x10^8$ on cv. Prisma and about $4.5x10^8$ on line B19909. Clearly B19909 supports more mildew development than cv. Prisma and much more than line I-17-40.

4.3. The effects of infection on plant growth and development

4.3.1. Effects of infection on shoot morphology

Measurements on shoot components of infected and uninfected plants of each line, at each harvest, are plotted graphically in Figs. 7, 8 and 10, while certain details of leaf development are recorded in Table 3.

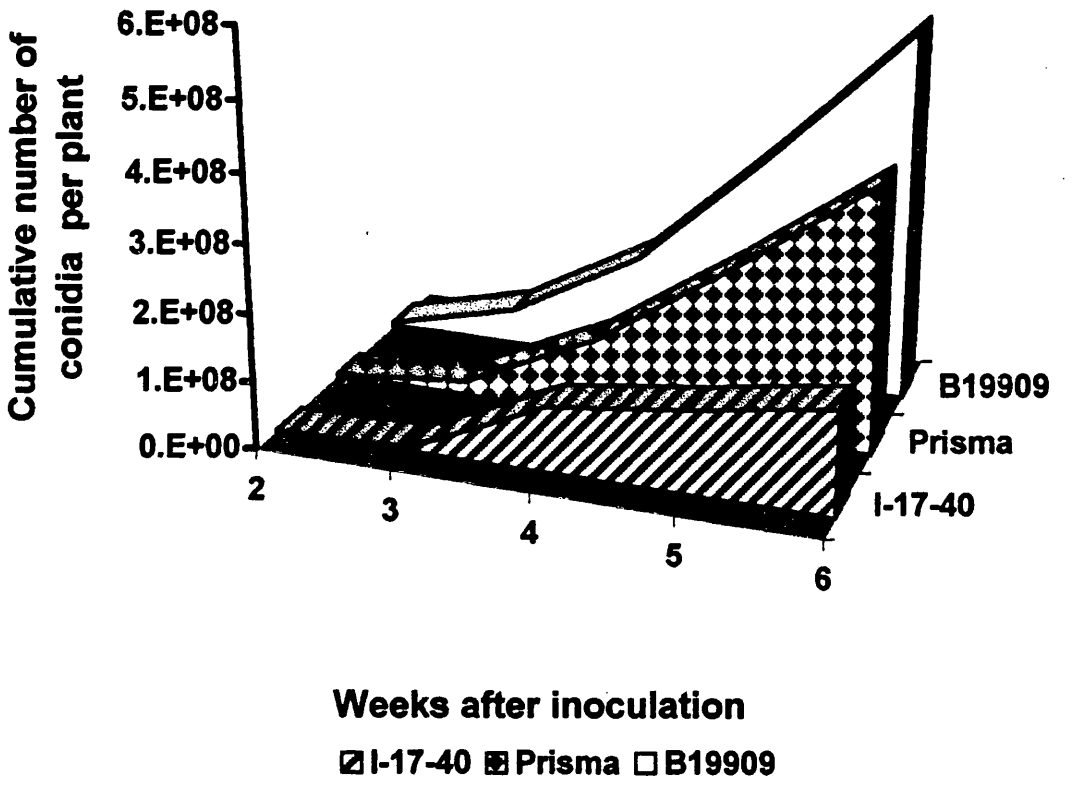


Fig. 6D: The cumulative number of conidia produced per plant.

4.3.1.1. Primary shoot growth

4.3.1.1.1. Primary shoot height

The primary shoot of uninfected plants continued to elongate throughout the experiment in both cv. Prisma and line B19909 (Fig. 7) with that of line B19909 showing accelerated growth from week seven after planting (five weeks after inoculation). The primary shoot of cv. Prisma and line B19909 showed relatively little increase in height after inoculation and were significantly shorter ($p < 0.05$) than those of uninoculated plants from four and six weeks after inoculation respectively. The primary shoot height of uninoculated plants of line I-17-40 was much shorter than those of the other two lines throughout the experiment. The primary shoot of this line did not begin to elongate significantly until a week after the experiment was terminated, and during the period of the experiment there was no significant difference in height between infected and uninfected plants.

4.3.1.1.2. Number of leaves expanded on the primary shoot

Both infected and uninfected plants of all three lines had expanded about the same number of leaves by seven weeks after planting (five weeks after inoculation) (Table 3), about 10 to 11 leaves on cv. Prisma and line B19909 and about 11 leaves on line I-17-40. By eight weeks after planting (six weeks after inoculation), uninfected plants of both cv. Prisma and line B19909 had expanded a further 1 to 2 leaves, but no further leaves expanded on the infected plants. An analysis of variance showed that the differences between infected and control plants were significant in cv. Prisma ($p < 0.05$) and in

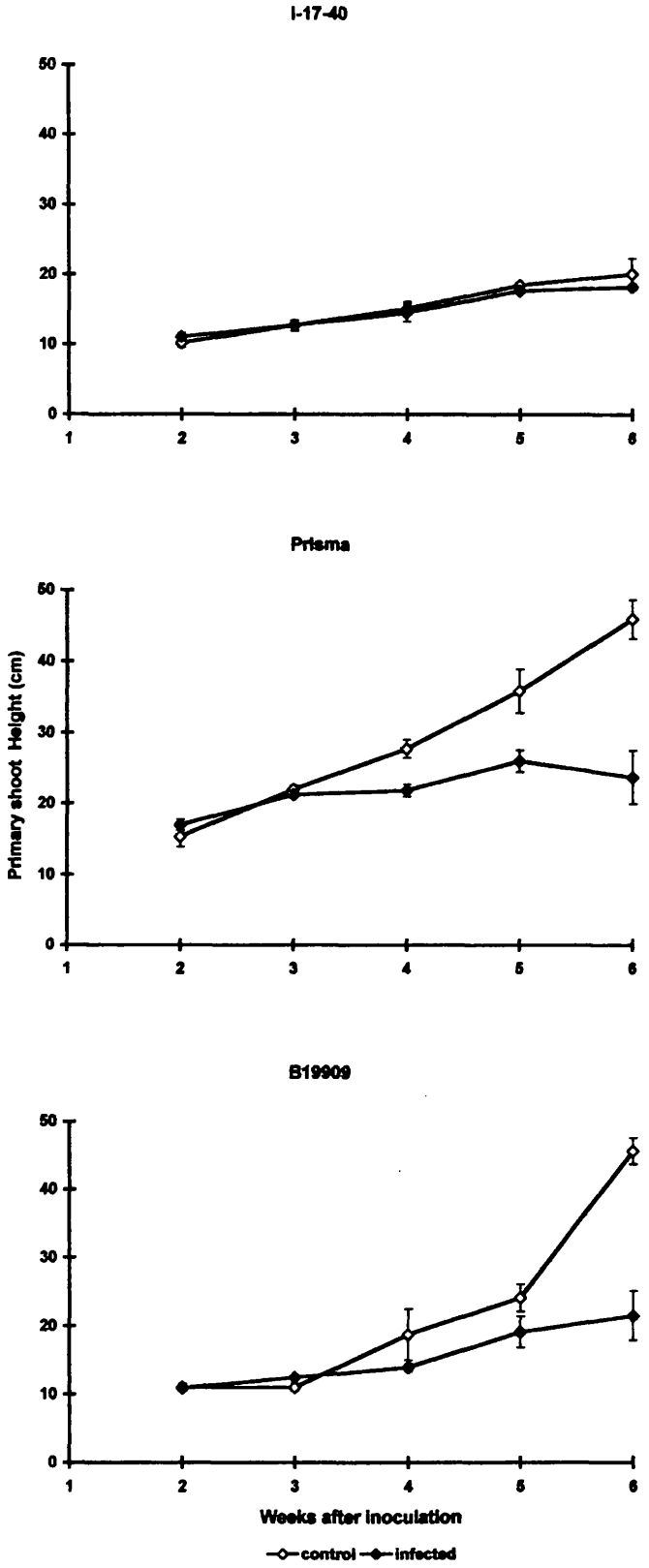


Fig. 7: The effects of infection on primary shoot height. Each datum is the mean of three replicates. Vertical bars are the standard errors.

Table 3: Leaf development on infected (I) and control (C) plants of the three barley lines at weekly intervals. Each reading is the mean of three replicates ± SE.

Weeks after inoculation	Total number of leaves			Number of senescent leaves		
	I-17-40	Prisma	B19909	I-17-40	Prisma	B19909
C	7.0 ± 0.0	7.0 ± 0.0	6.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2nd						
I	7.0 ± 0.0	7.0 ± 0.0	6.3 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
C	8.3 ± 0.3	8.3 ± 0.3	7.7 ± 0.3	1.0 ± 0.0	0.3 ± 0.3	1.0 ± 0.0
3rd						
I	9.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	3.3 ± 0.3	4.0 ± 0.0	3.0 ± 0.0
C	10.3 ± 0.3	9.3 ± 0.3	9.3 ± 0.3	3.0 ± 0.0	3.7 ± 0.3	4.0 ± 0.0
4th						
I	9.3 ± 0.3	9.0 ± 0.0	8.7 ± 0.3	6.3 ± 0.3	6.0 ± 0.0	6.0 ± 0.0
C	11.0 ± 0.0	10.3 ± 0.3	10.7 ± 0.3	6.0 ± 0.7	6.0 ± 0.0	6.0 ± 0.0
5th						
I	11.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0	7.7 ± 0.3	7.0 ± 0.0
C	11.3 ± 0.3	11.7 ± 0.3	11.7 ± 0.3	6.7 ± 0.3	7.0 ± 0.0	7.0 ± 0.0
6th						
I	11.3 ± 0.3	10.0 ± 0.0	10.3 ± 0.3	8.3 ± 0.3	8.0 ± 0.0	8.0 ± 0.0

line B19909 ($p < 0.01$). However, by the end of the experiment both inoculated and uninoculated plants of line B19909 had expanded the same number of leaves, the differences between the two groups being in the time of leaf expansion. In contrast, because of the heavy level of infection in the growth cabinet grown plants, the primary shoots of inoculated plants of cv. Prisma died just after the tenth leaf had expanded and so no further leaves were produced.

Infected and uninfected plants of line I-17-40 produced the same number of leaves on the primary shoot with no significant delay in leaf expansion in response to infection.

As infection progressed, the lower leaves on inoculated plants senesced earlier than on uninoculated plants. Senescence began in infected plants two weeks after inoculation and the number of leaves senescing was significantly higher than on uninoculated plants from the third week after inoculation (Table 3).

4.3.1.1.3. Green leaf blade area (GLA) on the primary shoot

Total GLA on the primary shoots of uninfected plants was generally higher in cv. Prisma than in the wild lines at all harvests (Fig. 8). In cv. Prisma, total GLA increased rapidly between the first and the second harvests, and then as plants matured it declined progressively due to the senescence of the lower leaves. In contrast, no changes were observed in GLA of the two wild lines during the course of the experiment.

Infection decreased total GLA in all three lines, with cv. Prisma being

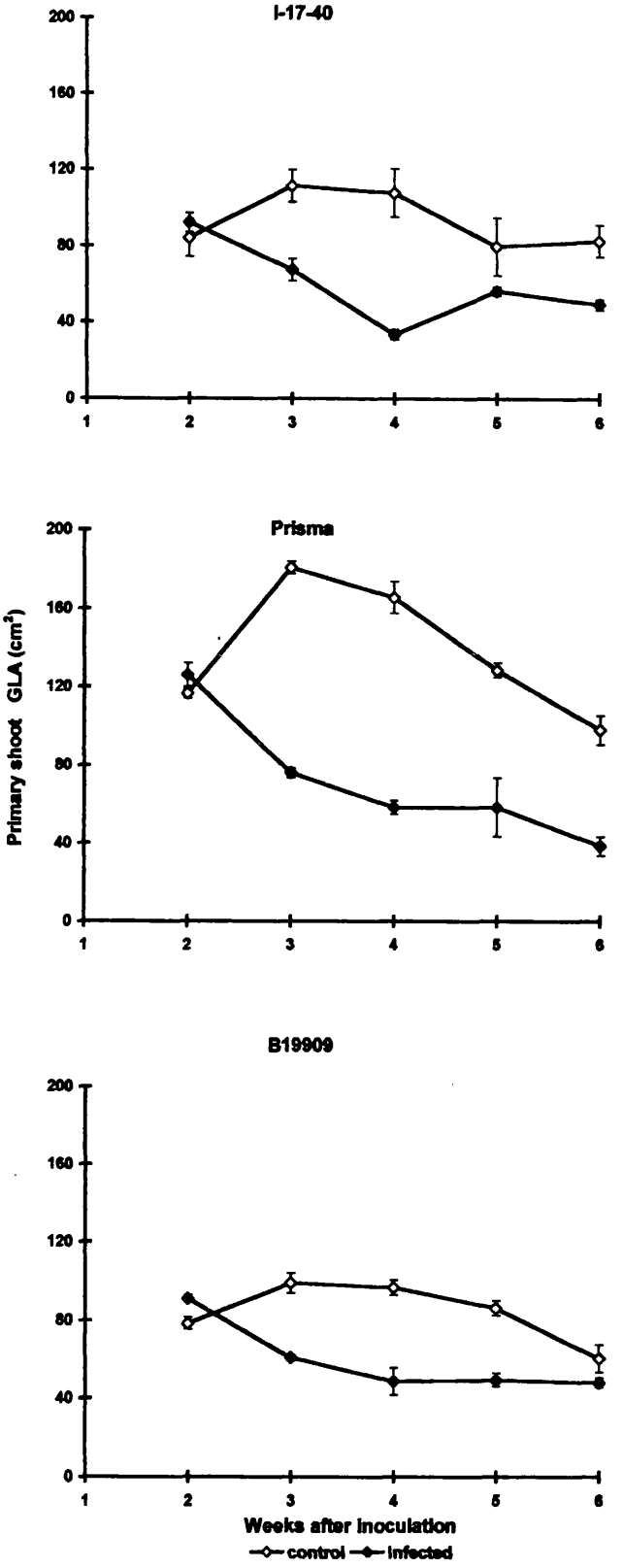


Fig. 8: The effects of infection on green leaf area of the primary shoot. Each datum point is the mean of three replicates. The vertical bars are the standard errors.

more affected than the wild lines. An analysis of variance showed highly significant ($p < 0.001$) differences for all lines between infected and uninfected plants from three weeks after inoculation. Four weeks after inoculation, the percentage reduction in total GLA over the controls was about 69% in line I-17-40, 65% in cv. Prisma and 50% in line B19909. At this stage, the percentage leaf area covered with mildew was about 27% in both cv. Prisma and line I-17-40 and about 34% in line B19909. Infection eventually progressed to the upper leaves where lower percentage mildew cover developed due to the greater resistance of the upper leaves than the lower leaves. Because of this, differences in total GLA between infected and controls became smaller and less significant, especially in the wild lines.

4.3.1.2. Tiller production

4.3.1.2.1. Number of tillers

The first tillers emerged before the first measurements were made. Fig. 9 shows that in all lines, tiller production by uninfected plants continued to increase during the course of the experiment with the exception of line B19909, which developed no further tillers after the sixth week from planting (fourth week after inoculation).

The number of tillers produced by inoculated plants of all three lines was lower than that on uninoculated plants with the differences becoming significant from three weeks after inoculation in line I-17-40 and line B19909 at a time when 17% of the leaf area was colonised in line I-17-40 ($p < 0.05$) and 26% in line B19909 ($p < 0.01$). The differences became

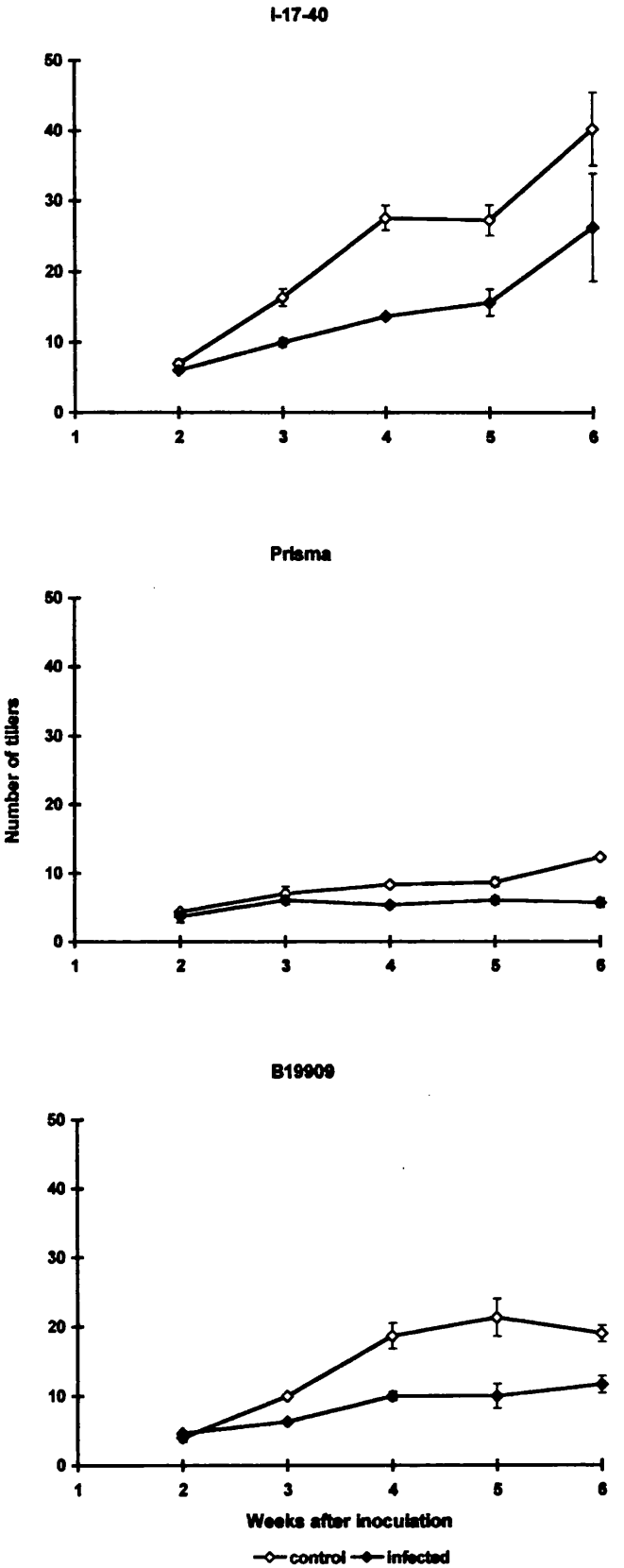


Fig. 9: The effects of infection on tiller production.
Each datum point is the mean of three replicates.
Vertical bars are the standard errors.

significant about a week later in cv. Prisma when 27 % of the leaf area was colonised ($p < 0.005$). Although, the difference in the number of tillers produced by infected and uninfected plants of line I-17-40 was not significant at the last harvest, this was probably due to the high variation between replicates at this harvest compared to the earlier harvests.

4.3.1.2.2. Total green leaf blade area (GLA) on the tillers

The production of leaf blades on the tillers (Fig. 10) showed a similar response to infection as leaf blade production on the primary shoots. Apart from the first harvest, infection lowered GLA on the tillers significantly ($p < 0.01$) in all three lines, with line I-17-40 and cv. Prisma being more affected than line B19909. At the final harvest, total GLA was reduced over the uninfected controls by about 76% in cv. Prisma, 49% in line I-17-40 and 32% in line B19909. The reduction in total GLA between the penultimate and the last harvest in the control plants of line B19909 was mainly due to the greater loss in this line of the lower leaves due to senescence.

4.3.2. Effects on root production

4.3.2.1. Seminal roots

Measurements of the different morphological components of the seminal roots of infected and uninfected plants are plotted graphically in Figs. 11 to 15.

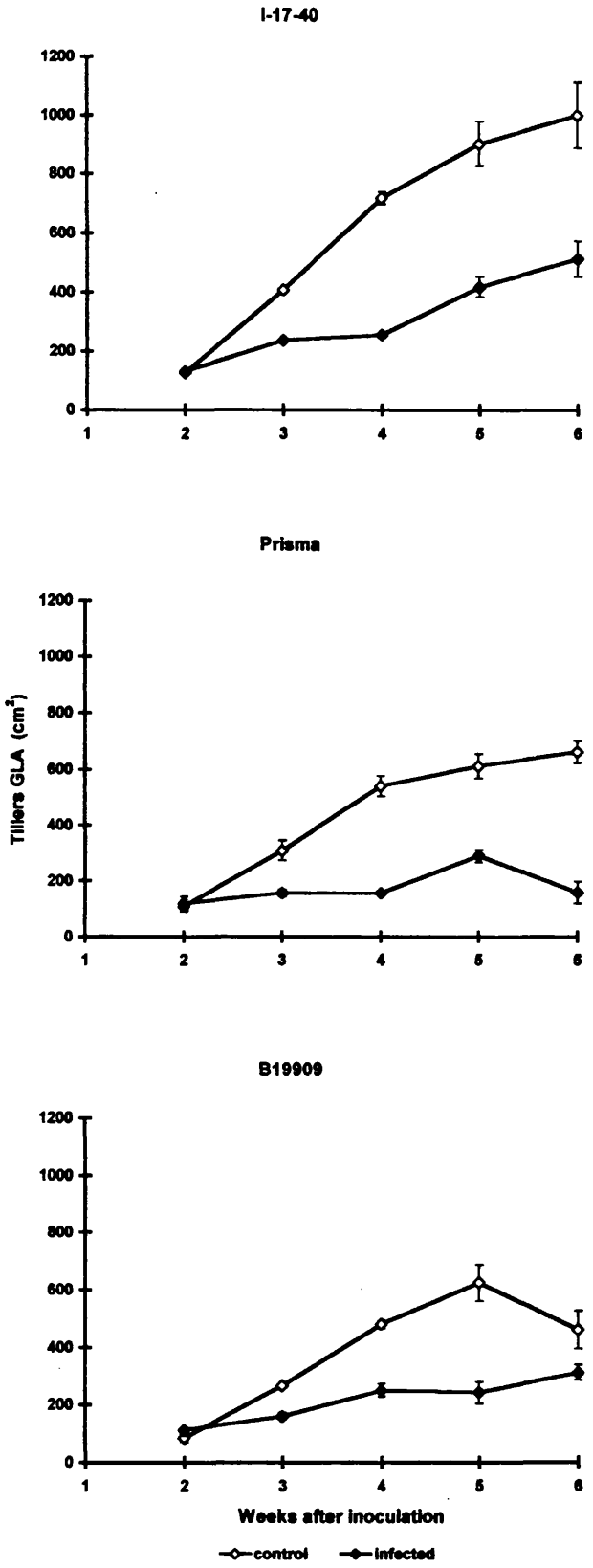


Fig. 10: The effects of infection on total green leaf area on all tillers. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

4.3.2.1.1 Number of seminal roots

Emergence of the seminal roots was completed before the plants were inoculated, and therefore their number shows only random variation between infected and uninfected plants at each harvest (Fig. 11). Plants of line I-17-40 and cv. Prisma produced between 6 to 8 seminal roots, while those of line B19909 produced between 5 to 8 seminal roots.

4.3.2.1.2. Number of laterals on the seminal roots

Uninfected and infected plants of all three lines produced both primary and secondary laterals on the seminal roots, with the numbers increasing with time (Fig. 12).

Infection reduced the number of lateral roots produced per plant in cv. Prisma and line B19909, but the reduction was more pronounced in the former. An analysis of variance shows that the differences between infected and uninfected plants became significant from around the fourth week after inoculation in both cv. Prisma ($p < 0.01$) and line B19909 ($p < 0.05$), and by the sixth week, cv. Prisma had produced about 51% fewer lateral roots and line B19909 about 27% fewer lateral roots.

None of the differences between infected and uninfected plants of line I-17-40 were significant at any harvest.

4.3.2.1.3. Total length of the seminal root system

The total length of the seminal root system of uninfected and infected plants increased as the experiment progressed in all three lines (Fig. 13). Infection reduced seminal root length significantly in both cv. Prisma ($p <$

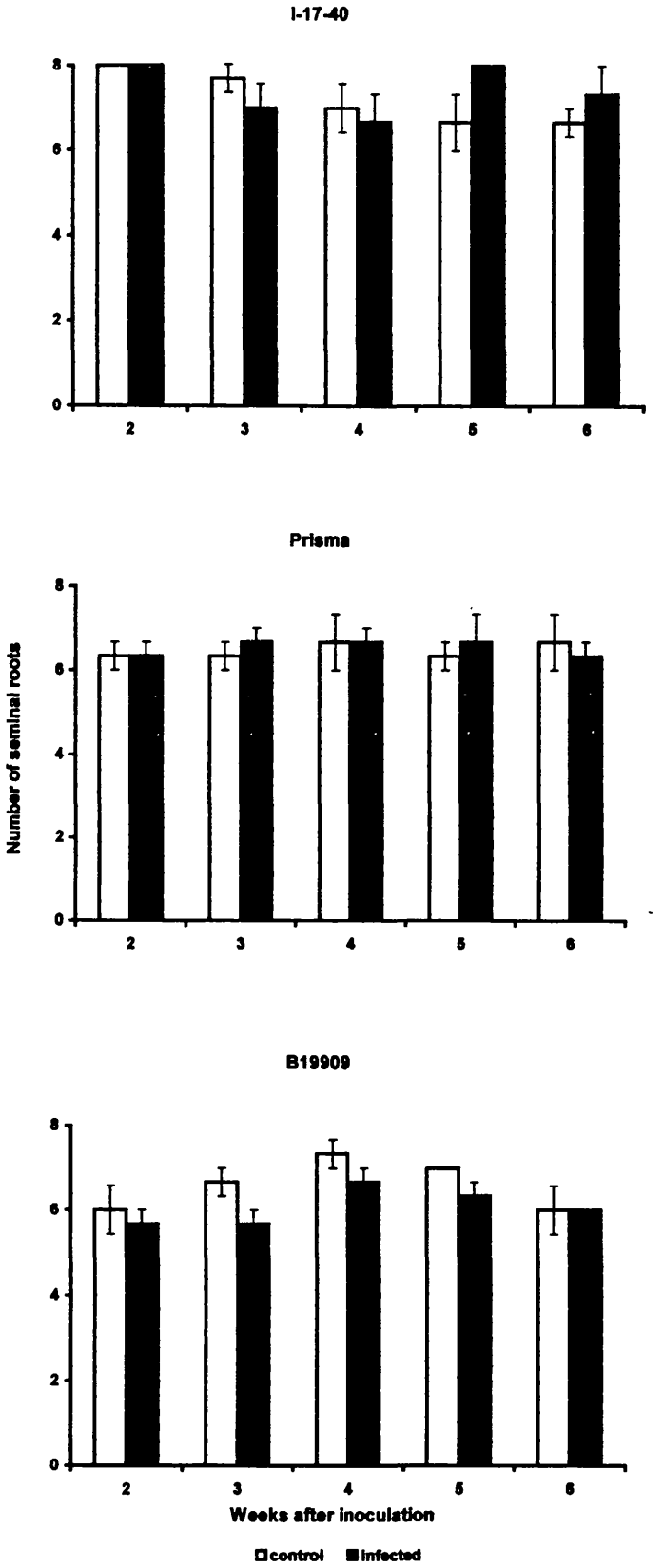


Fig. 11: The effect of infection on seminal root production. Each datum point is the mean of three replicates. vertical bars are the standard errors.

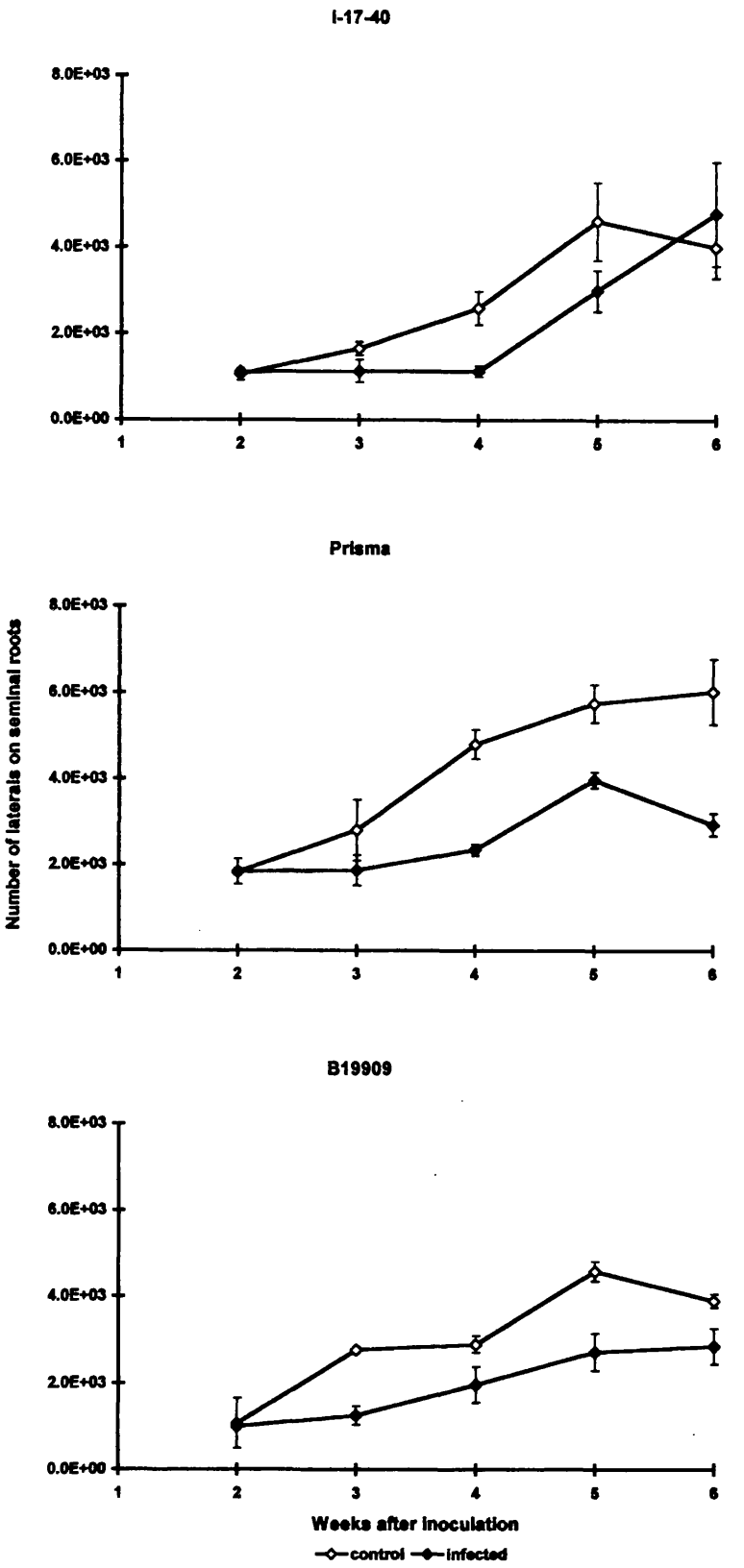


Fig. 12: The effects of infection on the development of seminal lateral roots.Each datum point is the mean of the three replicates. Vertical bars are the standard errors.

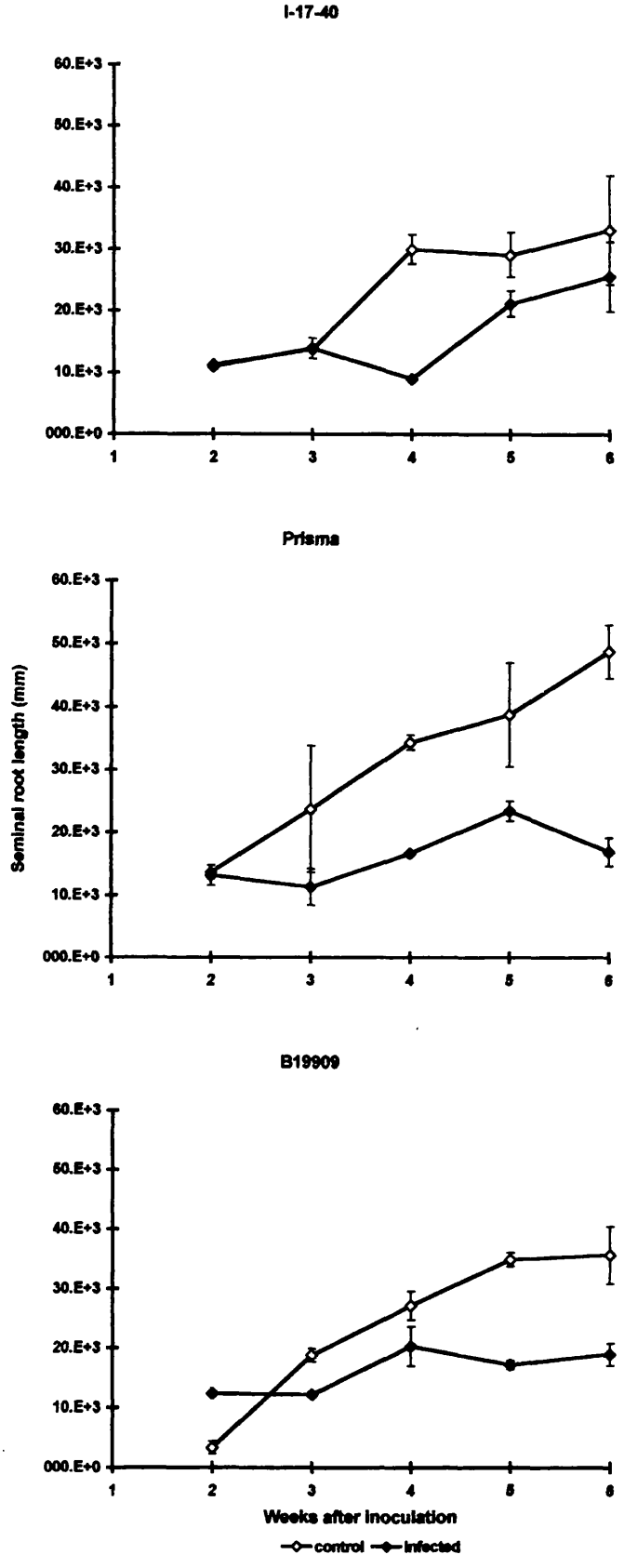


Fig. 13: The effects of infection on seminal root length. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

0.005) and line B19909 ($p < 0.05$). By the sixth week after inoculation the percentage reduction over the controls was about 65% in cv. Prisma but only 47% in line B19909. However, Seminal root length was not reduced significantly by infection in line I-17-40.

4.3.2.1.4. Mean seminal root diameter

The mean diameter of the seminal roots including laterals reduced with time due to the greater proportion of thinner laterals produced by the older plants (Fig. 14). Infection did not affect diameters significantly in any line at any harvest except at the last harvest in line I-17-40 ($p < 0.05$). The failure to show any effects of infection on seminal root diameters could be due to the large number of laterals with approximately the same diameters.

4.3.2.1.5. Surface area of the seminal root system

Total seminal root area (including laterals) of uninfected plants increased as the number and lengths of the seminal roots and their laterals increased during growth (Fig. 15). However, there were large fluctuations between harvests due to the high level of variability between replicates.

Infection decreased seminal root surface area significantly in both wild lines ($p < 0.01$) and in cv. Prisma ($p < 0.001$). This decrease become significant from the fourth week after inoculation in line I-17-40 and cv. Prisma, but not until the fifth week after inoculation in line B19909. It appears that cv. Prisma was more affected by infection than the two wild lines. The reduced seminal root surface area of infected plants was partly due to the reduction in seminal root length (Fig. 13) as well as in the number of laterals produced (Fig. 12).

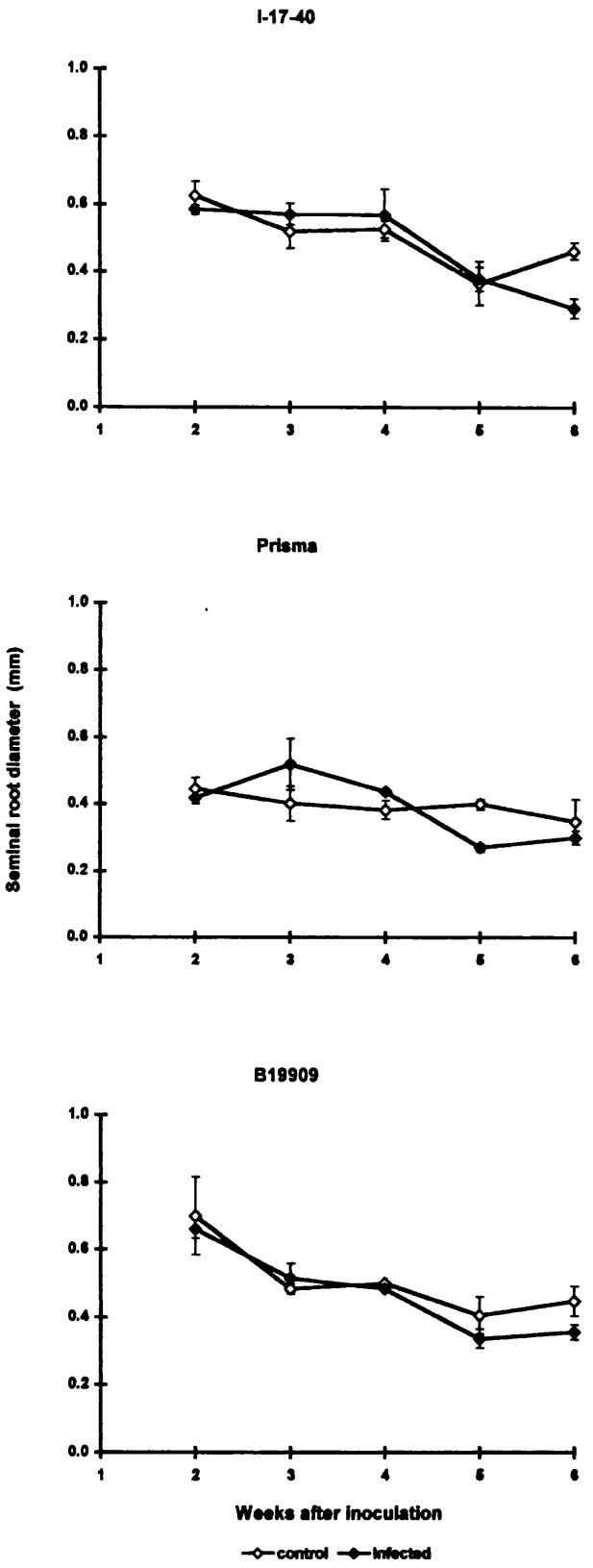


Fig. 14: The effects of infection on mean seminal root diameter. Each datum is the mean of three replicates. Vertical bars are the standard errors.

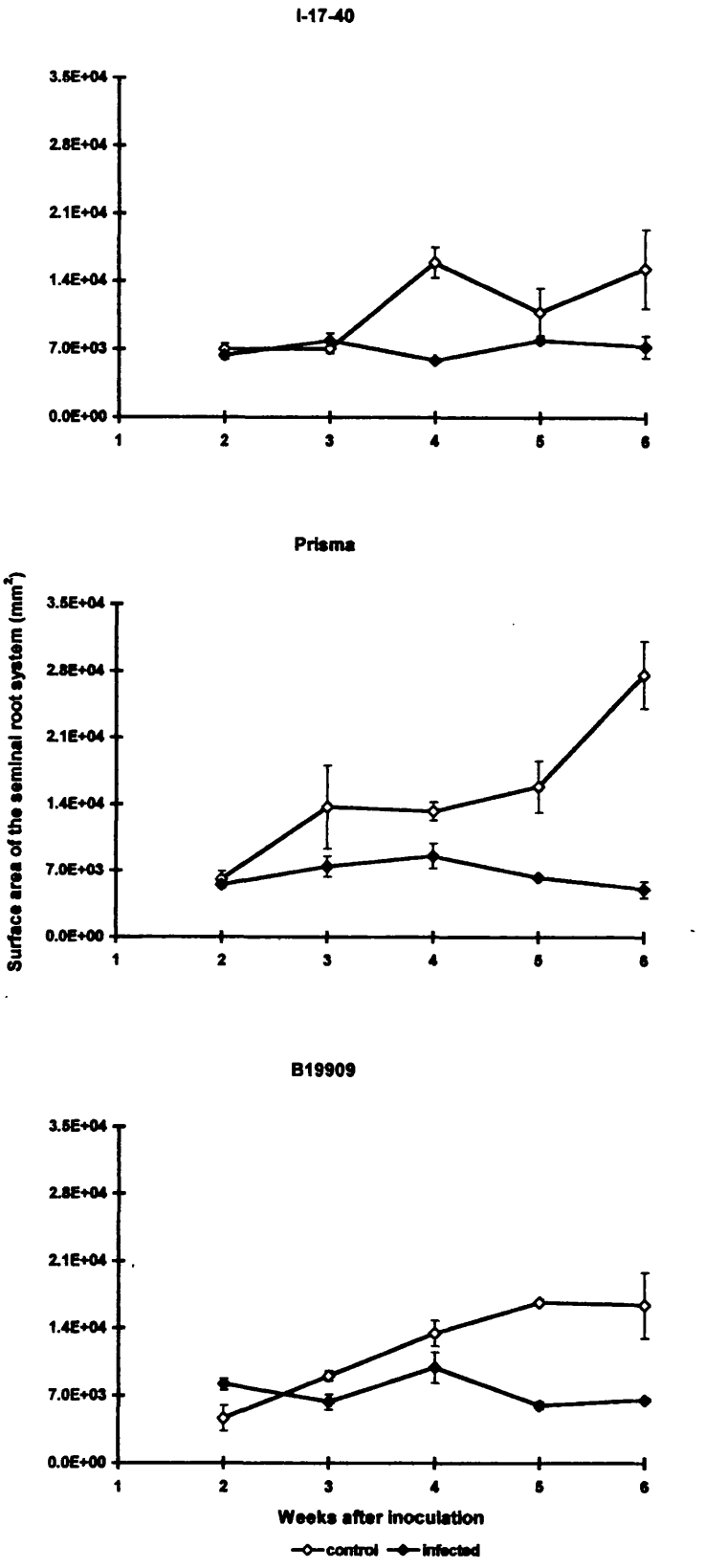


Fig. 15: The effects of infection on the surface area of the seminal root system. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

4.3.2.2. Nodal roots

Measurements of the different morphological components of the nodal roots of infected and uninfected plants are plotted graphically in Figs. 16 to 20.

4.3.2.2.1. Number of nodal roots

The number of nodal roots on uninfected plants increased progressively in all lines as the experiment progressed (Fig. 16). The slight decrease in the number of nodal roots shown by line B19909 at the end of the experiment was probably due to random variation.

Infection decreased the total number of nodal roots in all lines. An analysis of variance showed that the reduction was significant from three weeks after inoculation in cv. Prisma ($p < 0.0001$) and in wild lines B19909 and I-17-40 ($p < 0.05$). By the sixth week after inoculation, the percentage reduction was about 74% in cv. Prisma, 46% in I-17-40 but only 31% in line B19909.

4.3.2.2.2. Number of laterals on the nodal roots

Uninfected and infected plants of all three lines produced primary and secondary laterals on the nodal roots; tertiary laterals developed on all occasionally.

Infection reduced the total number of nodal root laterals produced per plant in cv. Prisma but not in the two wild lines (Fig. 17). An analysis of variance showed that by the sixth week after inoculation, infection had

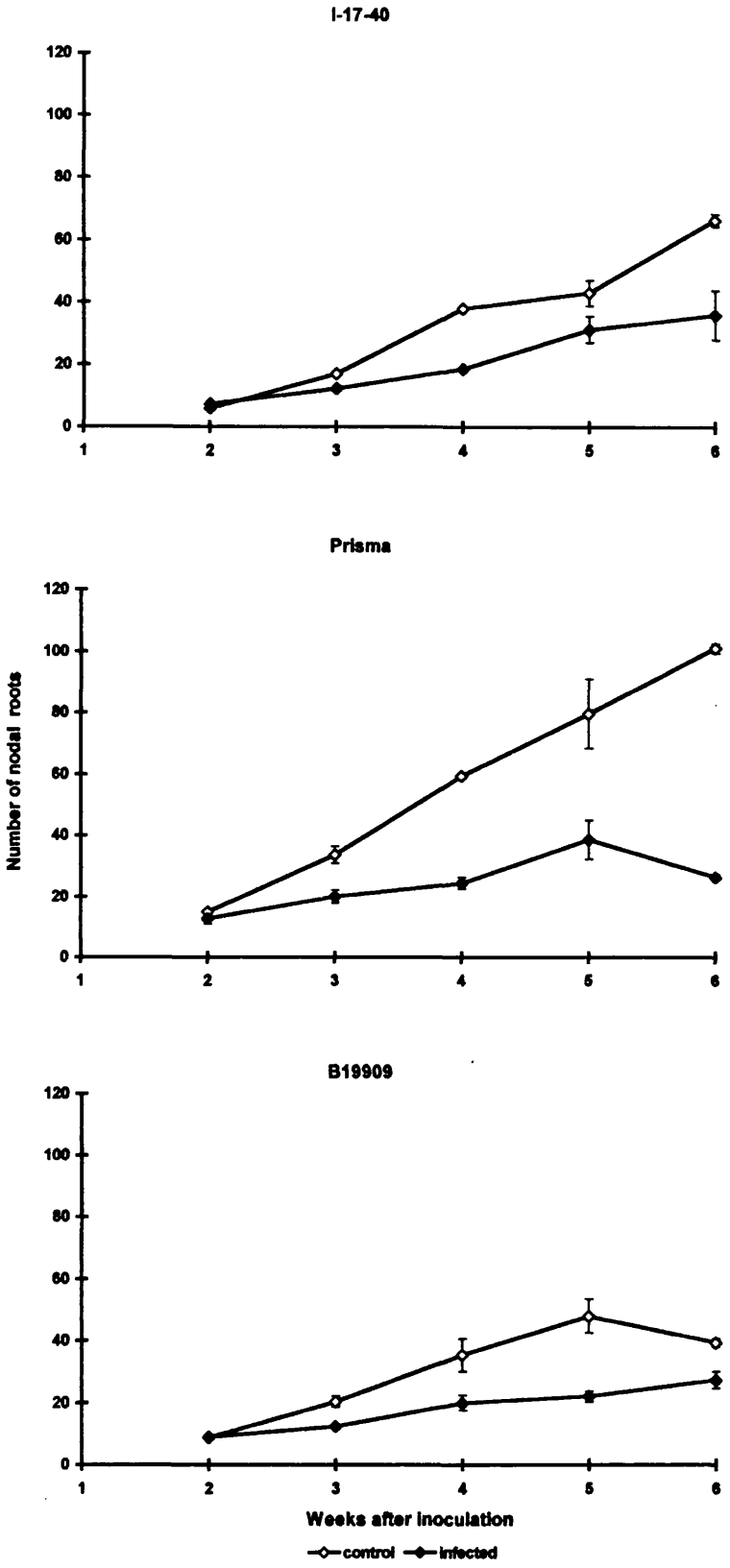


Fig. 16: The effects of infection on the production of nodal roots. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

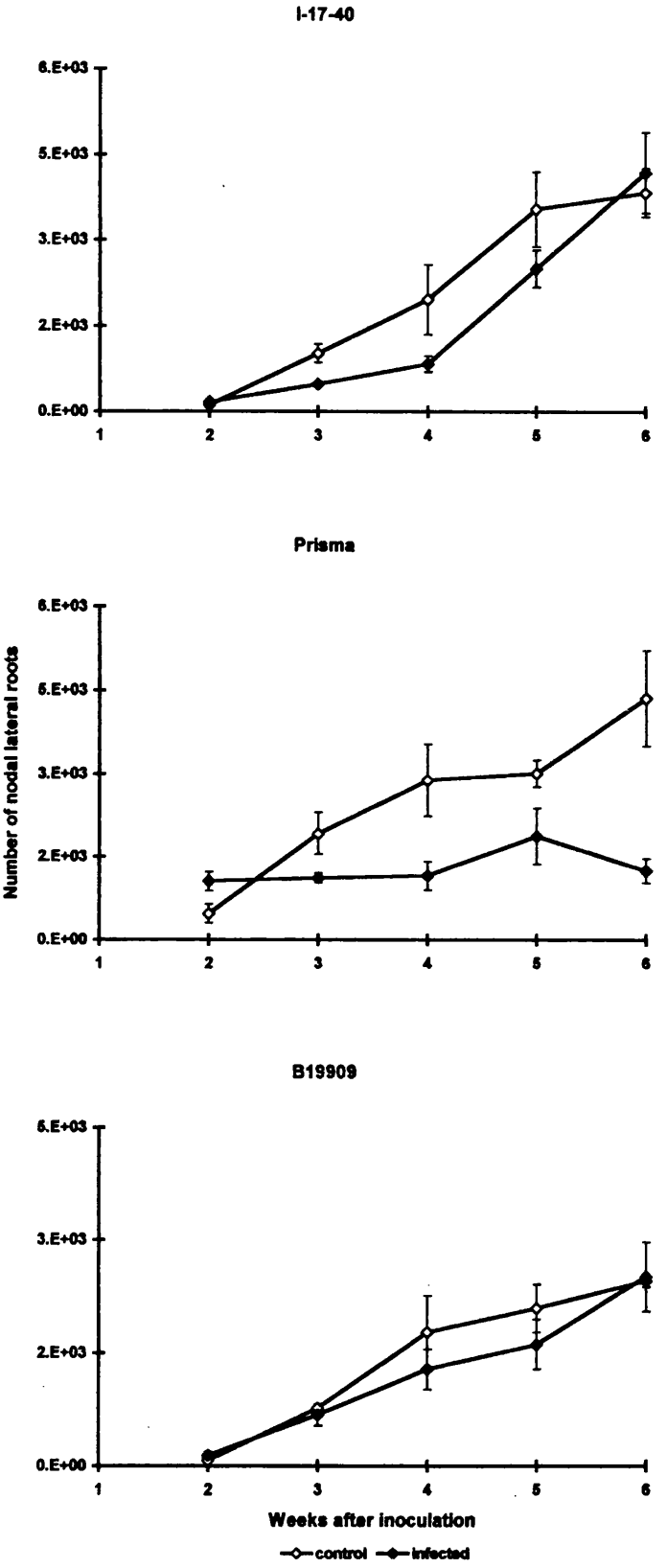


Fig. 17: The effects of infection on the production of nodal lateral roots. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

significantly reduced ($p < 0.01$) lateral root production on the nodal roots of cv. Prisma by up to 71%.

4.3.2.2.3. Total length of the nodal root system

The total length of the nodal root system of uninfected and infected plants continued to increase throughout the course of the experiment (Fig. 18). However, infection reduced the total length produced in all lines and an analysis of variance showed that the reductions were significant ($p < 0.005$) in cv. Prisma from the third week after inoculation, in line I-17-40 ($p < 0.05$) from the fourth week after inoculation but not until the sixth week after inoculation in line B19909 ($p < 0.05$). At the last harvest, total lengths of the nodal root system were reduced by 78% in cv. Prisma but only by about 39% in line I-17-40 and about 27% in line B19909.

4.3.2.2.4. Mean nodal root diameter

The mean diameters of the nodal roots, including laterals, in cv. Prisma and line I-17-40 appeared to be little affected by infection at any harvest probably because of the high level of variation (Fig. 19). Infection appeared to decrease significantly ($p < 0.001$) the diameter of nodal roots on infected plants of line B19909 at the first harvest, but from then on the differences were insignificant.

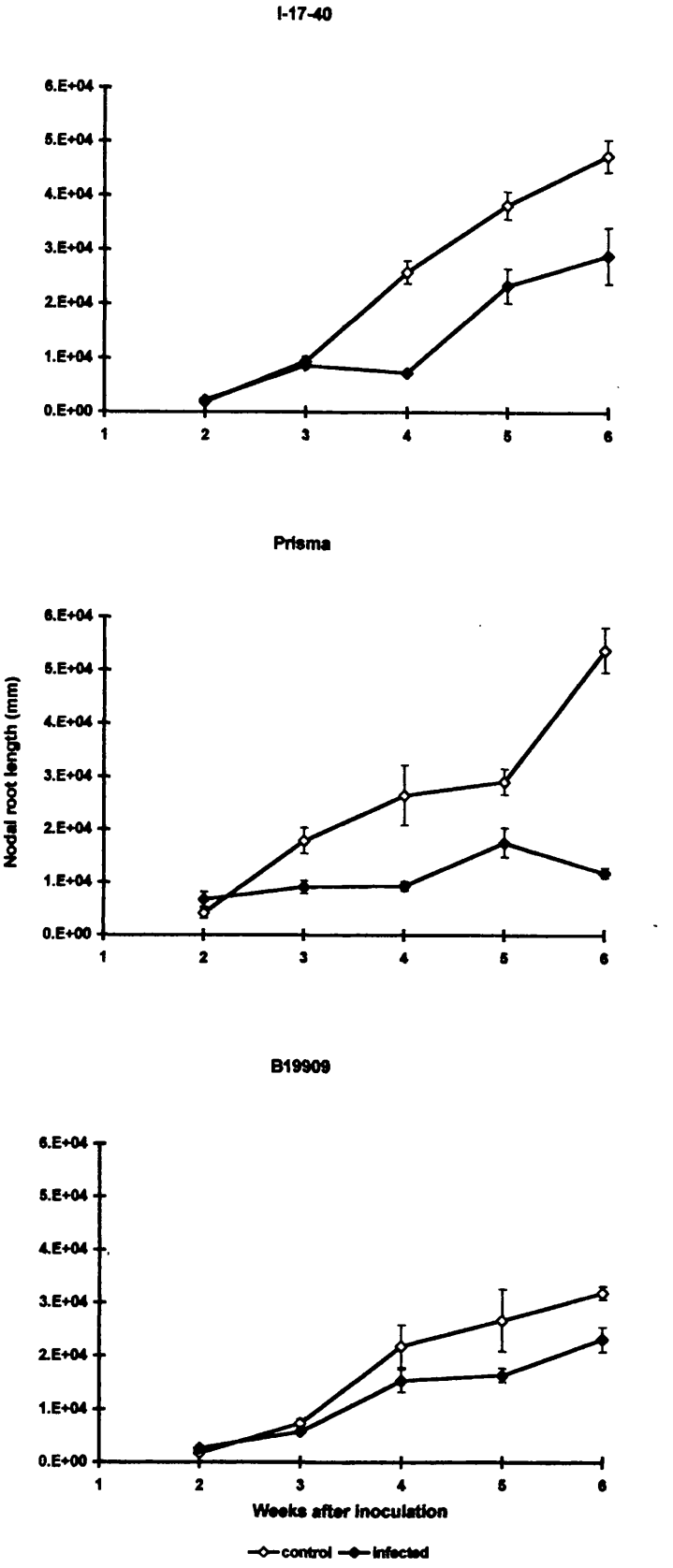


Fig. 18: The effects of infection on the nodal root length.

Each datum point is the mean of three replicates.

Vertical bars are the standard errors.

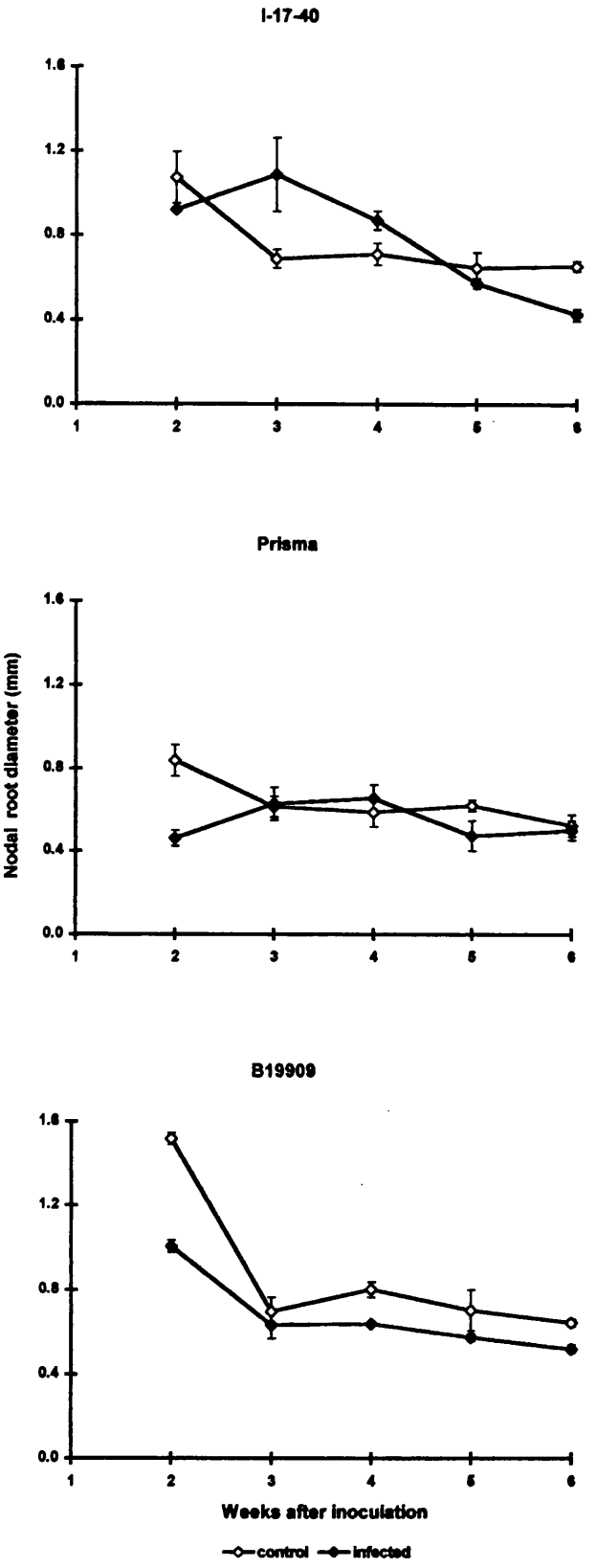


Fig. 19: The effects of infection on the diameter of the nodal root system. Each datum point is the mean of replicates. Vertical bars are the standard errors.

4.3.2.2.5. Total surface area of the nodal root system

The total surface area of the nodal root system on uninfected plants of all lines increased with time (Fig. 20).

Infection reduced total surface area, the reduction becoming significant from around the third week after inoculation in all lines. At the end of the experiment, an analysis of variance showed that the reductions were highly significant in cv. Prisma ($p < 0.0001$) but less so in the two wild lines ($p < 0.05$). At this stage, total surface area was reduced by about 80 % in cv. Prisma, 60% in line I-17-40 and 41% in line B19909.

4.3.2.3. Total number of lateral roots

Infection reduced the total number of all lateral roots per plant (sum of nodal and seminal laterals) in cv. Prisma more than in the other two lines (Fig. 21). An analysis of variance showed that the total number of lateral roots produced per plant in cv. Prisma was significantly reduced ($p < 0.005$) by up to 60% at the sixth week after inoculation, but infection had no significant effect on the number of lateral roots produced by the two wild lines at any harvest.

4.3.2.4. Total root length

Infection reduced total root length in all lines with the reductions becoming significant in cv. Prisma ($p < 0.002$) by the fourth week after inoculation and in line B19909 ($p < 0.05$) by the fifth week after inoculation (Fig. 22). As infection increased, the differences in total root length between infected and uninfected plants became more marked and by the end of the

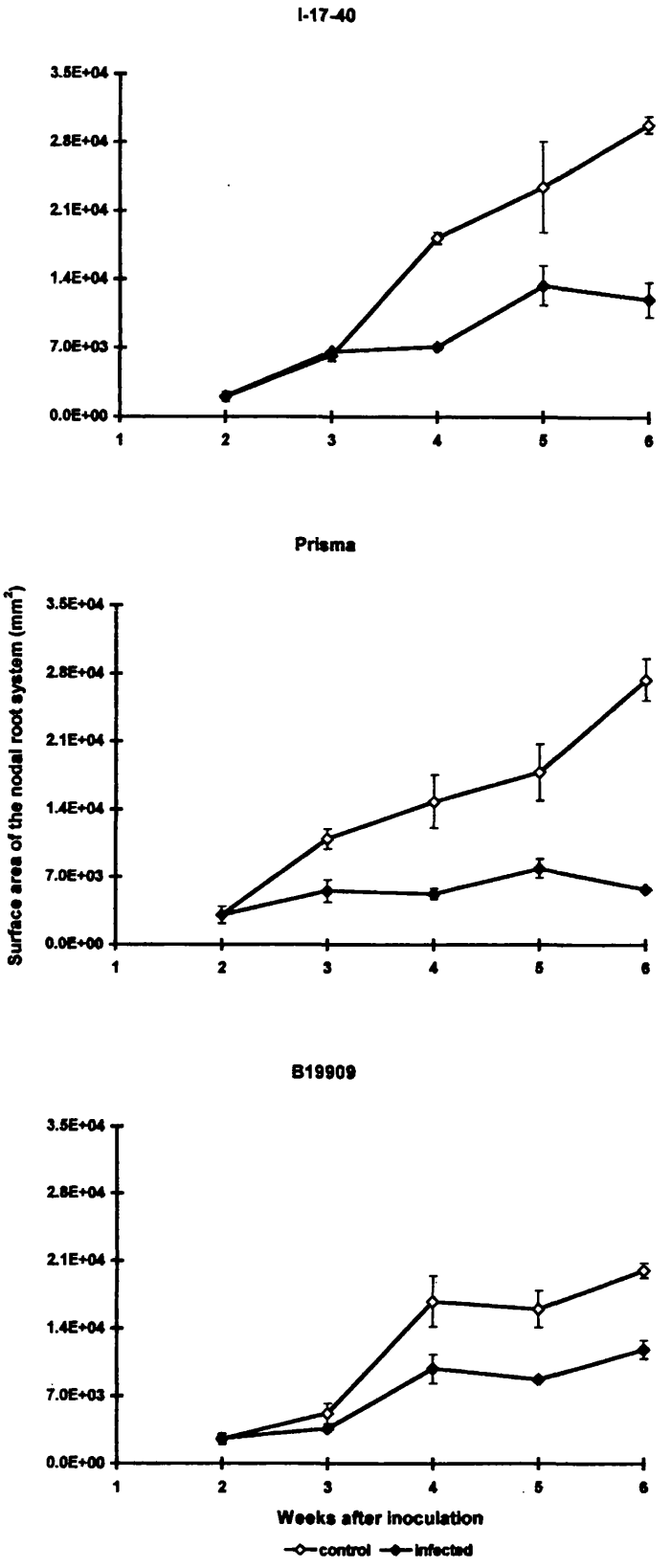


Fig. 20: The effects of infection on the total surface of the nodal root system. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

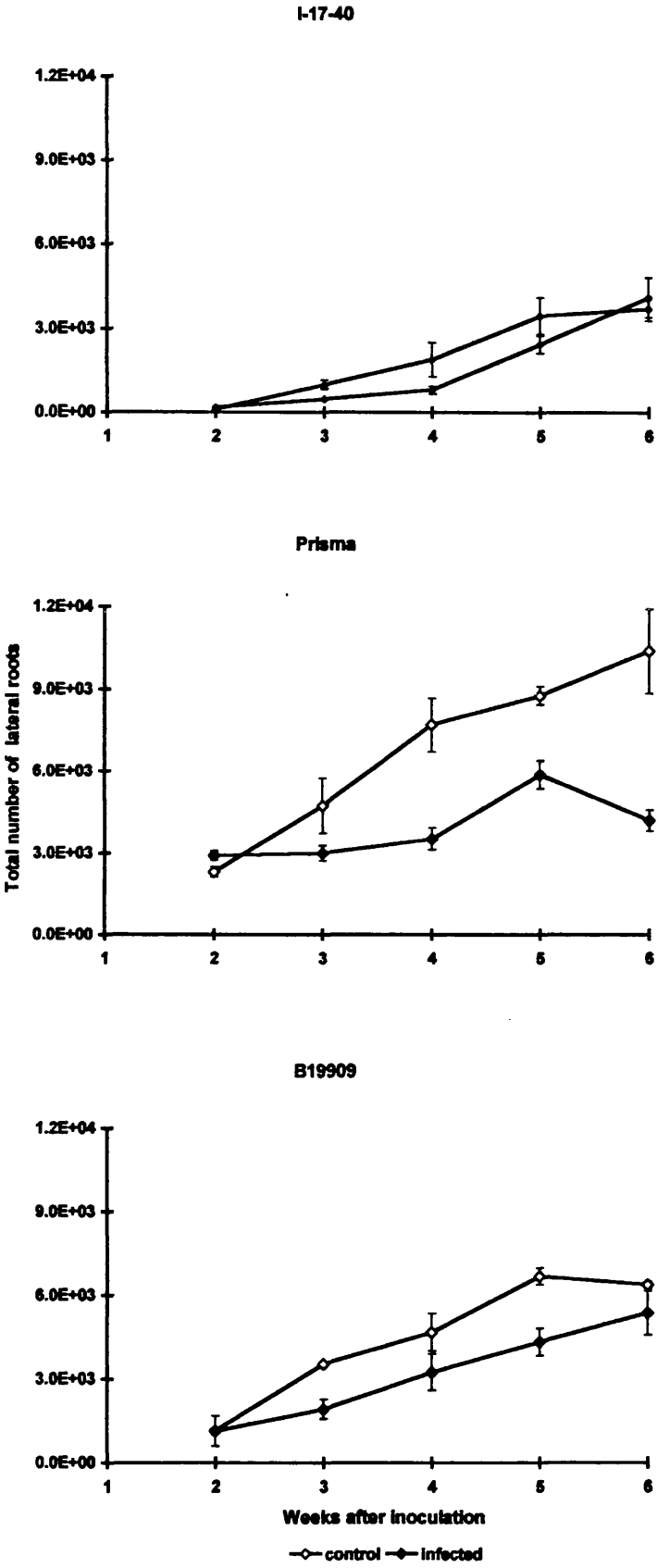


Fig. 21: The effects of infection on the development of lateral roots. Each datum point in the mean of three replicates. Vertical bars are the standard errors.

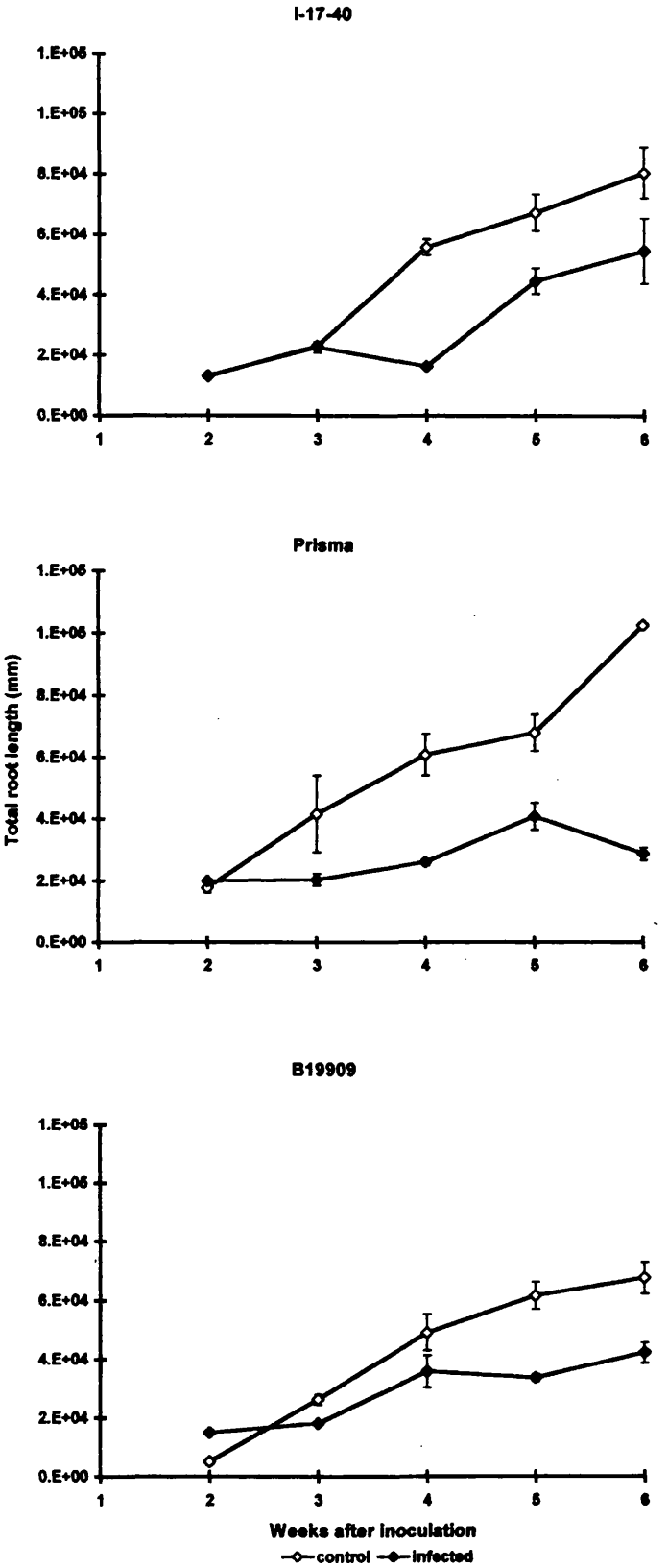


Fig. 22: The effects of infection on total root length. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

experiment (six weeks after inoculation), total root lengths of infected plants were reduced by about 72% in cv. Prisma and about 45% in line B19909. In contrast, at the end of the experiment there was no significant reduction in total root length in line I-17-40.

4.3.2.5. Total surface area of the whole root system

The total surface area of the whole root system was reduced by infection in all three lines (Fig. 23). An analysis of variance showed that these reductions were significant in cv. Prisma ($p < 0.0001$), line I-17-40 ($p < 0.01$) and line B19909 ($p < 0.01$). By the end of the experiment, the reductions were greatest in cv. Prisma where the total surface area was reduced by 80%. In line I-17-40, the total surface area was reduced by 57% and in line B19909 by 50%.

4.3.3. Effects on dry matter accumulation

The dry weights of shoots and roots and total plant dry weight of infected and uninfected plants of each line, at each harvest, are plotted graphically in Figs. 24A, 24B and 24C.

4.3.3.1. Shoot dry weight

Shoot dry weights of infected and control plants of all lines, continued to increase throughout the experiment (Fig. 24 A). Infection began to decrease shoot dry weight significantly ($p < 0.05$) in cv. Prisma from the fourth week after inoculation, and by the sixth week after inoculation, it was reduced by

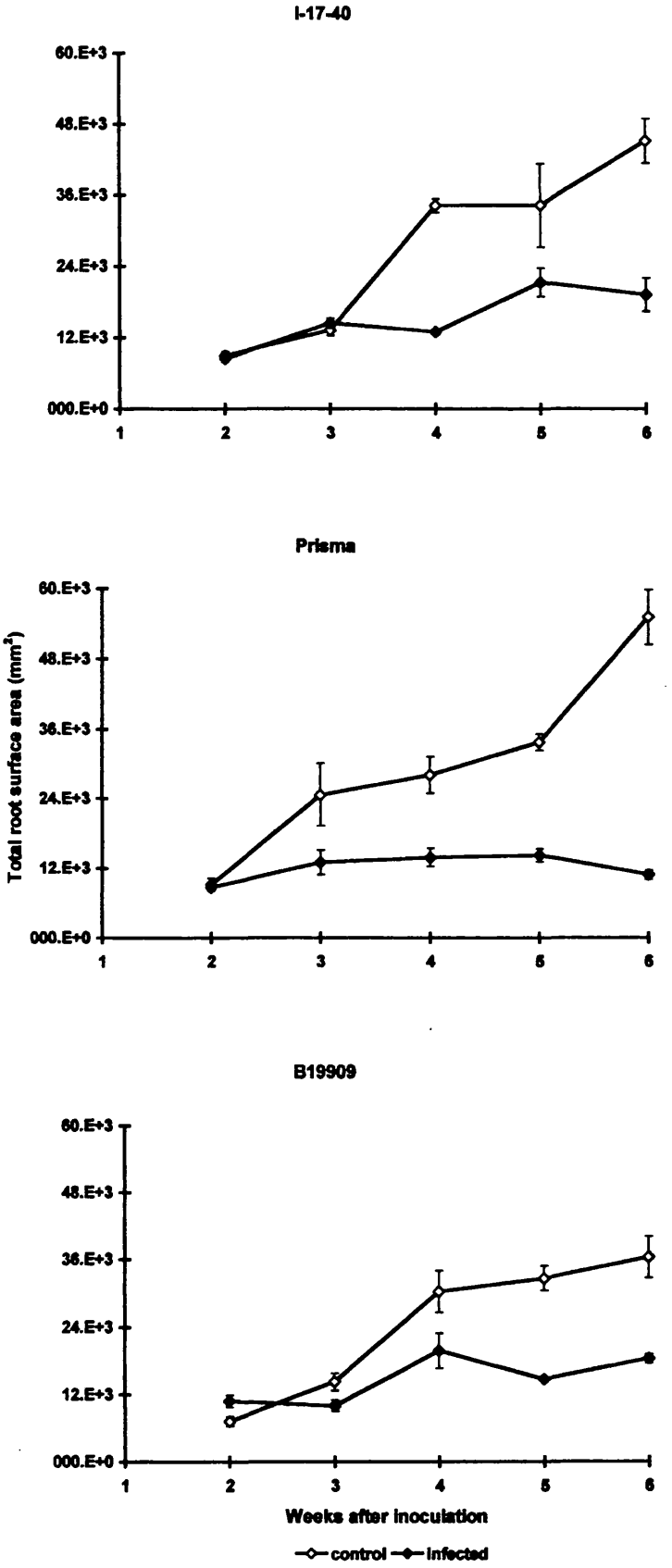


Fig. 23: The effects of infection on total root surface area. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

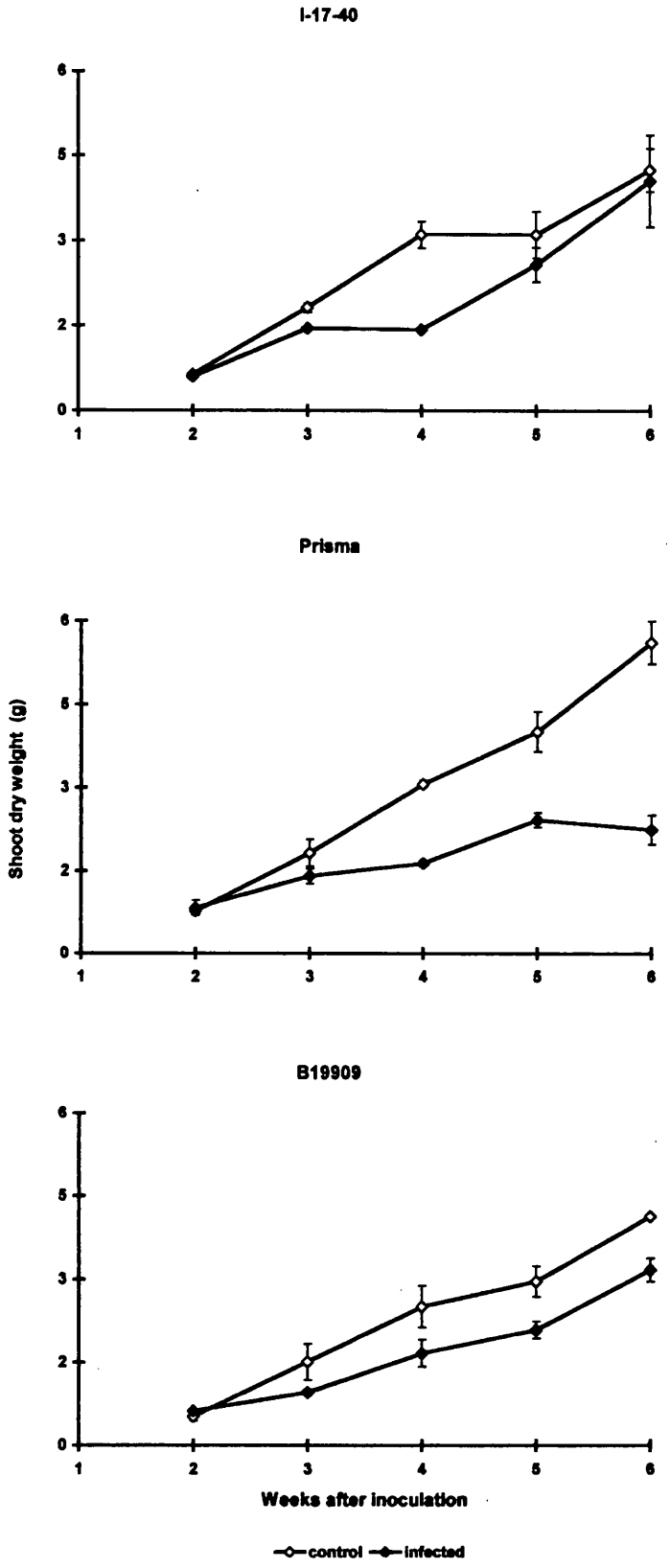


Fig. 24A: The effects of infection on shoot dry weight. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

about 60% of the levels of uninfected plants. Infection had also reduced shoot dry weight in line I-17-40 but only at the fourth week after inoculation but it had no significant effect on line B19909.

4.3.3.2. Root dry weight

Root dry weights of uninfected plants increased slowly with age in all lines. However, those of infected plants either showed no increase e.g. cv. Prisma and line B19909 or as in line I-17-40 increased only slightly over the period of the experiment (Fig. 24 B). The differences between infected and uninfected plants became significant from the fourth week after inoculation in cv. Prisma and the fifth week in line B19909. Line I-17-40 showed a high level of variation, particularly at the last two harvests and although root dry weights of infected plants were consistently lower than those of uninfected plants, the differences were significant ($p < 0.05$) only at the fourth week after inoculation. By the end of the experiment, the percentage reduction in root dry weight was about 30% in line B19909 but around 83% in cv. Prisma.

4.3.3.3. Total plant dry weight

Fig. 24 C. shows that the total dry weights of uninfected plants of all the three lines continued to increase throughout the experiment but no increase was observed in the infected plants except in line I-17-40. However, apart from the first harvest, two weeks after inoculation, the dry weights of infected plants were always lower than those of uninfected plants, although the differences did not become significant until four weeks after inoculation

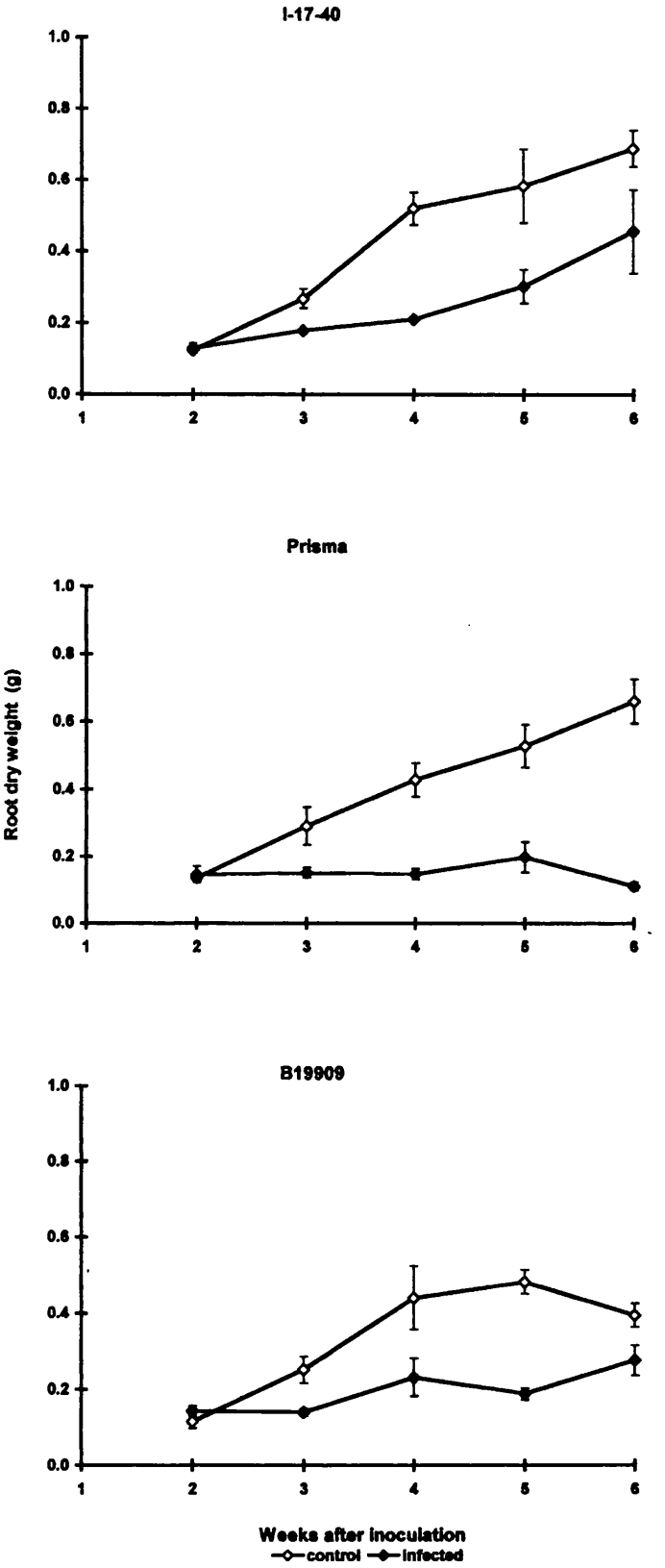


Fig. 24B: The effects of infection on root dry weight. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

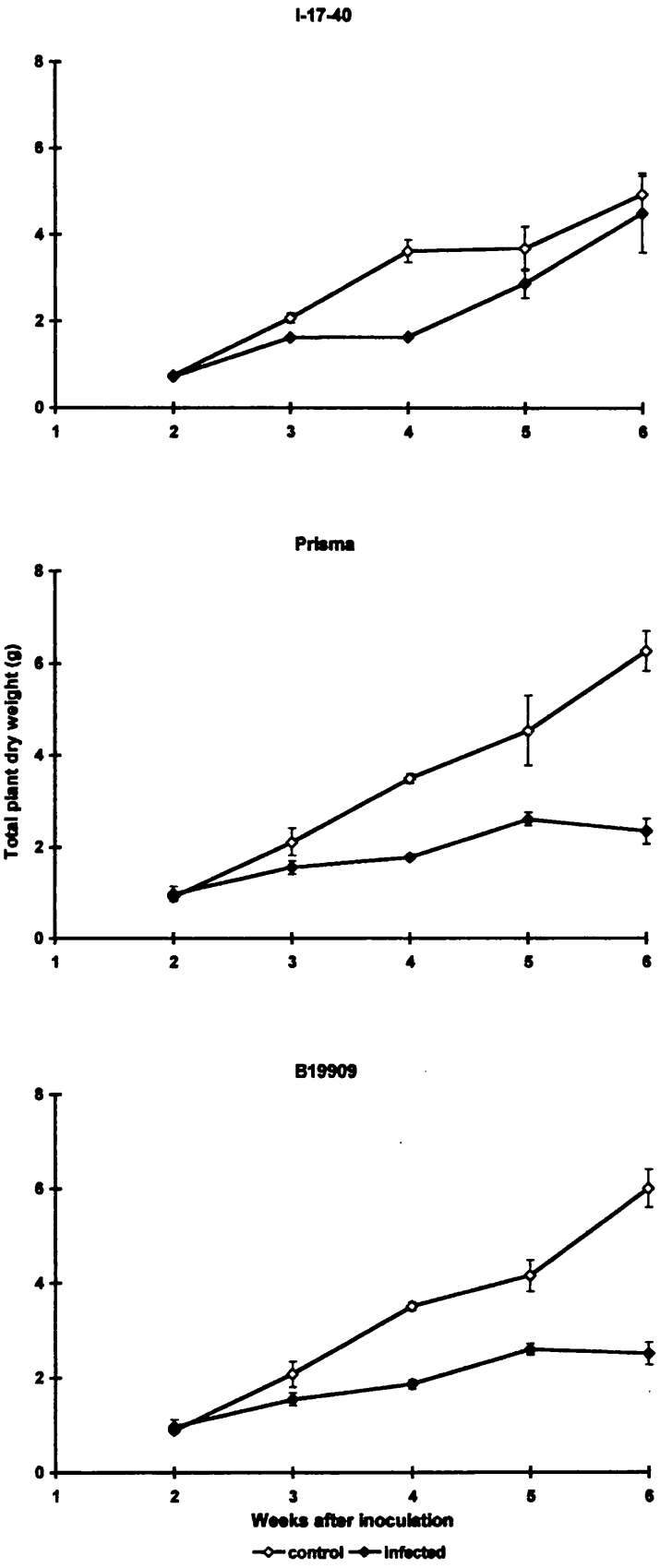


Fig. 24C: The effects of infection on total plant dry weight. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

in cv. Prisma and line B19909. By the end of experiment, the percentage reductions in the total dry weights of infected plants compared to uninfected plants was 63% in cv. Prisma but only 58% in line B19909. Although the total dry weights of infected plants of line I-17-40 were lower than the controls at all harvests, the differences were significant only at the fourth week after inoculation.

4.3.4. Effects of infection on the efficiency of growth

Changes in the efficiency of growth of infected and uninfected plants of each line, at each sampling time are plotted graphically in Figs. 25 A. and B.

4.3.4.1. Relative growth rates (RGR)

The rate of increase in biomass per unit of biomass (RGR) decreased with age in the uninfected plants of all lines between the fourth and seventh week after planting (the second and the fifth week after inoculation) (Fig. 25 A). This was probably due to a gradual increase in the proportion of non-photosynthetic tissues. Thereafter, the RGR increased slightly up to ear emergence.

Infection lead to a more rapid reduction in the RGR up to the period between the third and the fourth week after inoculation in all the lines. This greater reduction than in the controls probably reflected the increasing proportion of non-assimilatory tissues due to the senescence of infected leaves. However, from about the fourth week after inoculation, the RGR increased again, but this increase was short lived and from the fifth week

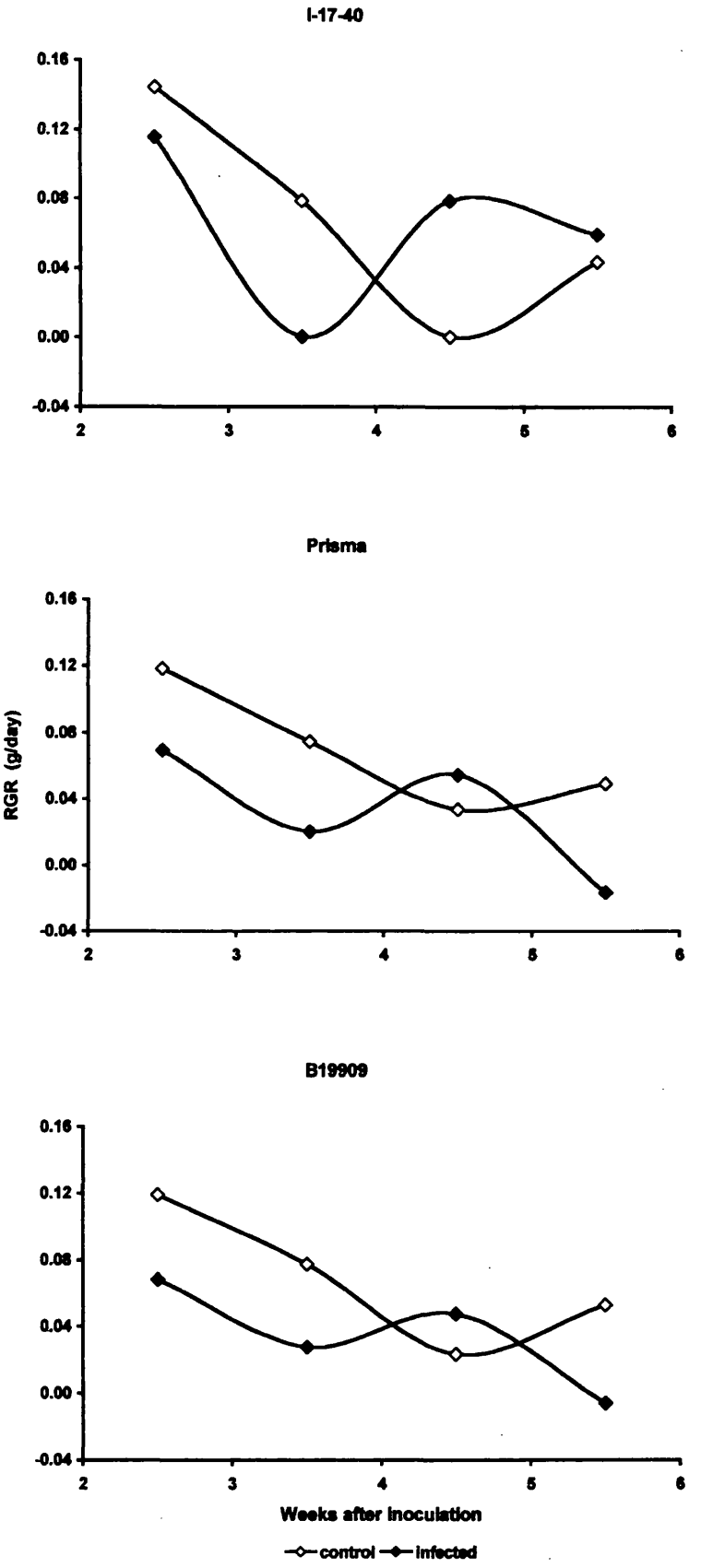


Fig. 25A: The effects of infection on the relative growth rate (RGR).

after inoculation, particularly in cv. Prisma and line B19909, it decreased again. The short period of increase in cv. Prisma and line B19909 coincided with the period of primary shoot elongation but the rapid final fall was probably due to the loss of senescent infected leaves, reduced tiller formation and root growth.

4.3.4.2. Unit leaf rate (ULR)

ULR is an estimate of the production of dry matter per unit leaf area. In uninfected plants of all three lines, ULRs decreased up to around the period between the sixth and the seventh week after planting (fourth and the fifth week after inoculation) after which it increased slightly (Fig. 25 B).

Infection lead to a more rapid reduction in ULR up to the period between the third and the fourth week after inoculation in all three lines. This rapid fall was followed by a transient but marked increase between the fourth and the fifth week followed by a further reduction particularly in cv. Prisma and line B19909. The marked increase in ULR from the fourth to the fifth week occurred when the level of infection was high. At this stage there were significantly lower green leaf areas on infected than uninfected plants and so that green leaf area must be more efficient than that of the controls probably reflecting compensatory photosynthesis.

4.3.5. Effects of infection on dry matter distribution

Changes in the distribution of dry matter in infected and uninfected plants of each line, at each harvest are plotted graphically in Figs. 26 to 33.

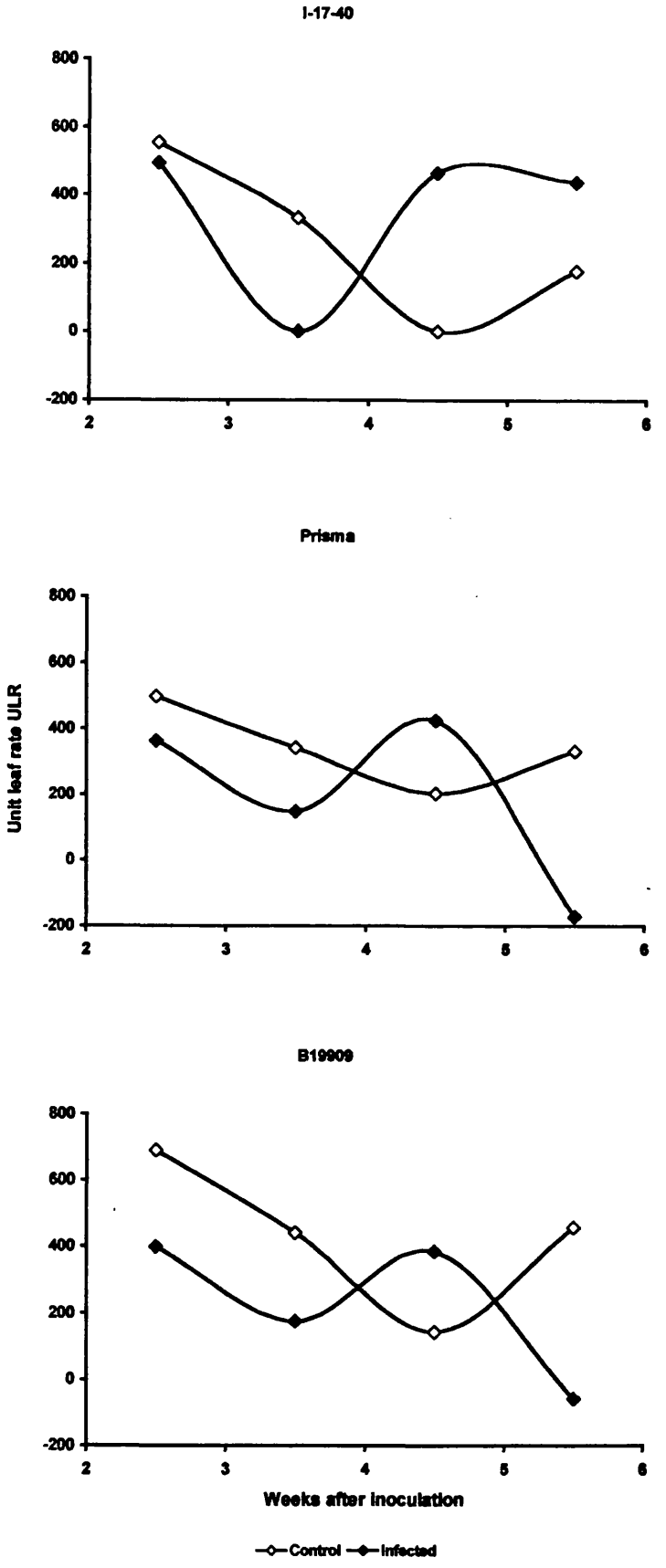


Fig. 25B: The effects of infection on the unit leaf rate (ULR).

4.3.5.1. Root : shoot ratio

In uninfected plants, the proportion of dry matter transported to the roots decreased with age in cv. Prisma and line B19909, but this trend was less clear in line I-17-40 (Fig. 26).

The proportion of dry matter partitioned to the roots compared to the shoots in infected plants had reduced significantly in cv. Prisma by the third week after inoculation ($p < 0.005$) and in line I-17-40 by the fifth week after inoculation ($p < 0.05$). In comparison, although infected plants of line B19909 showed a consistent, slight reduction in the root : shoot ratio, the differences were never significant at any stage.

4.3.5.2. Leaf weight ratio (LWR)

The LWR (ratio of leaf dry weight to total plant dry weight) of uninfected plants remained relatively constant during the whole period of the experiment in all three lines (Fig. 27). This constant LWR indicates that the proportion of dry matter allocated to the production of photosynthetic tissue (leaves) did not change significantly with age.

LWRs of infected plants were slightly higher than those of uninfected plants in all lines with the differences becoming significant ($p < 0.005$) by the fourth week after inoculation. The percentage increase in LWR of infected plants over uninfected plants at the last harvest was about 13% in line I-17-40, 36% in cv. Prisma and 132% in line B19909.

The increase in LWR in the infected plants indicates that the relative proportion of dry matter invested in largely respiring organs e.g. roots and

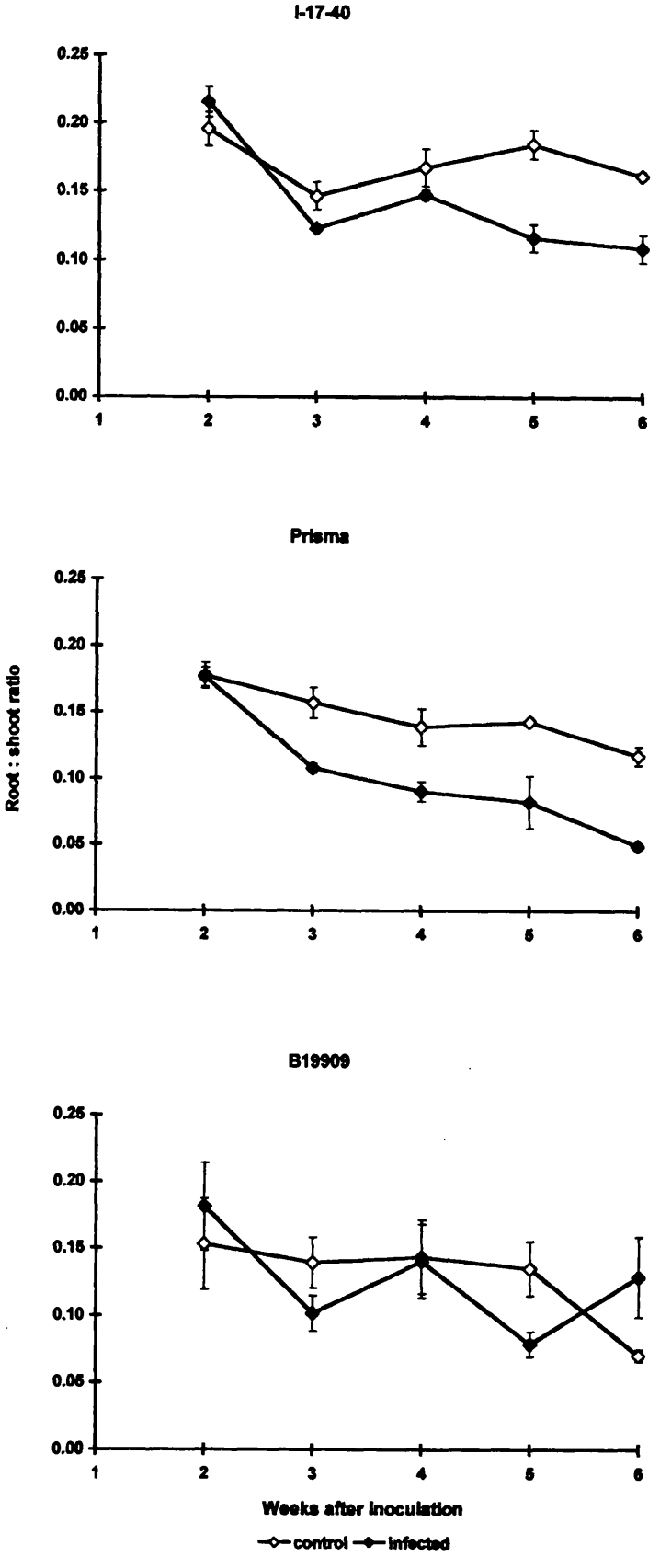


Fig. 26: The effects of infection on root : shoot ratio. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

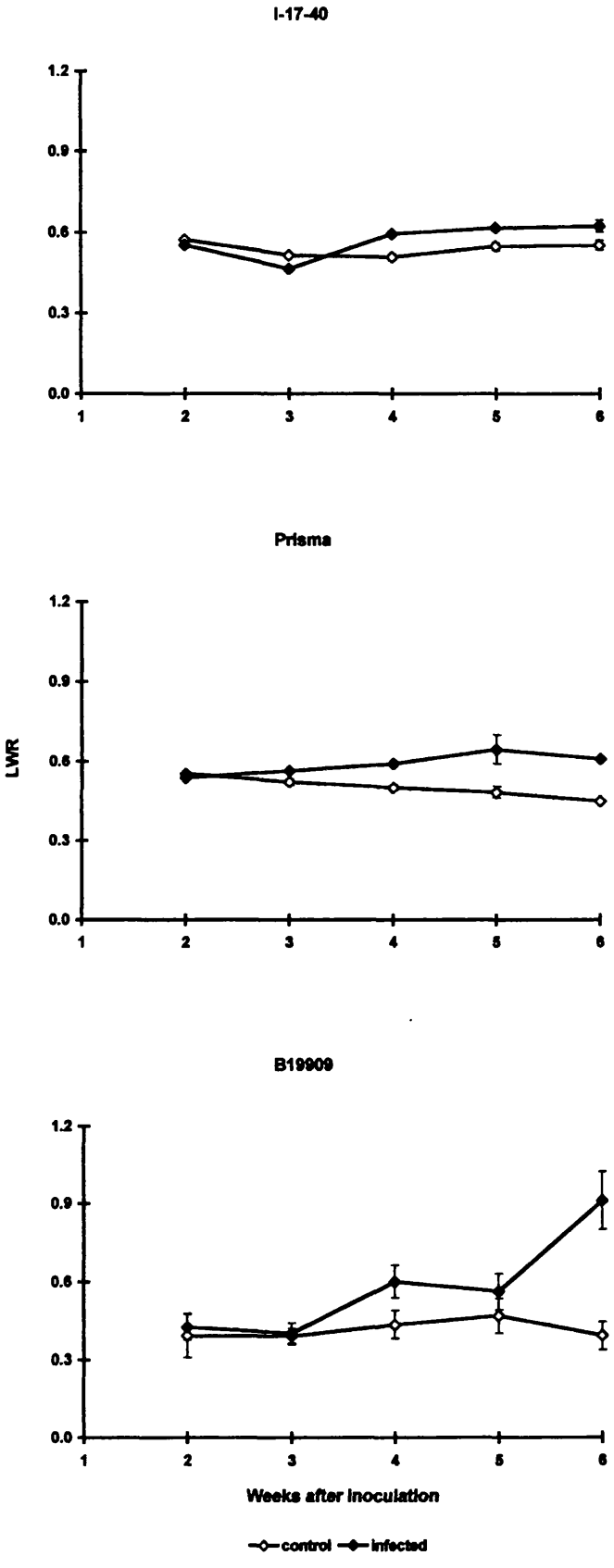


Fig. 27: The effects of infection on leaf weight ratio (LWR). Each datum point is the mean of three replicates. Vertical bars are the standard errors.

stems, was slightly smaller than the amount allocated to the photosynthesising organs (leaves) compared to the uninfected controls.

4.3.5.3. Leaf area ratio (LAR)

The LAR (ratio of leaf blade area to total plant dry weight, which is a measure of the leafiness of the plant) in uninfected plants remained relatively constant in the wild lines but decreased progressively in cv. Prisma over the period of the experiment. This indicates that the amount of dry matter allocated to the development of photosynthetic tissues (leaves) decreased with age in cv. Prisma but not in the two wild lines (Fig. 28).

LARs were reduced by infection in both cv. Prisma and line I-17-40, with the latter being more affected than the former. An analysis of variance showed that the LARs of these two lines were significantly reduced ($p < 0.05$) by three weeks after inoculation. Infection also consistently reduced the LAR in line B19909, but the differences were not significant at any time.

4.3.5.4. Specific leaf area (SLA)

The SLA (ratio of leaf area to leaf dry weight, which is a measure of leaf thickness) of the uninfected plants of all three lines decreased with age (Fig. 29). Thus as the plants aged, their leaves became heavier per unit area.

The SLA of infected plants, after a slightly significant increase ($p < 0.05$) in line I-17-40, decreased more rapidly than in the controls. The differences became significant from the third week after inoculation in both cv. Prisma and line I-17-40 ($p < 0.005$). Infection also lead to a reduced SLA in line

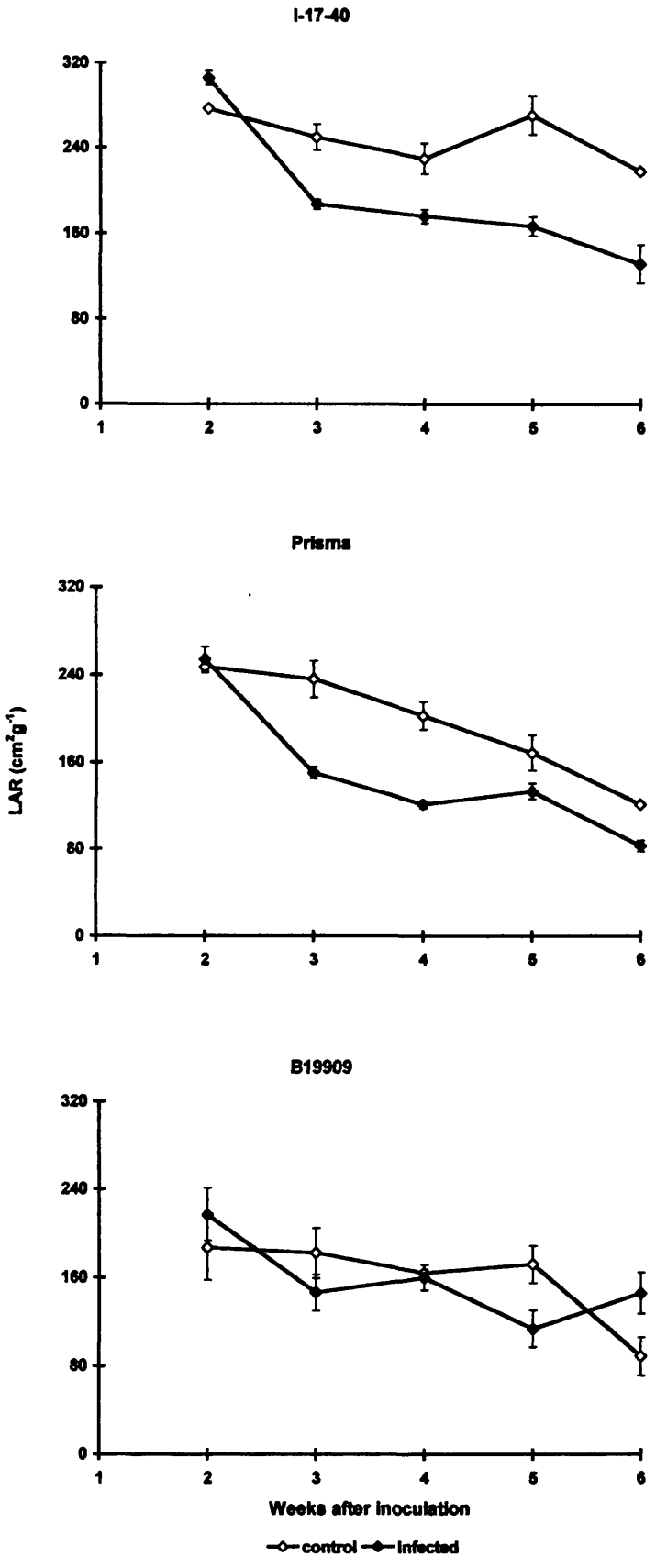


Fig. 28: The effects of infection on leaf area ratio (LAR). Each datum point is the mean of three replicates. Vertical bars are the standard errors.

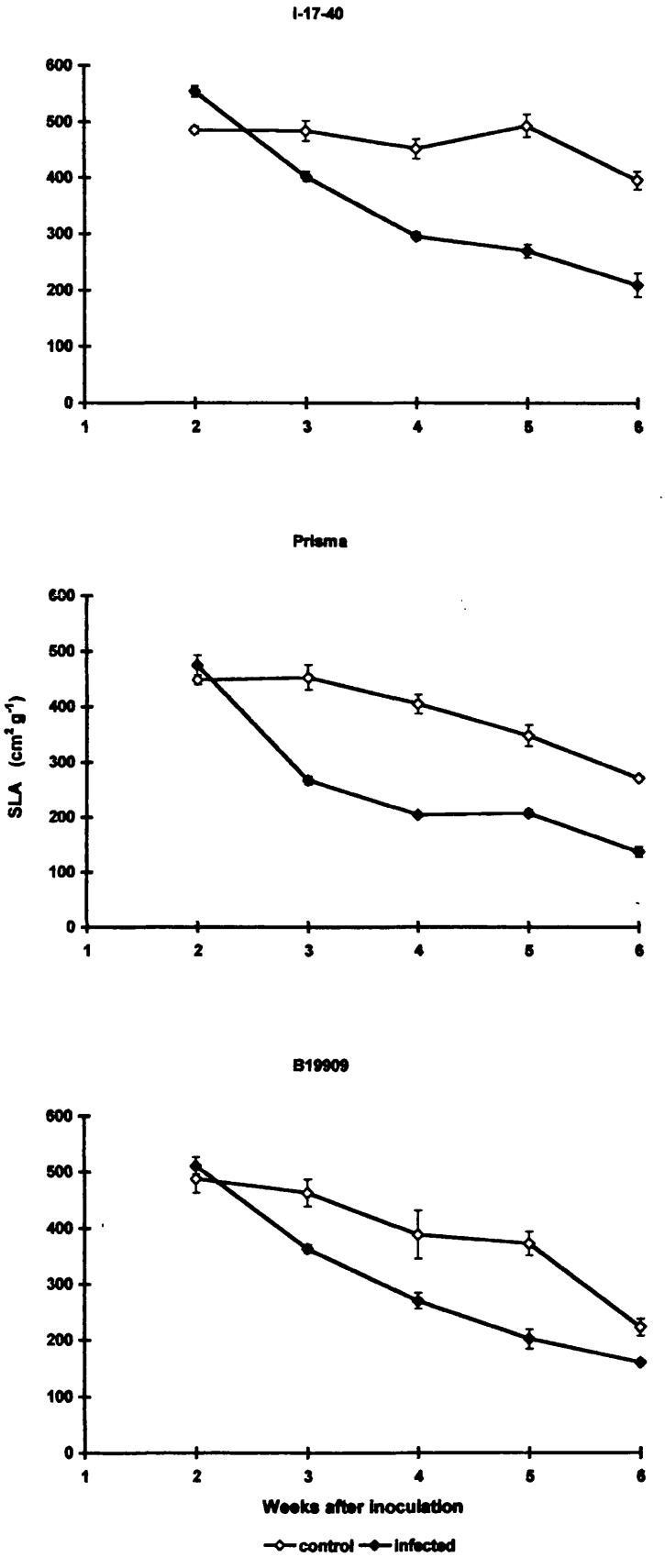


Fig. 29: The effects of infection on specific leaf area (SLA). Each datum is the mean of three replicates. Vertical bars are the standard errors.

B19909 ($p < 0.01$), but the differences were not so large as for the other two lines.

The reduction in SLAs of the infected plants was partly due to the greater loss of the lower leaves by senescence and partly to the accumulation of more dry matter in the relatively small remaining leaves. Whether the relative increase in dry matter in the leaves per unit area was structural (cell wall, vascular tissues...etc) or non-structural (storage carbohydrates) was not determined.

4.3.5.5. Root weight ratio (RWR)

In general, the RWR (ratio of root dry weight to total plant dry weight) of uninfected plants of all three lines decreased with time indicating that as plants aged less dry matter was allocated to the root system (Fig. 30).

Infection reduced the RWR compared to the controls in both cv. Prisma and line I-17-40 and an analysis of variance showed that the differences were significant in cv. Prisma ($p < 0.005$) and in line I-17-40 ($p < 0.05$). However, the RWRs of infected plants of line B19909 were not significantly different from the controls at any harvest.

4.3.5.6. Ratio of root weight to leaf area

The weight of roots per unit leaf area in uninfected plants increased progressively as the plants aged in all the lines (Fig. 31).

In infected plants, this ratio decreased in all three lines particularly in cv. Prisma. An analysis of variance showed that the differences between infected

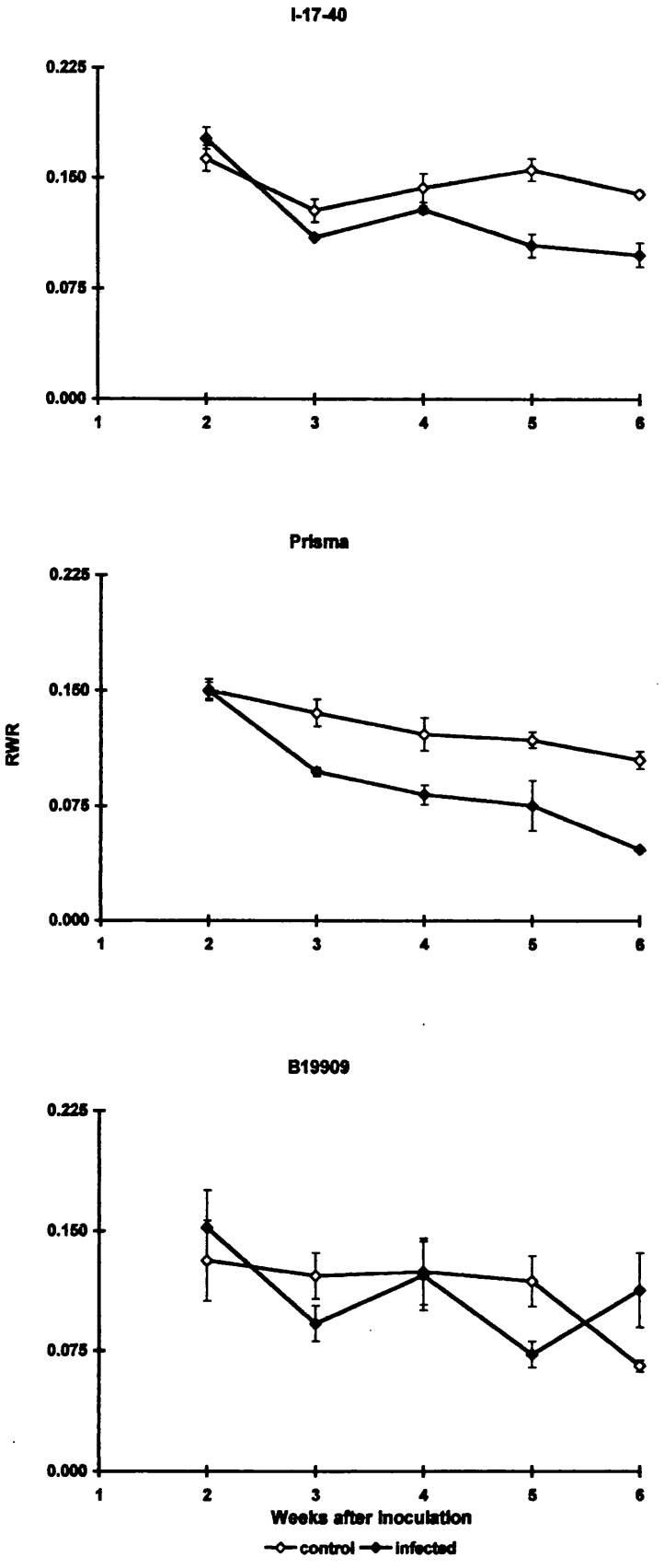


Fig. 30: The effects of infection on root weight ratio (RWR). Each datum is the mean of three replicates. Vertical bars are the standard errors.

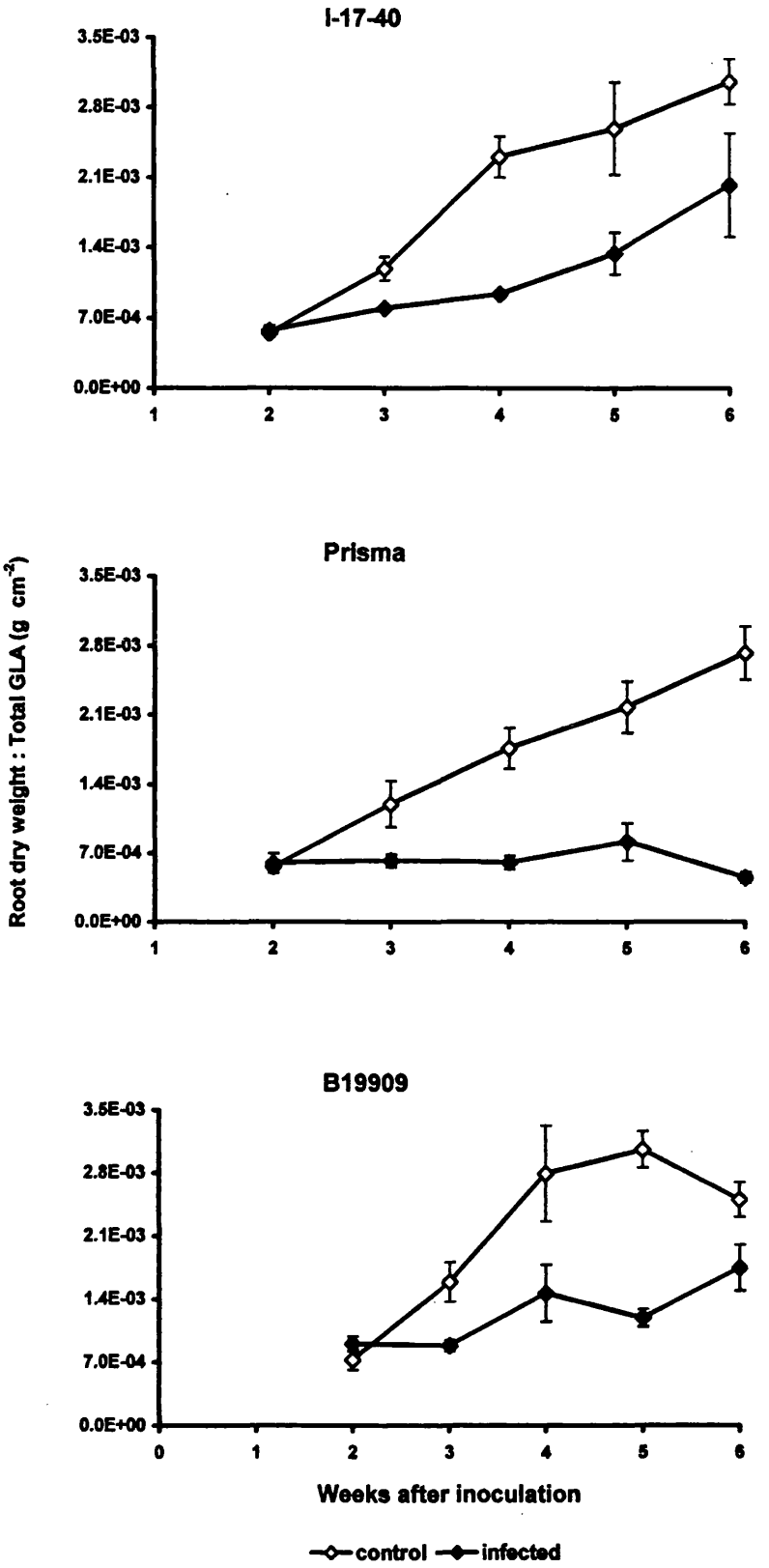


Fig. 31: The effects of infection on the ratio of root dry weight to total leaf area. Each datum point is the mean of three replicate Vertical bars are the standard errors.

and uninfected plants were highly significant in cv. Prisma ($p < 0.0001$) and line B19909 ($p < 0.005$), but less significant in line I-17-40 ($p < 0.05$). By the fifth week after inoculation, infection had reduced the dry weight of roots per unit leaf area by about 48% in line I-17-40, 61% in line B19909 and 63% in cv. Prisma. Thus although infection reduced GLA, it induced a relatively greater reduction in root development.

4.3.5.7. Root area ratio (RAR)

Fig. 32 shows that the RAR of uninfected plants remained constant throughout the course of the experiment. The RARs of infected plants of line I-17-40 and cv. Prisma were consistently lower than those of the controls from the third harvest, but an analysis of variance showed that the differences were only significant ($p < 0,05$) from the sixth week after inoculation. The RAR of infected plants of line B19909 was not significantly different from the controls at any harvest.

4.3.5.8. Specific Root Area (SRA)

Fig. 33 shows that the SRA remained relatively constant in both uninfected and infected plants throughout the period of experiment. The values were consistently higher for infected plants, but the differences were never significant.

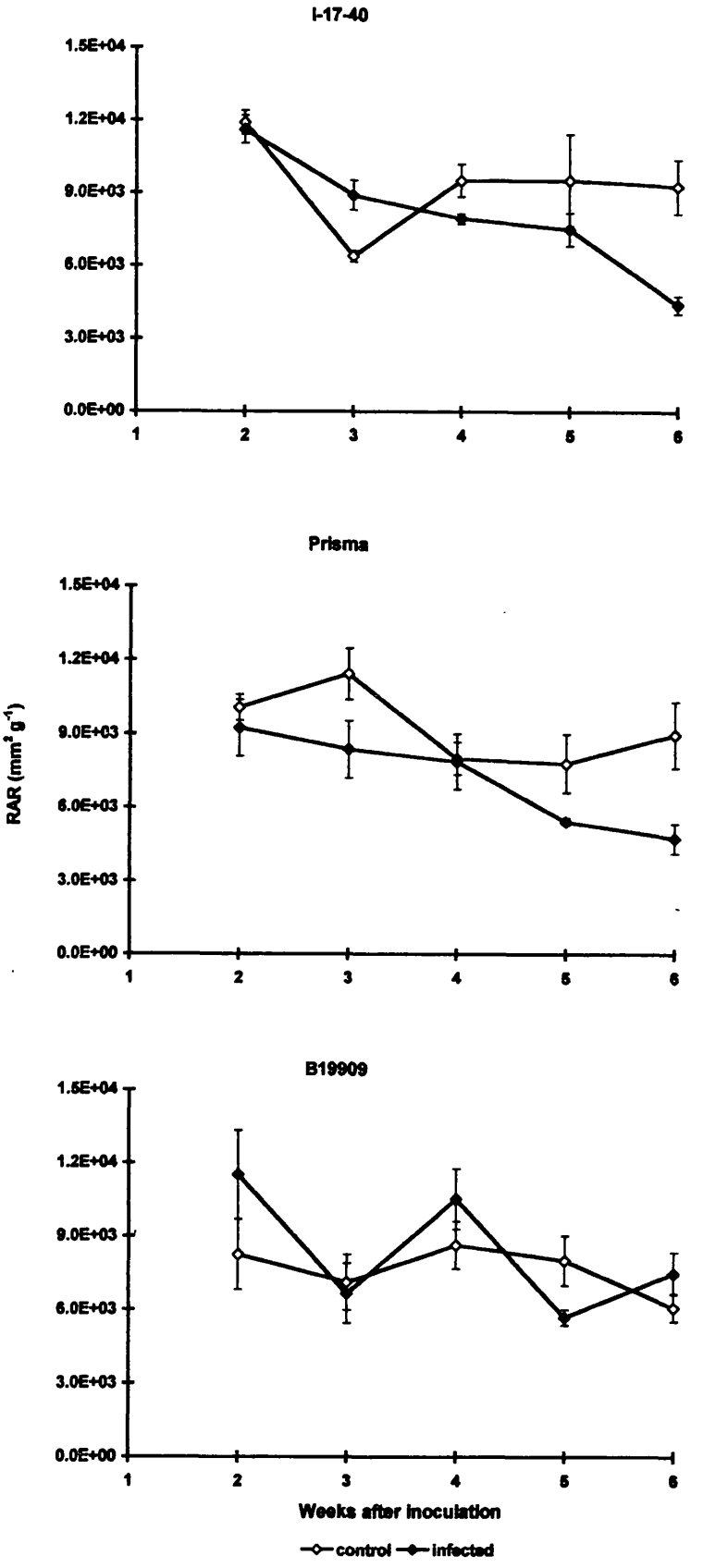


Fig. 32: The effects of infection on root area ratio (RAR). Each datum is the mean of three replicates. Vertical bars are the satandard errors.

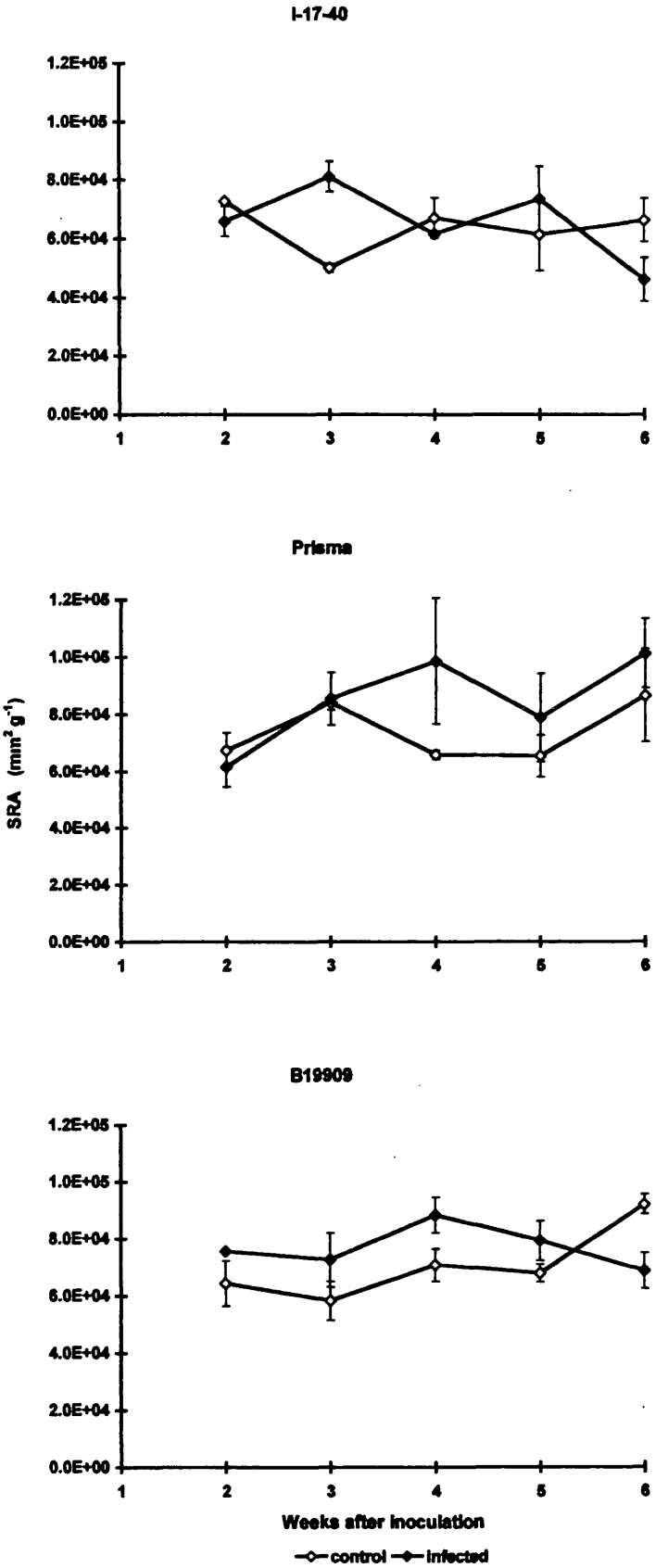


Fig. 33: The effects of infection on specific root area (SRA). Each datum point is the mean of three replicates. Vertical bars are the standard errors.

4.3.5.9. Specific root length (SRL)

Fig. 34 shows that the SRL (the ratio of total root length to total root dry weight) of uninfected plants generally remained constant in line I-17-40 and cv. Prisma throughout growth. In contrast, line B19909 showed a slight increase over the course of the experiment.

The SRLs of infected plants of both cv. Prisma and line B19909 were consistently higher than those of uninfected plants, but the difference was only significant ($p < 0.05$) in cv. Prisma from the sixth week after inoculation; it was never significant in line B19909 and no significance was measurable in line I-17-40.

The increase in SRL of infected plants in cv. Prisma and to an extent in line B19909, indicates that the roots of infected plants tended to be thinner than those of the uninfected plants.

4.3.6. Effects of infection on cell division in the apical meristems of the roots

In order to compare the rates of mitotic cell divisions in the apical meristems of the nodal root tips of infected and uninfected plants, the mitotic index of seedlings eight days after inoculation were compared with controls. The results are summarised graphically in Fig. 35.

Eight days after inoculation, the mitotic indices of uninfected control plants were 9.75 for cv. Prisma, 7.0 for line I-17-40 and 11.5 for line B19909. In contrast, the mitotic indices of infected plants were about half these values 4.75 for cv. Prisma, 3.75 for line I-17-40 and 5.5 for line

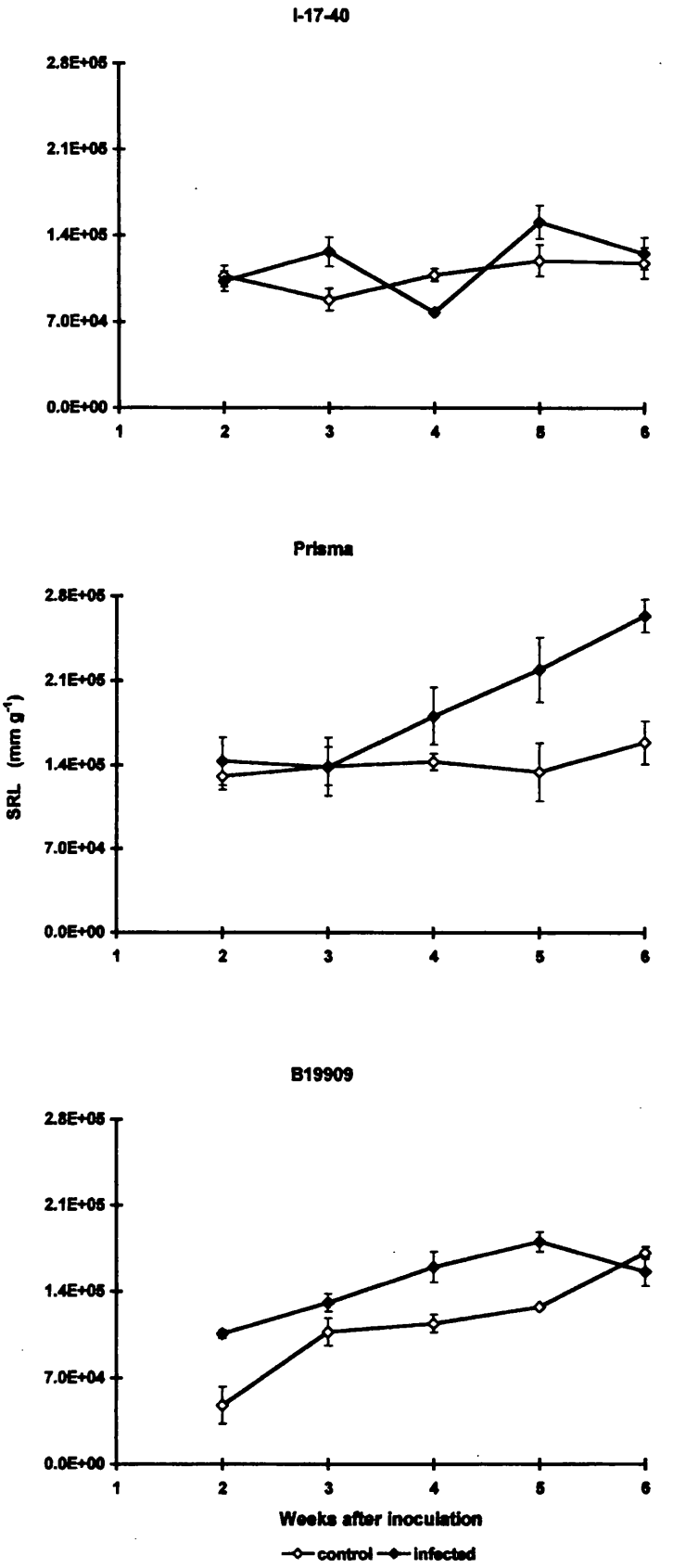


Fig. 34: The effects of infection on specific root length (SRL). Each datum point is the mean of three replicates. Vertical bars are the standard errors.

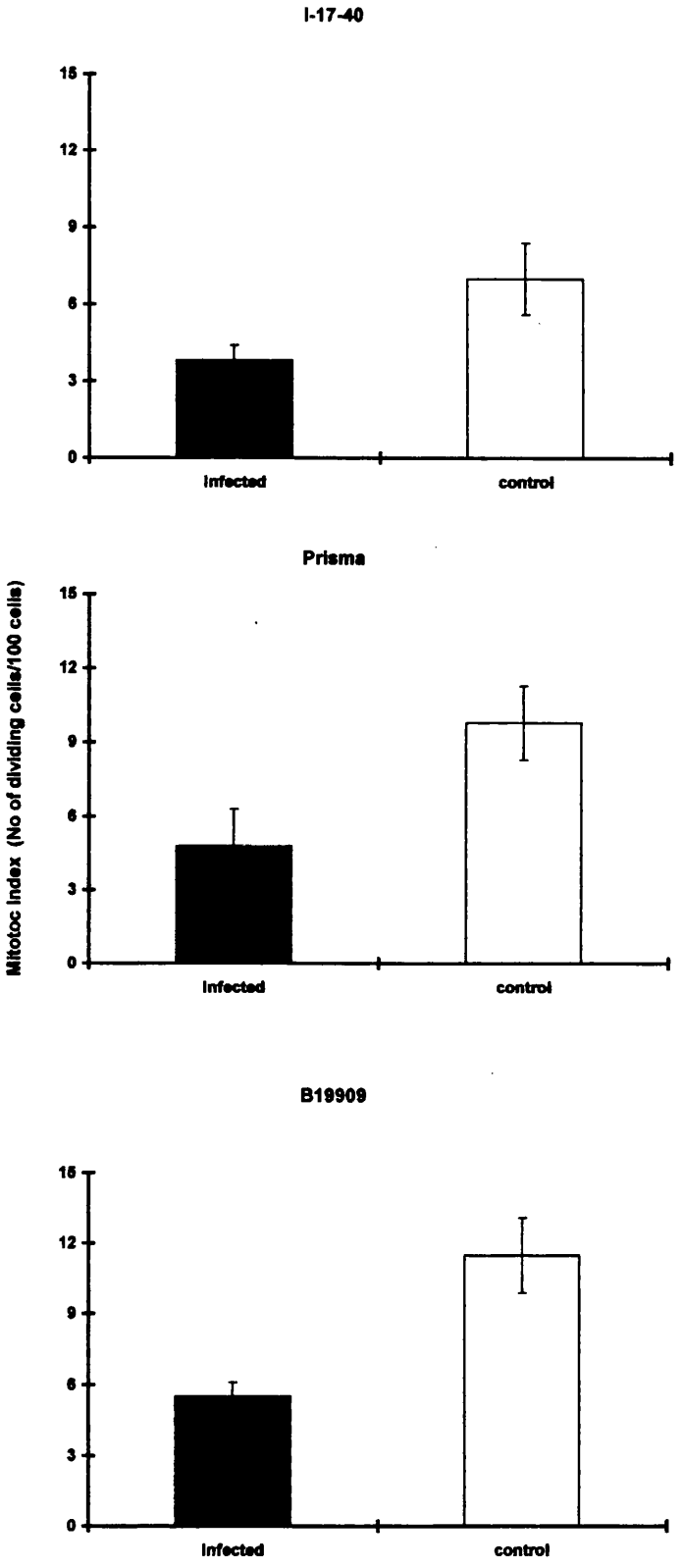


Fig. 35: The percentage of meristematic cells in mitosis (mitotic index) of 20 day-old seedlings of the three barley lines, following 8 days after inoculation with powdery mildew conidia. Each datum point is the mean of three replicates. Vertical bars are the standard errors.

B19909. However, because of the variability statistical analyses demonstrated that the differences in mitotic indices between infected and uninfected plants were significant ($p < 0.05$) for line B19909 only.

4.3.7. Effects of infection on the components of plant yield

Yield components of infected and uninfected plants of each line are plotted graphically in Figs. 36 to 44.

4.3.7.1. Grain production by the primary shoot

4.3.7.1.1. Total number of grains

Fig. 36 shows that on uninfected plants most grains were produced per primary shoot ear by line I-17-40 and the least by line B19909; cv. Prisma was intermediate for this character.

Infection reduced the number of grains per primary shoot ear in cv. Prisma by about 36%, and a statistical analysis showed that this difference was highly significant ($p < 0.01$). However, infection did not significantly affect the number of grains per primary shoot ear in the two wild lines.

4.3.7.1.2. Total dry weight of grains

Fig. 37 shows that the total dry weight of grain produced per primary shoot ear on infected plants of cv. Prisma was significantly reduced by about 41% ($p < 0.005$). In contrast the total dry weight of grains per primary shoot ear in line I-17-40 was not altered by infection, while in infected plants of B19909 it was significantly greater by about 24% ($p < 0.05$).

Fig. 36

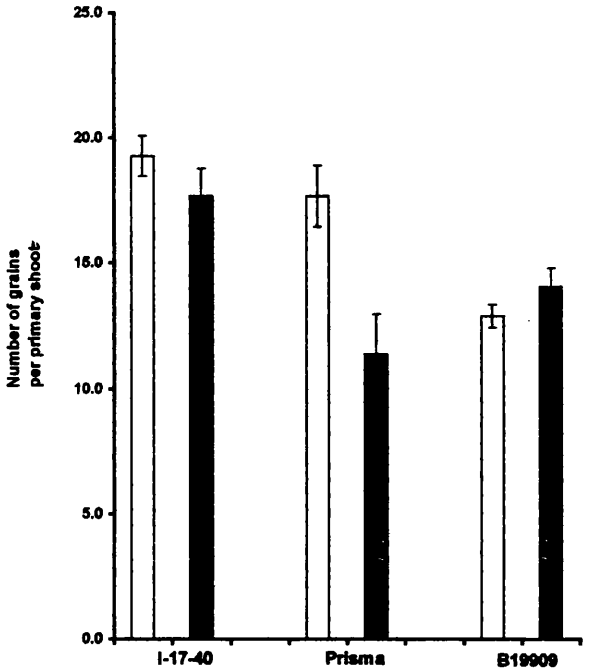


Fig. 37

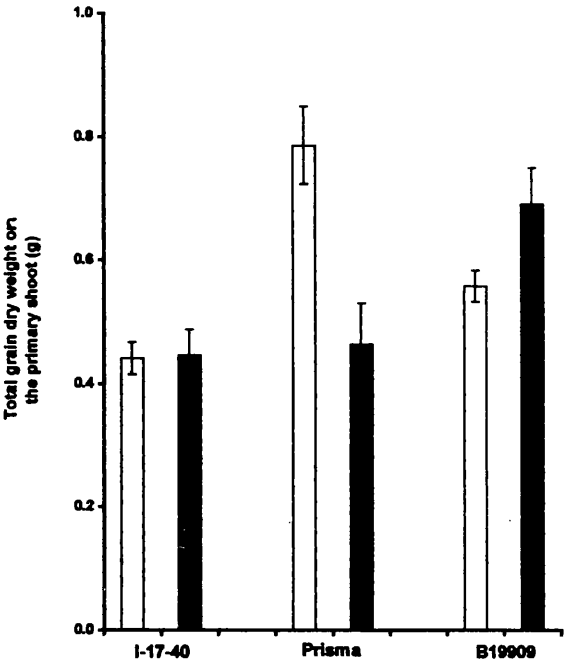


Fig. 38

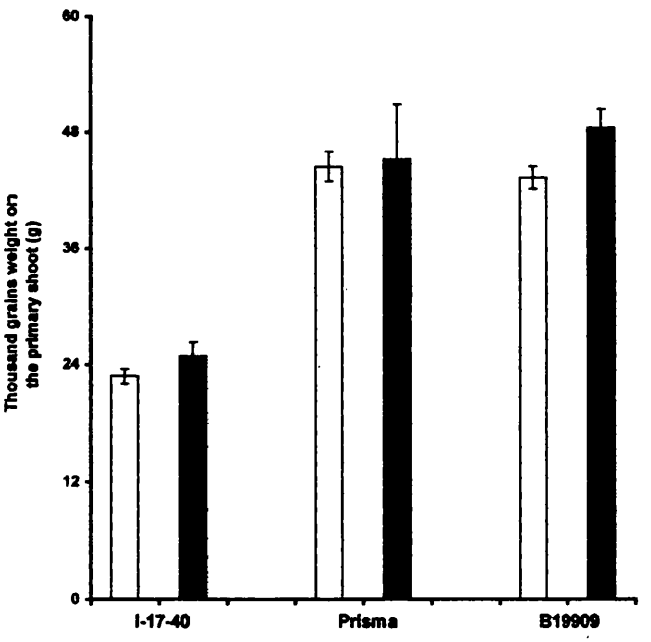


Fig. 36: Total number of grains produced on the primary shoot.

Fig. 37: Total grain dry weight produced on the primary shoot.

Fig. 38: 1000-grains dry weight on the primary shoot.

Uninfected □ Infected ■

4.3.7.1.3. Thousand-grain weight

Infection slightly increased the thousand-grain weight of line B19909, by about 12% ($p < 0.05$), but it had no effect on thousand grain weights of the other two lines (Fig. 38).

4.3.7.2. Grain production by the tillers

4.3.7.2.1. Number of fertile tillers

Uninfected plants of the two wild lines produced higher numbers of fertile tillers than cv. Prisma (Fig. 39A).

The infected plants of the three lines produced slightly fewer fertile tillers than the uninfected plants, although the differences were not significant, probably because of the large variation between plants in fertile tiller production. The number of sterile tillers produced per plant was not affected by infection in any line (Fig. 39B).

4.3.7.2.2. Total number of grains

The total number of grains produced by all fertile tiller ears was consistently reduced by infection in all three lines (Fig. 40). However, the reduction was only significant in cv. Prisma ($p < 0.02$). The decrease in the total number of grains in the tiller ears of cv. Prisma was mainly due to fewer grains per ear.

Fig. 39 A

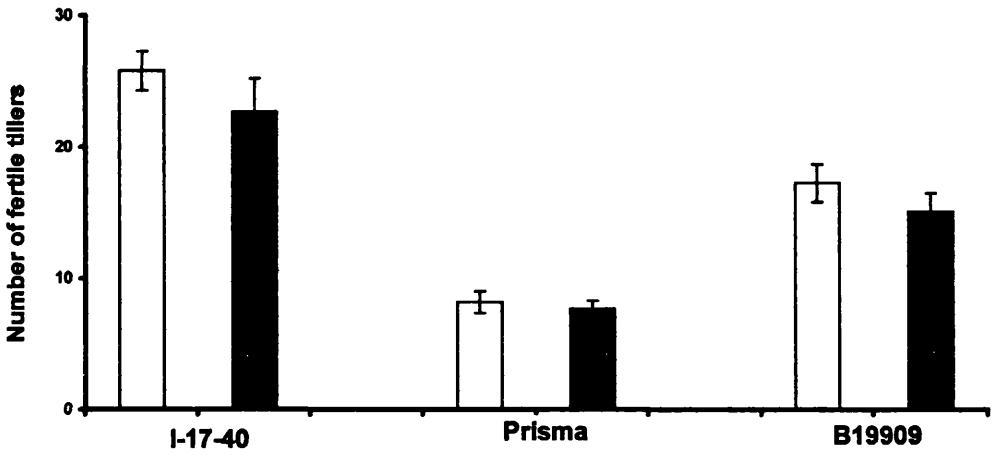


Fig. 39 B

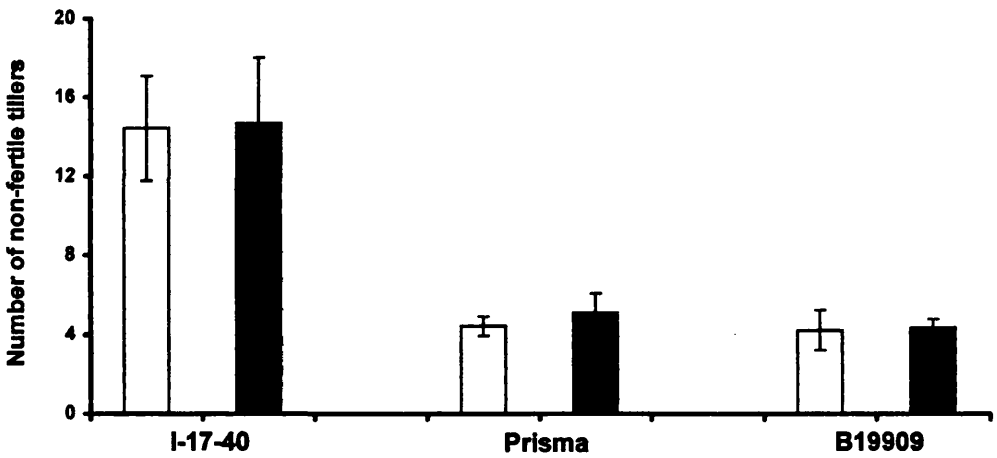


Fig. 39: Number of fertile (A) and unfertile (B) tillers per plant.

Uninfected □ **Infected** ■

4.3.7.2.3. Total dry weight of grains

Fig. 41 shows that infection slightly reduced the total dry weight of grain produced by all tillers of lines I-17-40 and B19909, but the reductions were not significant. In contrast, total grain production by cv. Prisma was reduced by about 55%, a reduction that was highly significant ($p < 0.0001$).

4.3.7.2.4. Thousand-grain weight

Infection had no significant effect on the thousand-grain weight of any line (Fig. 42).

4.3.7.3. Grain production on the whole plant

4.3.7.3.1. Total grain dry weight per plant

Infection reduced total grain dry weight per plant of lines I-17-40 and B19909, but the reductions were not significant (Fig. 43). In contrast, infection significantly reduced total grain dry weight of cv. Prisma by 52% ($p < 0.001$). The decrease in total grain dry weight per plant was mainly due to fewer grains per ear, particularly in tiller ears.

4.3.7.3.2. Harvest Index

Fig. 44 shows that the ratio of dry weight of grain to total dry matter (harvest index) was higher in uninfected plants of line B19909 than in the other two lines. Infection significantly reduced ($p < 0.01$) the harvest index of cv. Prisma by about 43%, but it had no effect on the harvest index of either wild line. The lower harvest index of cv. Prisma was due mainly to fewer grains per ear and a lower total grain dry weight.

Fig. 40

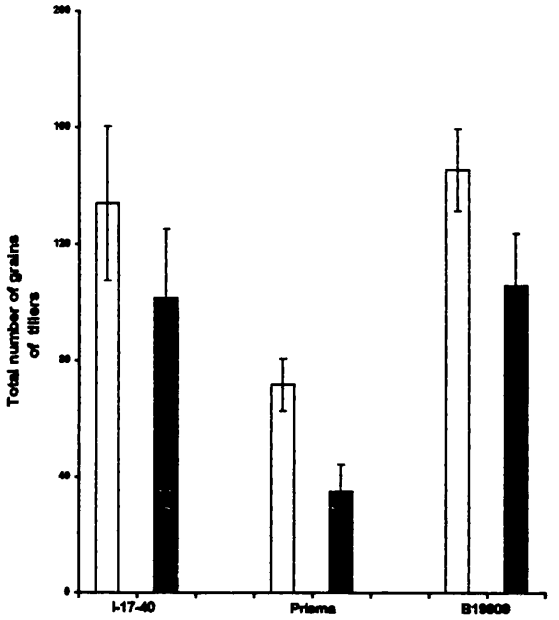


Fig. 41

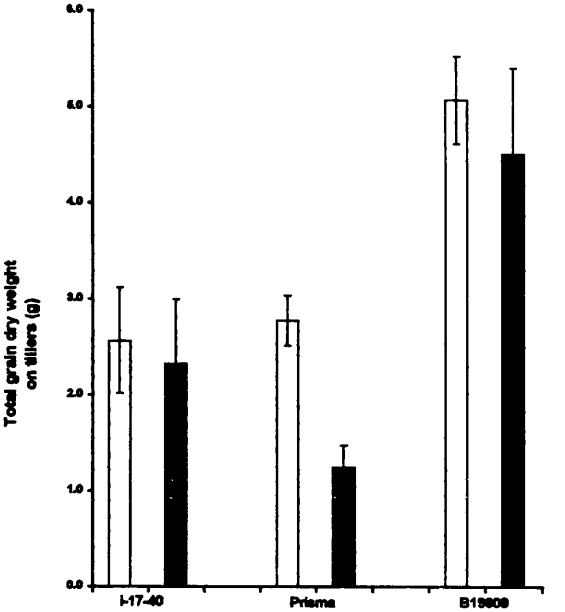


Fig. 42

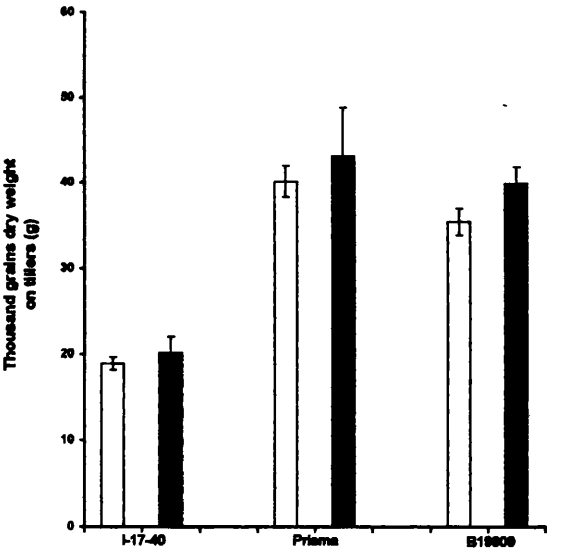


Fig. 40: Total number of grains produced on all tillers.

Fig. 41: Total grain dry weight produced on all tillers.

Fig. 42: 1000-grains dry weight on tillers

Uninfected ☐ Infected ■

Fig. 43

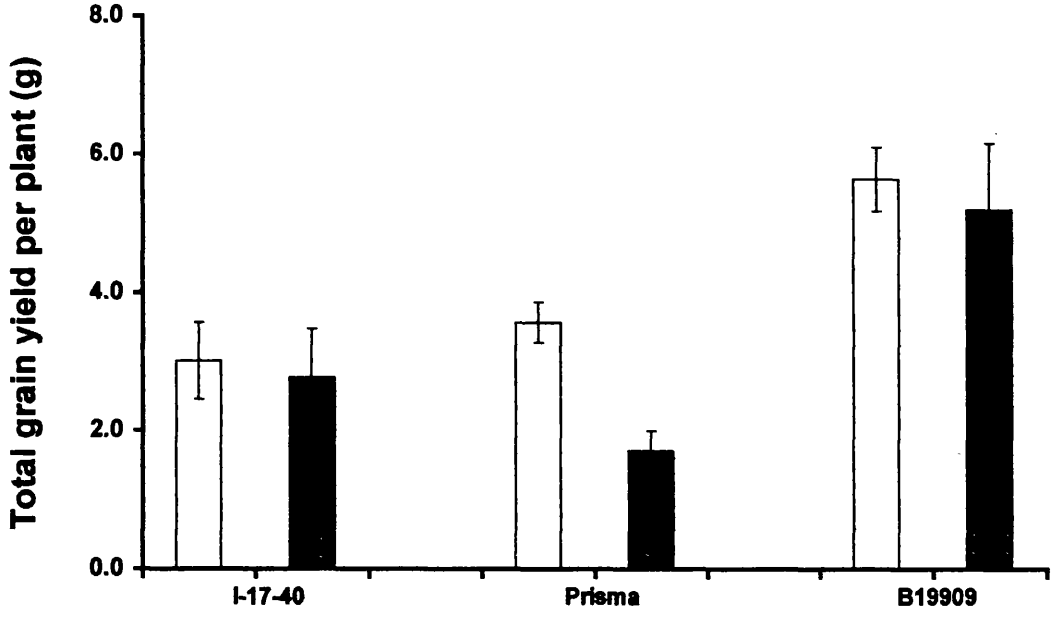


Fig. 44

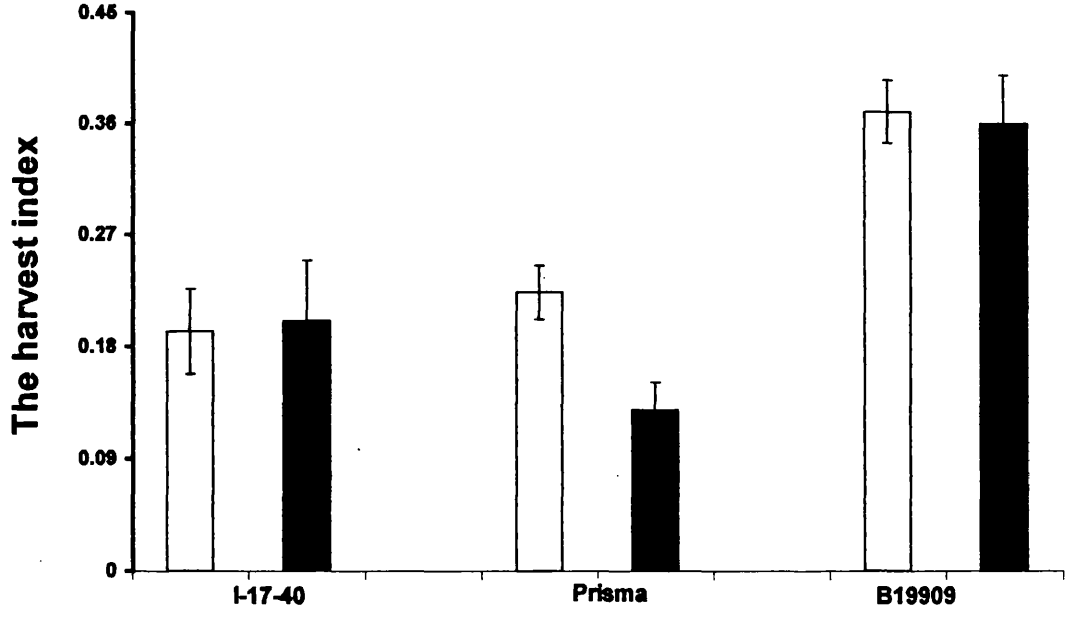


Fig. 43: Total grain yield produced per plant

Fig. 44: Harvest index

Uninfected ☐ Infected ■

Chapter 5

THE EFFECTS OF INFECTION ON LEAF EXPANSION

5.1. Introduction

The reduced expansion of leaf blades formed after inoculation could be due to either a reduction in cell expansion in the leaf or to a reduction in the numbers of cells produced from the leaf blade meristem or both.

Since the ratio between stomatal number and epidermal cell number (stomatal index) is a constant (Wilkinson, 1979), counts of stomata and epidermal cells per unit area of leaf, together with measurements of total leaf area, were used to determine the approximate numbers of stomata and epidermal cells per leaf.

5.2. Results

Counts of stomata and epidermal cells per field of view are presented in Table 4. The ratio between numbers of stomata and numbers of epidermal cells per field of view was relatively constant and the stomatal indices are presented in Table 4.

The number of epidermal cells per field of view were higher in the uninfected lower epidermis of the infected third leaf than in the corresponding uninfected leaf in all three lines. An analysis of variance showed that the differences between uninfected and infected leaves in each line, were significant ($p < 0.001$). The higher number of stomata per field of view for the infected leaves in the three lines is due to the reduced expansion of the epidermal cells with the relative increase in number indicating the extent of this reduction. The percentage reductions were between 13 and

Table 4: Mean number of stomata and epidermal cells per field of view in the lower epidermis of the third leaf of cv. Prisma and the two wild lines B19909 and I-17-40.

Lines		Uninfected plants			Infected plants		
	Days after inoculation	8	11	13	8	11	13
	% Mildew	0	0	0	25	50	75
I-17-40	Stomata	15.6±0.581	15.0±0.843	15.1±0.458	18.9±0.433	17.9±0.348	18.8±0.359
	Epidermal cells	59.9±2.861	52.5±1.851	51.4±1.318	69.2±0.712	62.3±2.216	68.7±2.140
	Stomatal Index	0.26±0.013	0.29±0.012	0.30±0.011	0.27±0.004	0.29±0.009	0.27±0.005
Cv. Prisma	Stomata	14.2±0.573	14.7±0.253	16.0±0.494	17.8±0.573	17.3±0.199	18.0±0.537
	Epidermal cells	50.4±0.819	59.7±1.267	57.7±1.571	68.3±1.944	71.4±0.914	69.2±2.308
	Stomatal Index	0.28±0.010	0.25±0.004	0.28±0.007	0.26±0.007	0.24±0.003	0.26±0.012
B19909	Stomata	16.9±0.640	14.9±0.586	14.6±0.221	18.3±0.597	19.5±0.582	17.7±0.396
	Epidermal cells	51.7±1.415	49.6±1.024	51.2±0.467	57.6±0.636	63.4±1.634	58.6±0.670
	Stomatal Index	0.33±0.017	0.30±0.009	0.29±0.003	0.32±0.009	0.31±0.009	0.30±0.005

25% in line I-17-40, between 16 and 26% in cv. Prisma and between 10 and 22% in line B19909 (Table 5).

The approximate number of epidermal cells per leaf in each line was calculated from counts of epidermal cells per field of view together with measurements of total leaf area (Table 5). The results show that there were about 5% fewer cells in the lower epidermis of infected than uninfected leaves of line I17-40, between 2 and 7% in cv. Prisma and between 5 and 10% in line B19909. Thus the reduced leaf area was due to a reduction in cell division in the leaf blade meristem as well as to reduced cell expansion.

Table 5: The effects of infection on the size and number of epidermal cells of uninfected and infected third leaves of cv. Prisma and the two wild lines B19909 and I-17-40.

Lines	Days after inoculation	Uninfected plants		Infected plants			% Change		
		Leaf area (cm^2)	Number of epidermal cells per leaf	% Mildew	Leaf area (cm^2)	Number of epidermal cells per leaf	Leaf area	Number	Size
I-17-40	8	13.86	133260.7	25	12.76	141732.3	- 7.9	+ 6.0	- 13.4
	11	13.85	116713.5	50	11.63	116300.0	- 16.0	- 0.4	- 15.7
	13	15.09	124498.6	75	10.67	117661.2	- 29.3	- 5.5	- 25.2
Cv. Prisma	8	20.75	167865.2	25	15.07	165213.6	- 20.2	- 1.6	- 26.2
	11	19.67	188491.0	50	15.80	181078.7	- 15.0	- 3.9	- 16.4
	13	18.00	166709.5	75	14.00	155505.6	- 11.1	- 6.7	- 16.6
B19909	8	17.81	147797.3	25	16.79	155233.4	- 5.7	+ 5.0	- 10.2
	11	18.68	148720.4	50	13.70	139418.9	- 26.7	- 6.3	- 21.8
	13	17.94	147436.3	75	14.14	133002.2	- 21.2	- 9.8	- 12.6

-: A decrease
+: An increase

Chapter 6

EFFECTS OF INFECTION ON THE PHOTOSYNTHESIS AND RESPIRATION OF THE THIRD AND FOURTH LEAVES OF SIX BARLEY LINES.

6.1. INTRODUCTION

The growth analyses reported in chapter 4 showed that infection altered the growth and development of both the wild and the cultivated barley lines. The reduced dry matter accumulation in different organs was related to reductions in photosynthetic tissue due to the premature senescence of the infected leaves, reduced leaf expansion and to reductions in unit leaf rate, which is a measure of net photosynthesis. This section describes a series of experiments on the effects of infection on photosynthesis, specifically the following: (1) gross and net photosynthesis, (2) quantum efficiency, which is a measure of the number of moles of O_2 evolved per mole quanta of absorbed photosynthetically active radiation, (3) the ratio of physical to total resistance of CO_2 diffusion into the leaf and (4) the chlorophyll content of the leaf. In addition dark respiration in the leaf was measured. Changes in the measured parameters were related to the amount of fungal biomass produced on the leaf during the course of the infection.

Two experiments were carried out. In the first, the effects of infection on the different photosynthetic parameters were measured in the third leaf of the three lines used for the growth analysis study, the two wild lines B19909 and

I-17-40 and cv. Prisma. Line I-17-40 has a high level of adult plant resistance, while line B19909 and cv. Prisma are relatively susceptible, line B19909 being the most susceptible.

In the second experiment, the effects of infection were measured on the fourth leaf of three barley lines, which exhibited different levels of partial resistance: cv. Golden Promise a highly susceptible cultivated barley, cv. Triumph showing intermediate resistance in response to inoculation, and the wild line B8893, which has a high level of resistance. A summary of the results is presented in the Appendix Table 14.

6.2. RESULTS

EXPERIMENT –I

6.2.1. Development of fungal biomass on the third leaf of cv. Prisma and the two wild lines I-17-40 and B19909.

The cumulative number of conidia produced per unit leaf area (cm^2) on each barley line, by each harvest is plotted in Fig. 45.

Four to six days after inoculation the mildew had developed a uniform mycelial cover over the inoculated leaf surface, but little sporulation was occurring. Conidial production increased rapidly from the sixth day after inoculation onwards on all three lines with that on cv. Prisma and line B19909 continuing to increase up to sixteen days after inoculation. In contrast, the leaf of line I-17-40 ceased to support conidial production from about twelve days after inoculation due to leaf senescence. By the twelfth day after inoculation, line B19909 had produced about 31.6×10^5 conidia cm^{-2}, cv. Prisma about 16×10^5 conidia cm^{-2} while line I-17-40 had produced only 10.4×10^5 conidia cm^{-2}. The differences were significant ($p < 0.05$) between the three lines. By the sixteenth day after inoculation, the number of conidia had increased per cm^2 leaf area to about 41.6×10^5 conidia on line B19909 and about 26×10^5 conidia on cv. Prisma.

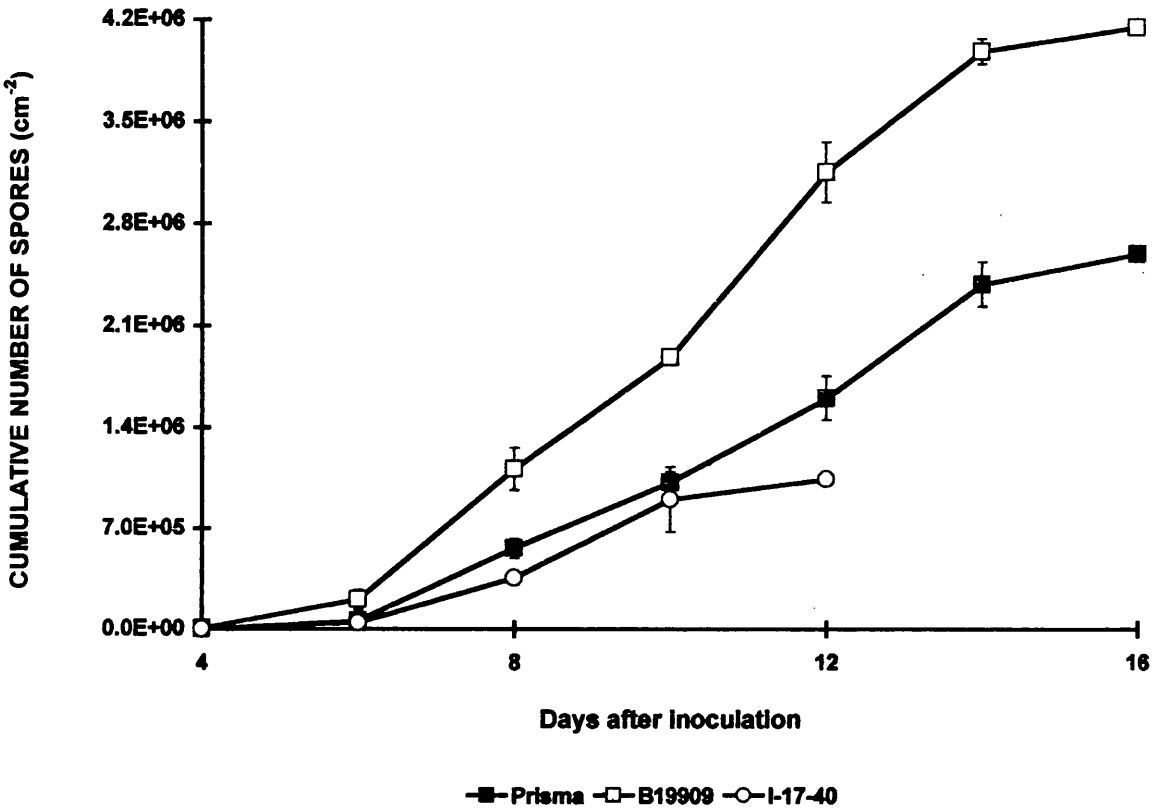


Fig. 45: **The cumulative number of spores produced by each harvest (days after inoculation) on the third leaves of three barley lines. Each datum point is the mean of three replicates.**

6.2.2. Effects of infection on photosynthesis

6.2.2.1. Maximum rates of gross photosynthesis (Pg_{max})

Pg_{max} (μmol of O_2 evolved m^{-2} s^{-1}) in uninfected leaves was relatively constant for up to ten days after the third leaf was fully expanded in all lines and then, it declined progressively in cv. Prisma (Fig. 46A). After ten days, measurements were not continued in line I-17-40 because the infected leaves had senesced.

Pg_{max} in the infected leaves of each line was not affected by infection until six days after inoculation when it began to decline in all three lines and from then on declined rapidly. By ten days after inoculation, Pg_{max} in the infected leaves had reduced significantly to about 17% of the levels in uninfected leaves in line I-17-40 ($p < 0.01$), to about 26% of the levels in uninfected leaves in cv. Prisma ($p < 0.001$) and to about 30% of that in uninfected leaves in line B19909 ($p < 0.001$). After ten days, the infected leaves of line I-17-40 had senesced to such an extent that no further measurements were possible. However, measurements were possible with the other two lines up to fourteen days after inoculation, but then senescence of their leaves also precluded any further measurements.

At fourteen days after inoculation, infection had reduced gross photosynthesis to about 20% of the level in uninfected leaves in both cv. Prisma and line B19909 ($P < 0.002$).

When related to the amount of chlorophyll present in the leaf (Fig. 46B), although Pg_{max} fluctuated between sampling times due to random variation no significant differences were observed in any line at any stage.

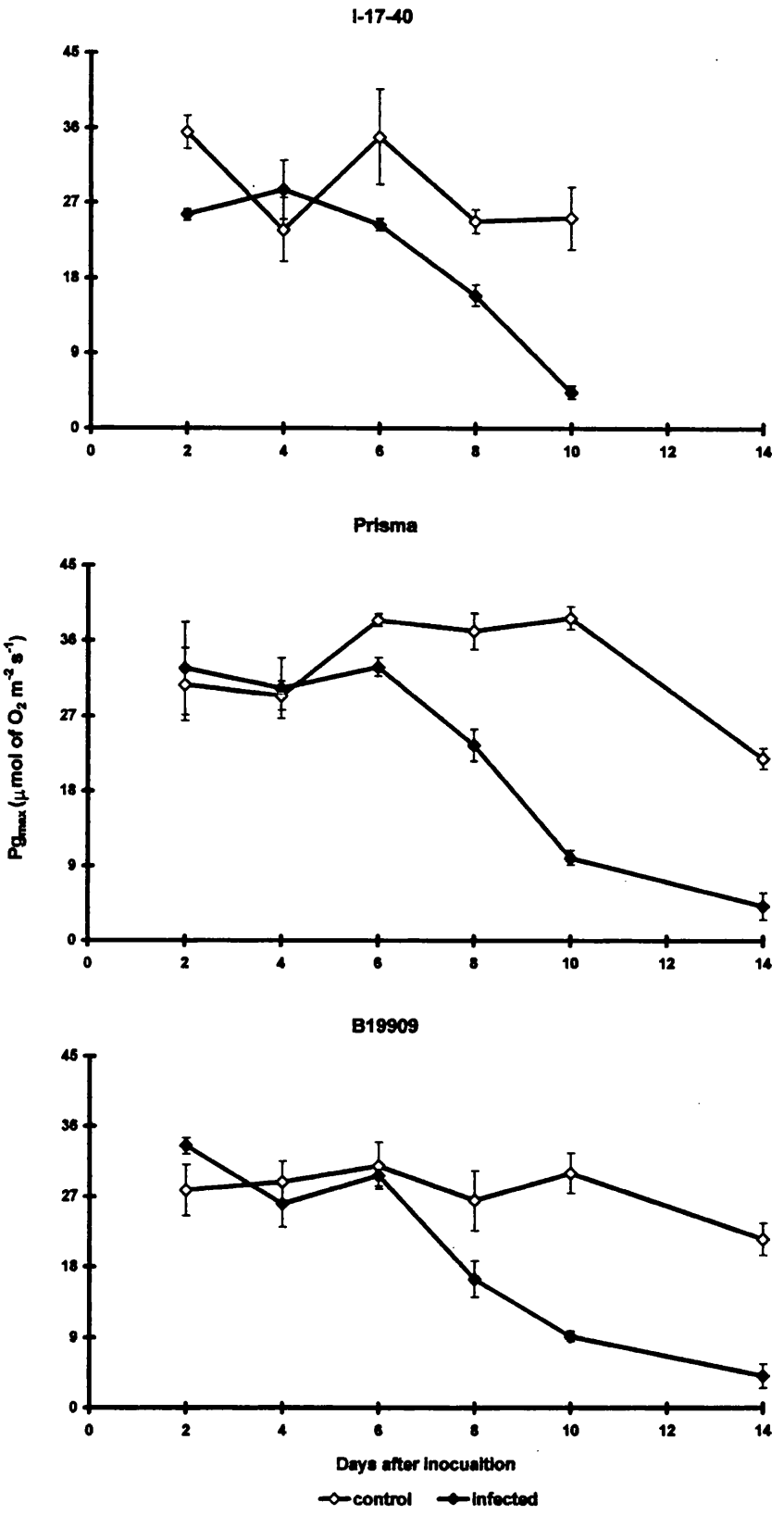


Fig. 46A : Effects of infection on maximum gross photosynthesis per unit area of infected and uninfected third leaves of the three barley lines. Each datum point is the mean of three replicates.

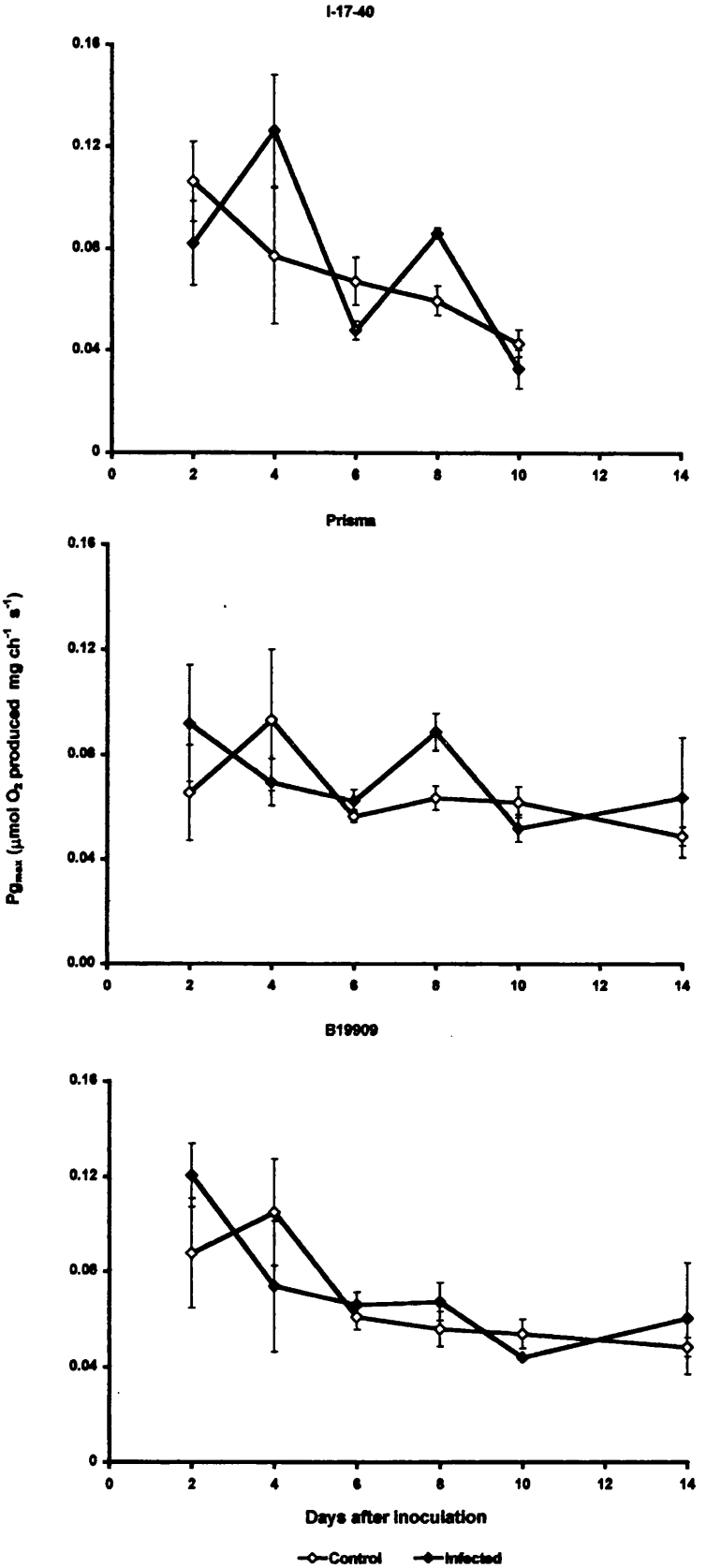


Fig. 46B: Effects of infection on maximum gross photosynthesis per unit mass of total chlorophyll of infected and uninfected third leaves of the three barley lines. Each datum point is the mean of three replicates.

6.2.2.2. Maximum rates of net photosynthesis (Pn_{max})

Pn_{max} per unit leaf area of uninfected leaves followed the same pattern as Pg_{max} declining progressively as leaves aged (Fig. 47A). Infection reduced Pn_{max} in all three lines in the same way as Pg_{max}. The reductions became significant ($p < 0.02$) from 6, 8 and 10 days after inoculation in cv. Prisma, I-17-40 and B19909 respectively.

As with Pg_{max}, Pn_{max} per milligram of chlorophyll (Fig. 47B) fluctuated between harvests but at no stage were any significant differences found between infected and uninfected lines.

6.2.2.3. Relationship between Pg_{max} and fungal biomass

The reduction in the rate of maximum gross photosynthesis during infection in each line was linearly correlated ($p < 0.01$) with increasing fungal biomass, measured as conidial production, in the infected third leaves (Table 6 and Fig. 48).

The slopes of the regression lines of cv. Prisma and the wild line I-17-40 were similar, but that of line B19909 was significantly different ($p < 0.05$) from both.

6.2.2.4. Quantum efficiency of photosynthesis (α)

The quantum efficiency (or quantum yield) of photosynthesis (α) in the uninfected leaves of each line remaining relatively constant up to the fourth day after the third leaf was fully expanded (fourth day after inoculation) in line

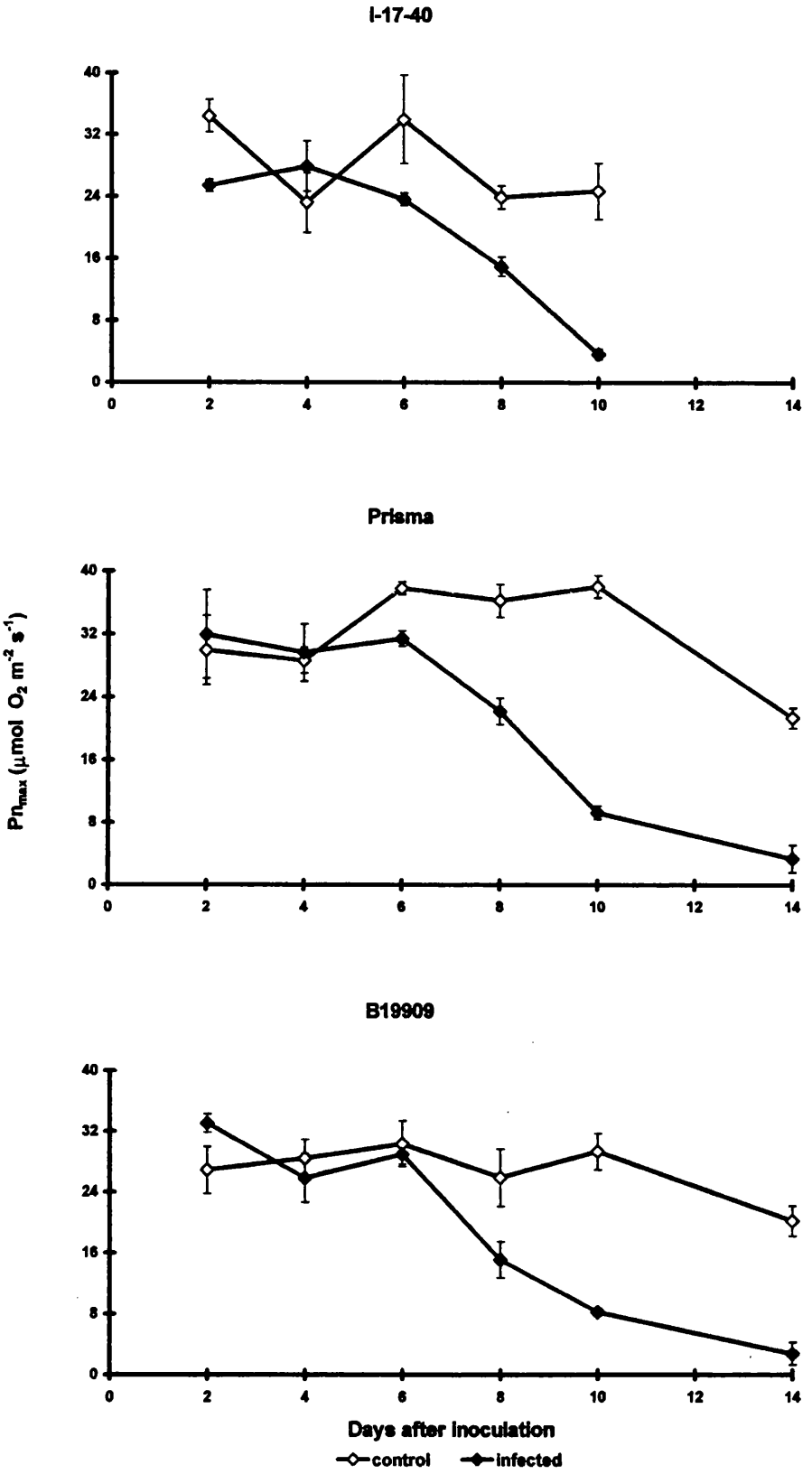


Fig. 47A: Effects of infection on maximum net photosynthesis per unit area of infected and uninfected third leaves of the three barley lines. Each datum point is the mean of three replicates.

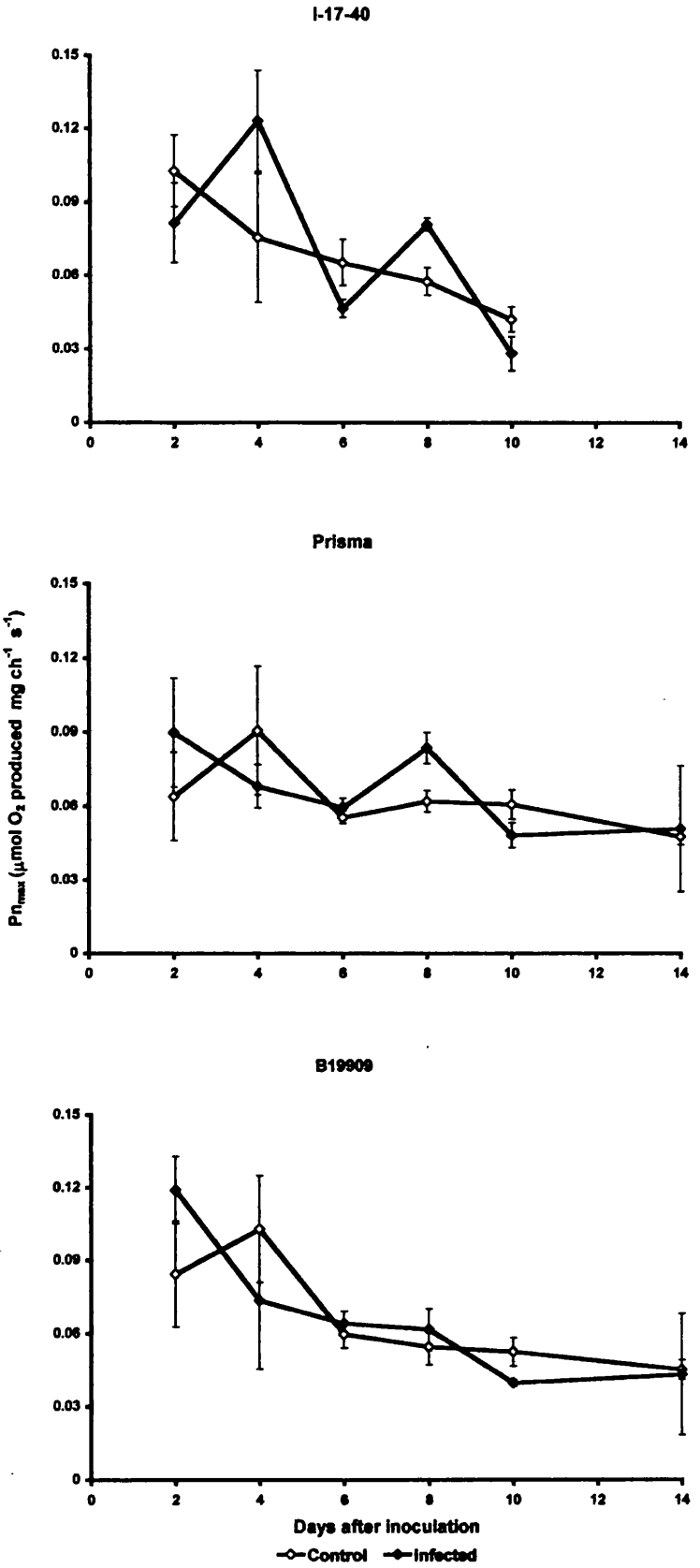


Fig. 47B: Effects of infection on maximum net photosynthesis per unit mass of total chlorophyll of infected and uninfected third leaves of the three barley lines. Each datum point is the mean of three replicates.

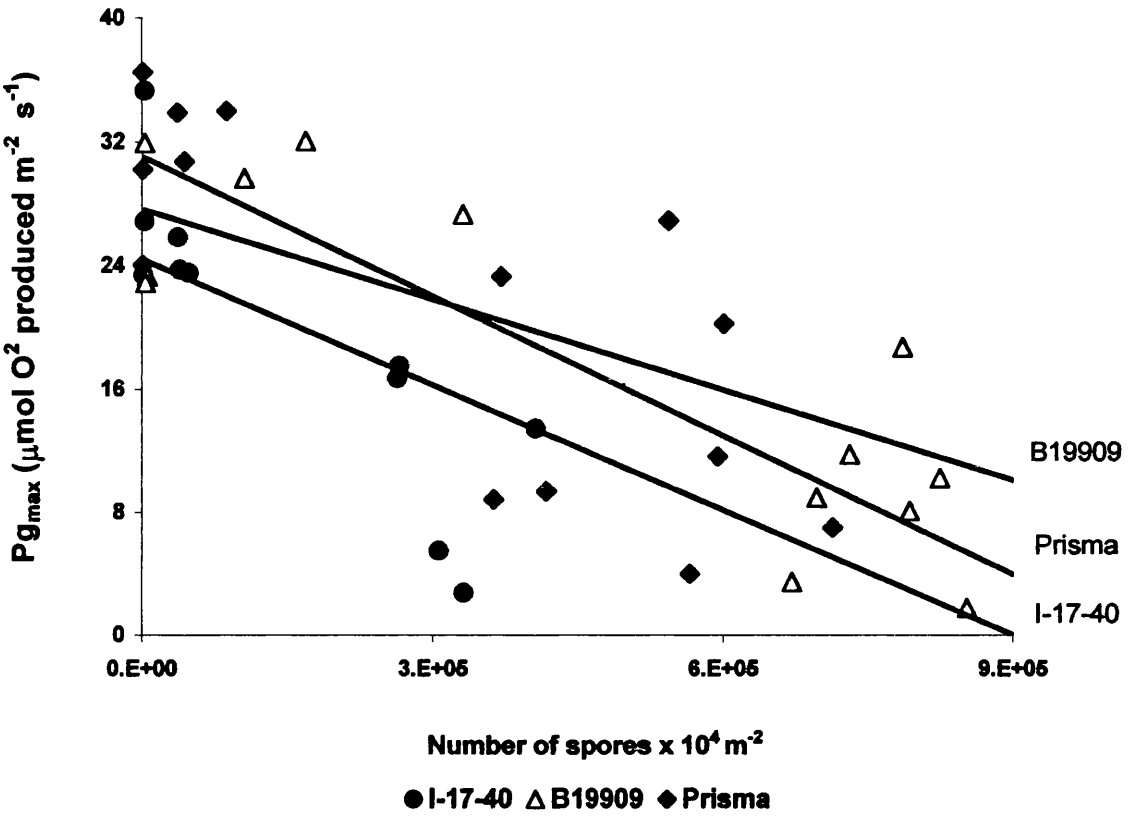


Fig. 48: Relationship between gross photosynthesis and conidial production on the infected third leaf of the three lines.

Table 6.

Regression analyses of maximum gross photosynthesis (Pg_{max}) and fungal spore production in cv. Prisma and the two wild lines I-17-40 and B19909.

Regression Parameters	**I-17-40**	**Prisma**	**B19909**
Intercept (a)	24.402	31.043	27.655
Slope (b)	-3×10^{-5}	-3×10^{-5}	-2×10^{-5}
Coefficient of Correlation (R^2)	0.5905	0.6704	0.5534
Degrees of Freedom	11	14	14
F ratio	14.42	26.44	16.11
p	< 0.01	< 0.001	< 0.01

I-17-40 and up to the tenth day in cv. Prisma and line B19909. From then on α declined slowly (Fig. 49).

Infection decreased α in all three lines, but the reduction was less marked in line B19909 than in the other two lines. Ten days after inoculation, α had been reduced to about 23% of the value in uninfected leaves in line I-17-40 ($p < 0.05$), to about 21% in cv. Prisma ($p < 0.005$) and to about 35% of the value in uninfected leaves in line B19909 ($p < 0.05$). From the tenth day after inoculation, cv. Prisma showed a further decrease to about 20% of the control value, but no further decrease over the 35% occurred in line B19909. In this line the values in the uninfected leaves also declined and the difference at the last harvest was no longer significant.

6.2.2.5. The convexity (θ) or the ratio of physical to total resistance to CO_2 diffusion.

Fig. 50 Shows that θ began to increase in all three lines very soon after inoculation and the higher rate than the uninfected controls persisted more or less throughout the period of measurement. However, an analysis of variance showed that only the differences for cv. Prisma were significant ($p < 0.005$) becoming so from the fourth day after inoculation and remained so until fourteen days after inoculation when the differences were no longer significant because of an increase in the values in the controls.

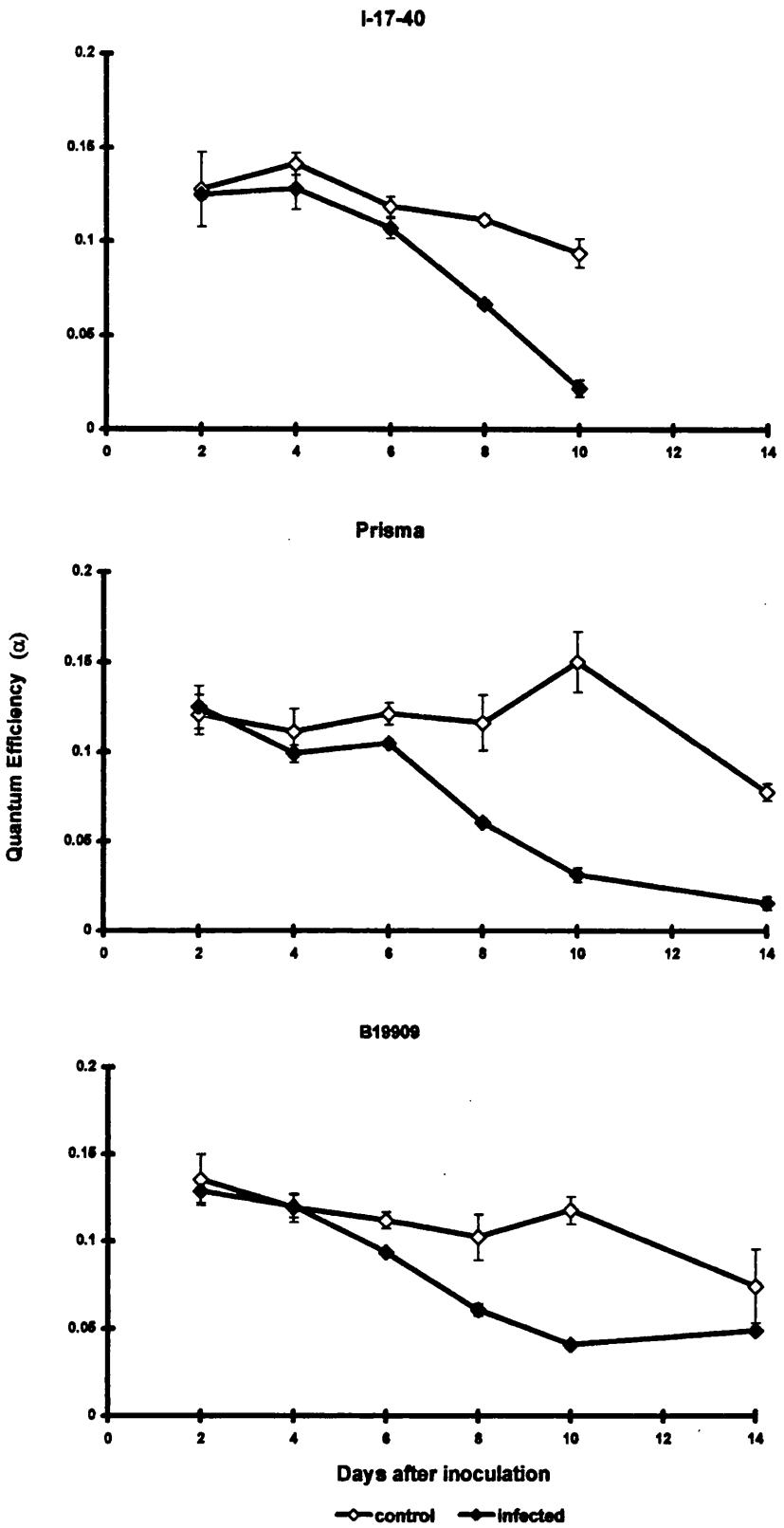


Fig. 49: Effects of infection on quantum efficiency of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

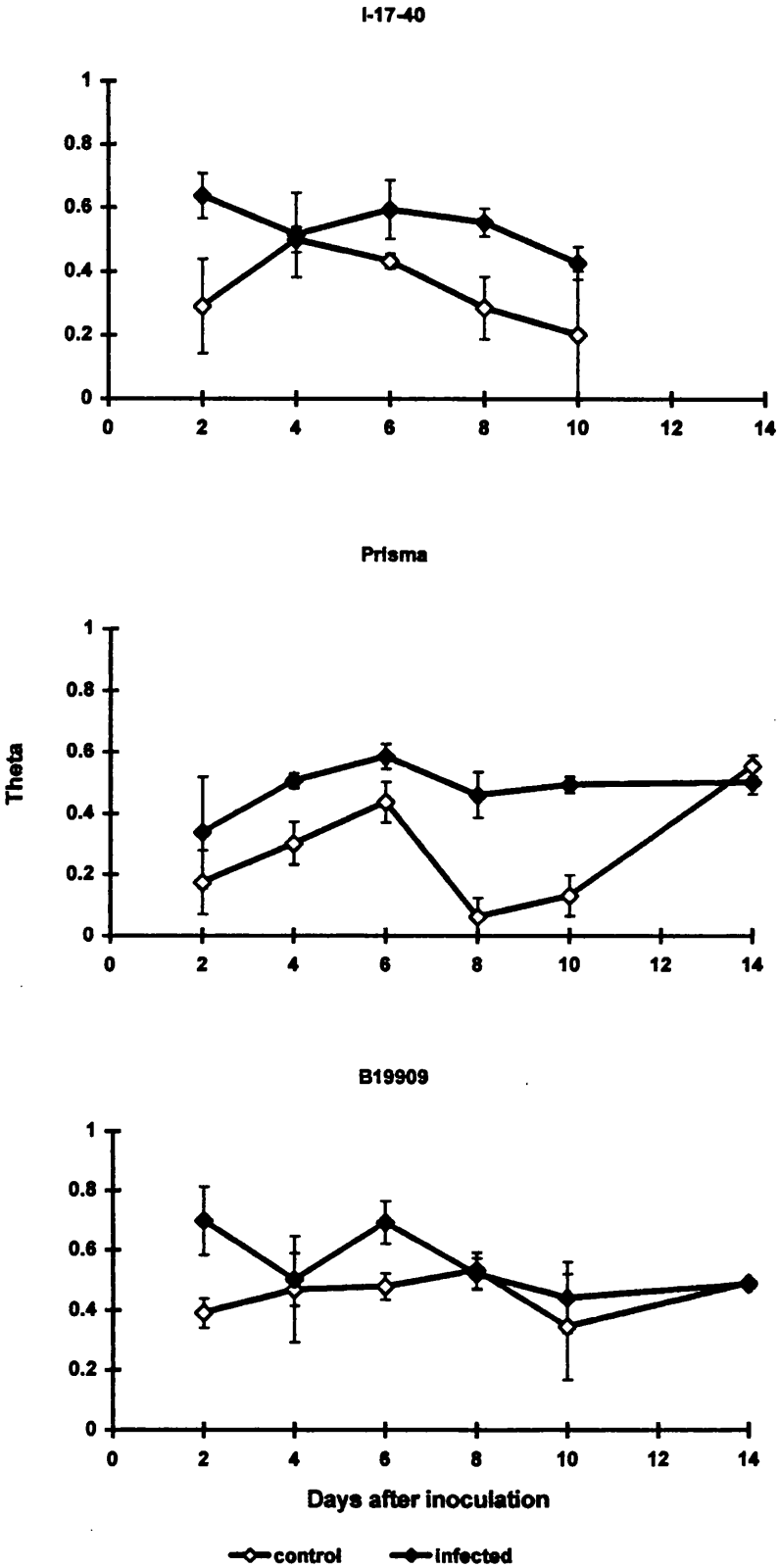


Fig. 50: Effects of infection on theta of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

6.2.3. Effects of infection on chlorophyll content

6.2.3.1. Total chlorophyll content

Fig. 51A shows that total chlorophyll levels in uninfected leaves increased during the early stages of the experiment in all three lines but decreased from about ten days onwards in both cv. Prisma and line B19909. Because of senescence in the infected leaves, total chlorophylls in the infected leaves of all three lines were reduced by infection with the reductions becoming significant ($p < 0.05$) slightly earlier in cv. Prisma than in the two wild lines, and the overall reductions were greater in cv. Prisma than in the wild lines. The overall effect of infection, at ten days after inoculation, was to reduce total chlorophyll levels to about 23% of the controls in infected leaves in line I-17-40, to about 30% in cv. Prisma and to about 37% in line B19909. Because of senescence no further measurements were made on line I-17-40, but fourteen days after inoculation, chlorophyll levels in infected leaves of both cv. Prisma and the wild line B19909 had fallen to 15% of the levels in uninfected leaves.

6.2.3.2. Chlorophylls a and b

Chlorophylls a and b increased slowly in the uninfected control leaves in all three lines during the course of the experiment. In contrast, levels reduced dramatically in infected leaves in all lines (Figs. 51B and 51C). An analysis of variance showed significant reductions in both chlorophylls a and b as infection progressed.

For chlorophyll a, the differences became significant from four to six days

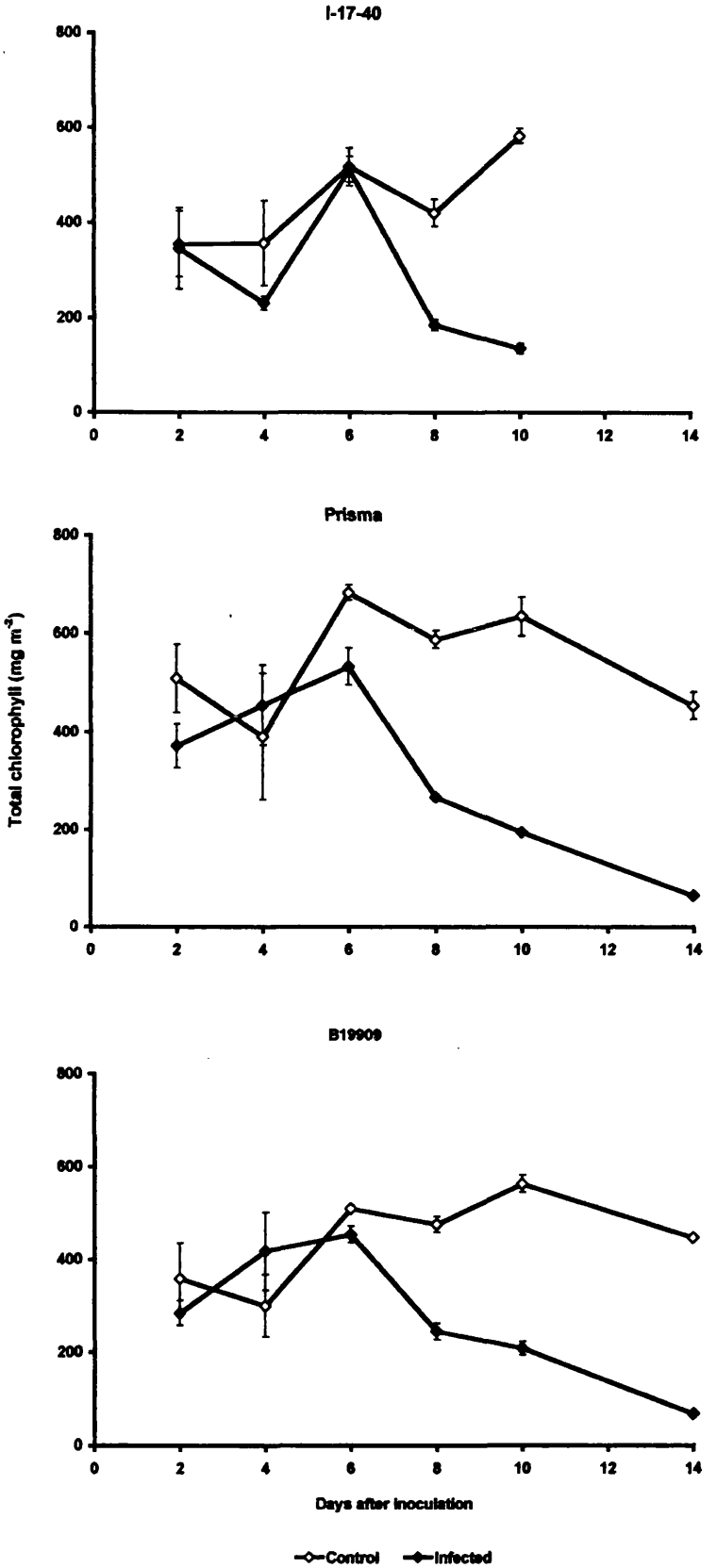


Fig. 51A: Effects of infection on total chlorophyll per unit area of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

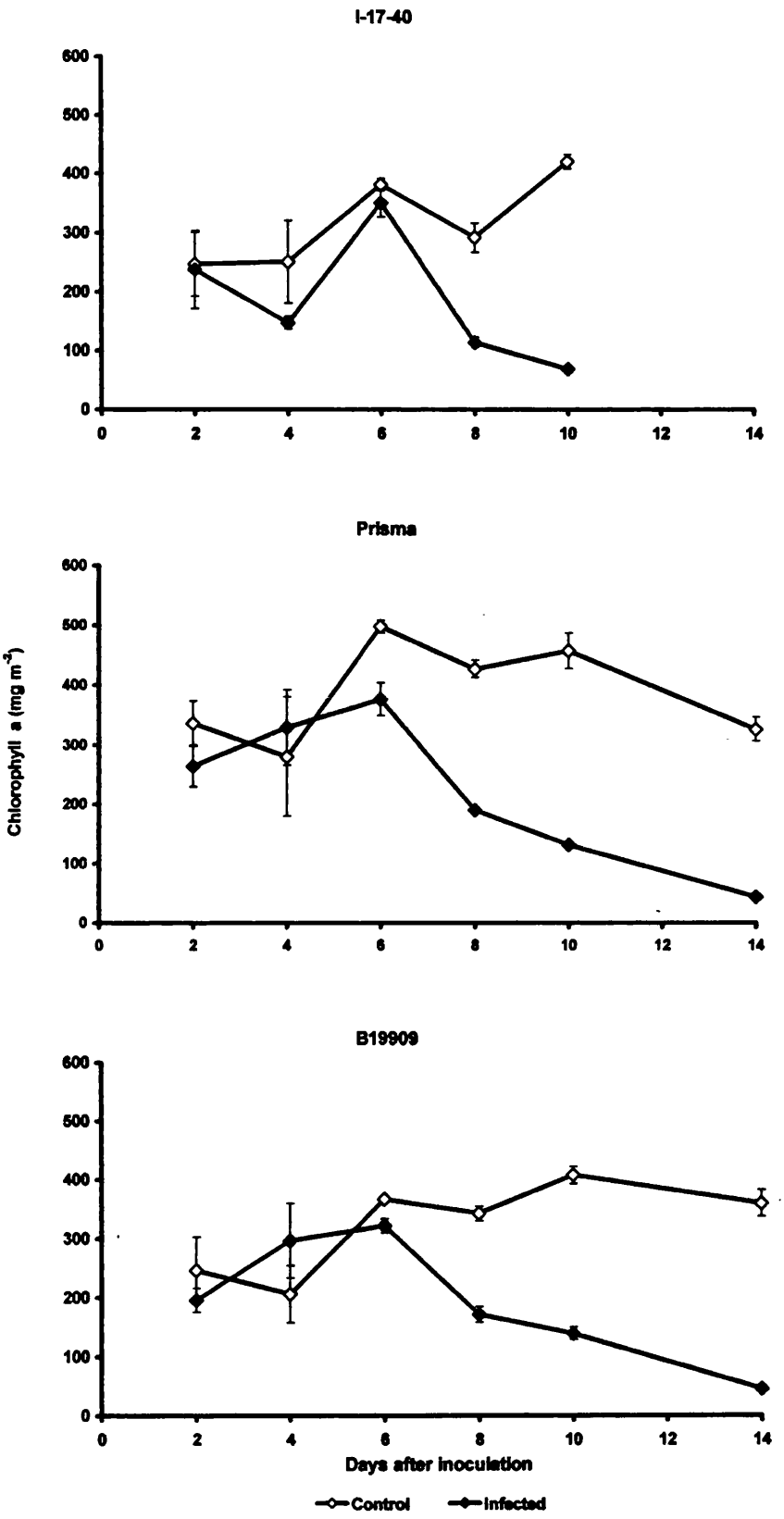


Fig. 51B: Effects of infection on chlorophyll a per leaf area of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

after inoculation in cv. Prisma and from six days after inoculation in the two wild lines (Fig. 51B). Ten days after inoculation, the percentage reductions of chlorophyll a in infected leaves compared to the uninfected leaves, were about 66% in line B19909, 71% in cv. Prisma and 84% in line I-17-40. By the fourteenth day after inoculation, about 87% of chlorophyll a was lost in infected leaves of both cv. Prisma and the wild line B19909.

For chlorophyll b (Fig. 51C), the differences between infected and uninfected leaves were not significant until about eight days after inoculation in all three lines ($p < 0.05$). By the tenth day after inoculation, infection had reduced levels by about 55% in line B19909, 60% in line I-17-40 and 65% in cv. Prisma. By the fourteenth day after inoculation, levels were down to 26% of the control levels in line B19909 and 16% in cv. Prisma.

6.2.3.3. Ratio of chlorophyll a : b

Infection consistently reduced the ratios of chlorophyll a : b in the leaves of all three lines but the reductions were only significant in line I-17-40 from the eighth day after inoculation(Fig. 51D).

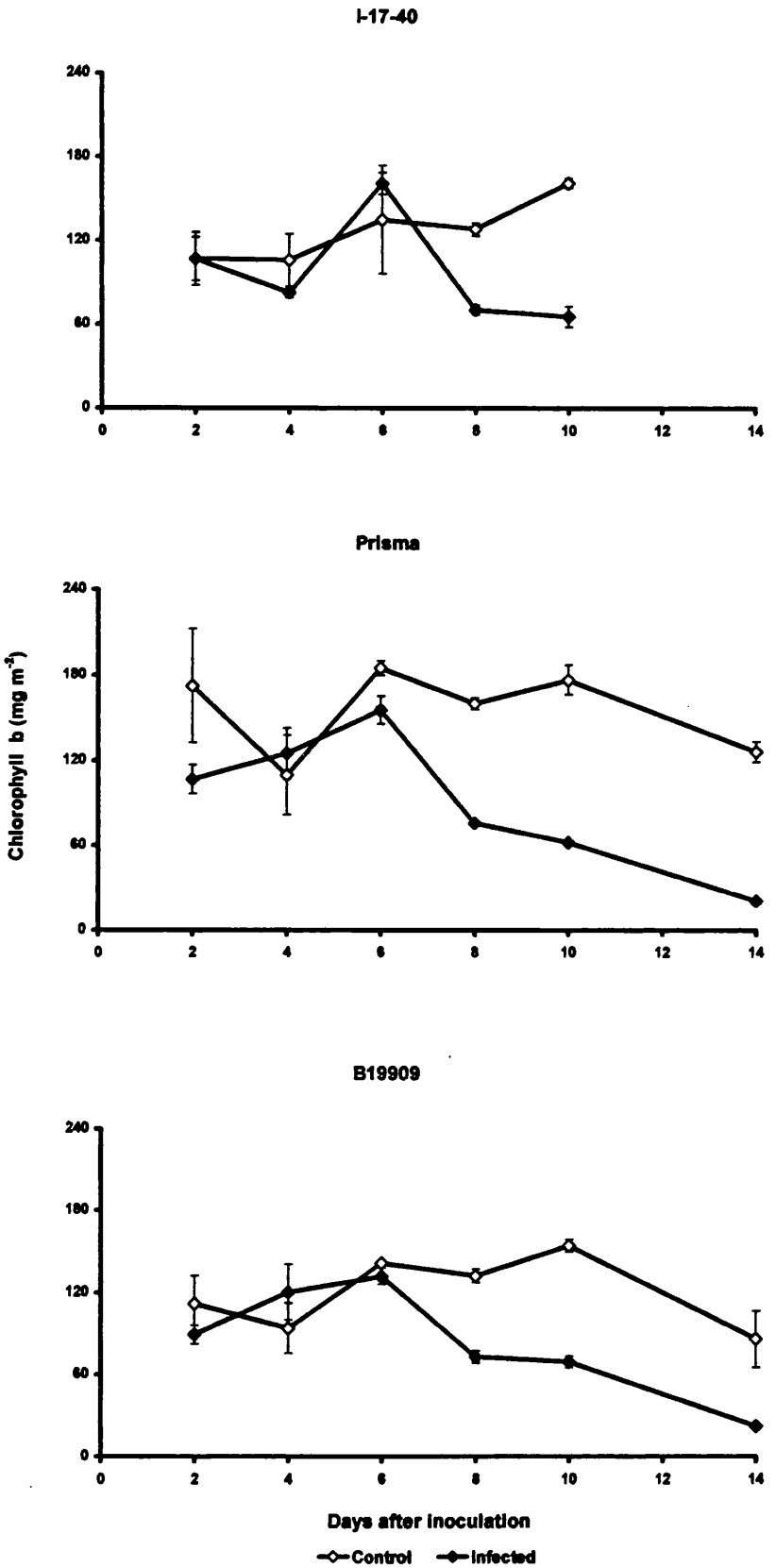


Fig. 51C: Effects of infection on chlorophyll b per leaf area of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

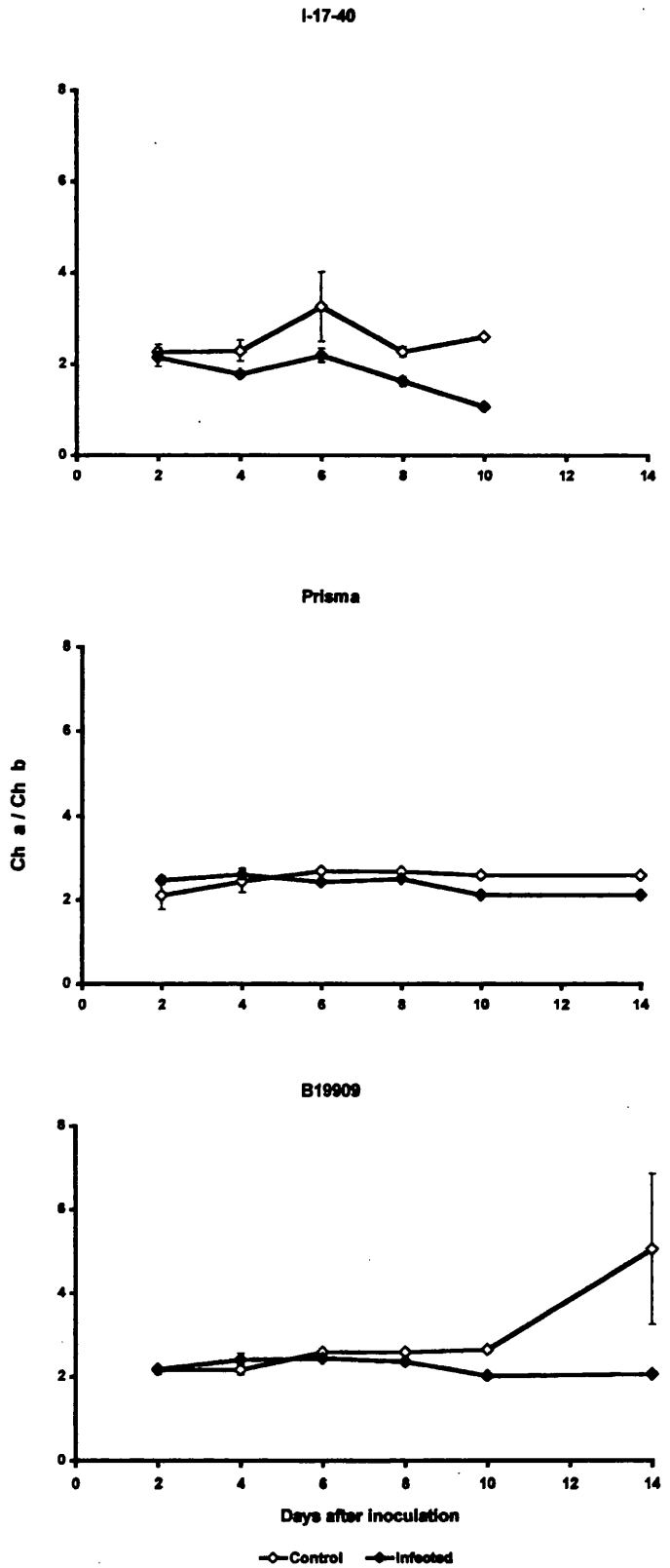


Fig. 51 D: Effects of infection on the ratio of chlorophylls a:b of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

6.2.4. Effects of infection on dark respiration (Rd)

The rates of **Rd** in the third leaf of infected and uninfected plants of the three lines are shown in Fig. 52.

Dark respiration in uninfected control leaves of the three lines remained relatively constant throughout the course of the experiment. However, **Rd** began to increase in infected leaves of the three lines from the fourth day after inoculation onwards. As infection progressed, the rates continued to increase reaching a maximum in cv. Prisma six days after inoculation and in the two wild lines six to eight days after inoculation, after which **Rd** decreased in all lines. An analysis of variance showed that the differences between infected and uninfected plants were significant in all lines with the level of significance being $p < 0.05$ in line I-17-40 and $p < 0.005$ in the other two lines. The percentage increase in **Rd** of infected compared to the uninfected leaves was about 70% in line I-17-40 and about 106% in line B19909 eight days after inoculation, and about 178% in cv. Prisma six days after inoculation.

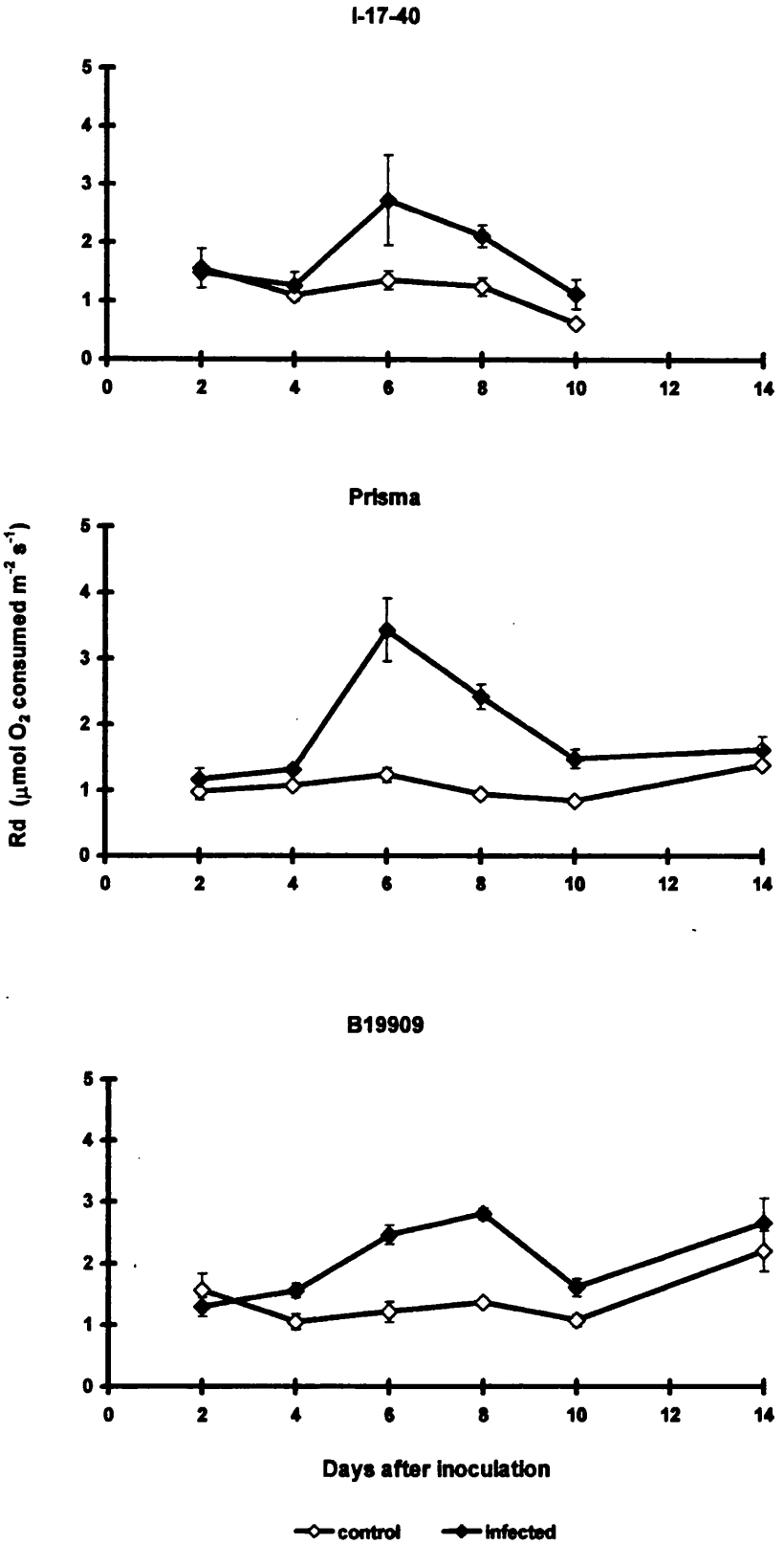


Fig. 52: Effects of infection on dark respiration per unit area of infected and uninfected third leaves of the three lines. Each datum point represents the mean of three replicates.

EXPERIMENT -II

6.2.6. Development of fungal biomass on the fourth leaf of cvs Golden Promise and Triumph and wild line B8893.

Conidial production per cm^2 leaf area was determined on the fourth leaf of each line, at each sampling time and the results are presented graphically in Fig. 53.

Conidial production was highest on cv. Golden Promise and lowest on line B8893 with intermediate numbers being produced on cv. Triumph. The differences between the lines were statistically significant ($p < 0.05$) from the eighth day after inoculation onwards. Line B8893, reacted to the inoculum, by producing small necrotic lesions, although much less necrosis than cv. Triumph and very little mildew mycelium developed. Mildew developed freely over the leaf surface of cv. Golden Promise, but cv. Triumph reacted to the inoculum with a high level of necrosis, and the amount of mildew mycelium developed was lower.

6.2.7. Effects of infection on photosynthesis

6.2.7.1. Maximum rates of gross photosynthesis (Pg_{max})

Rates of Pg_{max} were higher in uninfected leaves of cv. Golden Promise and line B8893 than in cv. Triumph and showed little decrease throughout the experiment (Fig. 54A).

From about six days after inoculation, Pg_{max} began to reduce in cvs

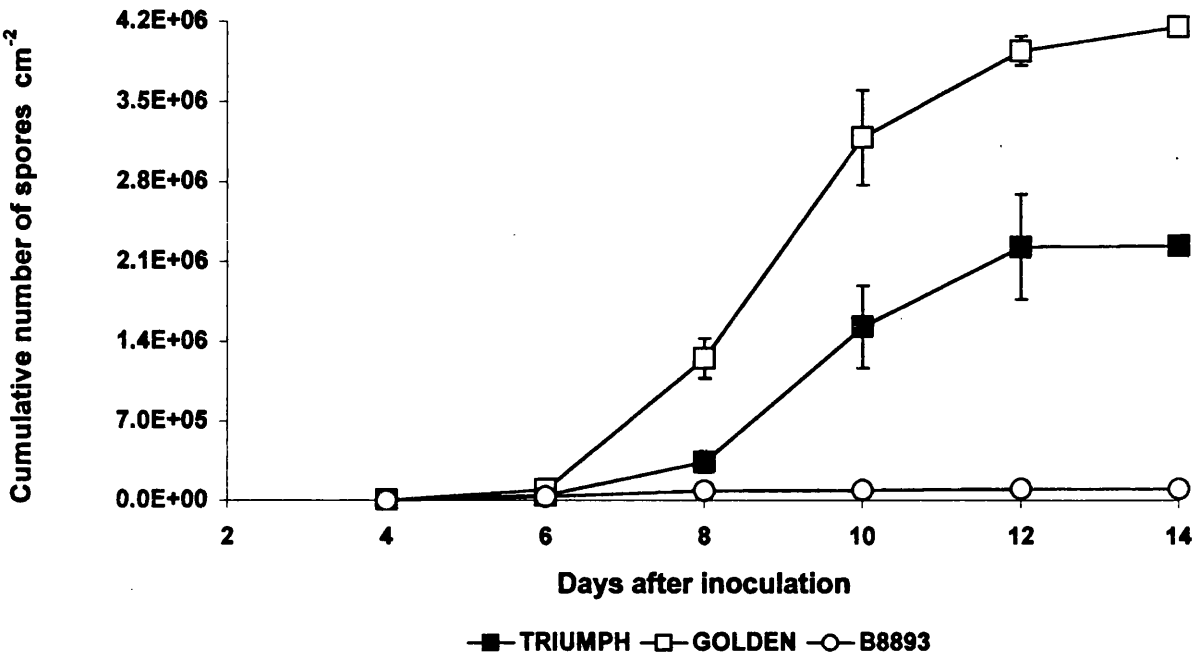


Fig. 53: The cumulative number of spores produced on the fourth leaf of three barley lines differing in their susceptibility to mildew infection.

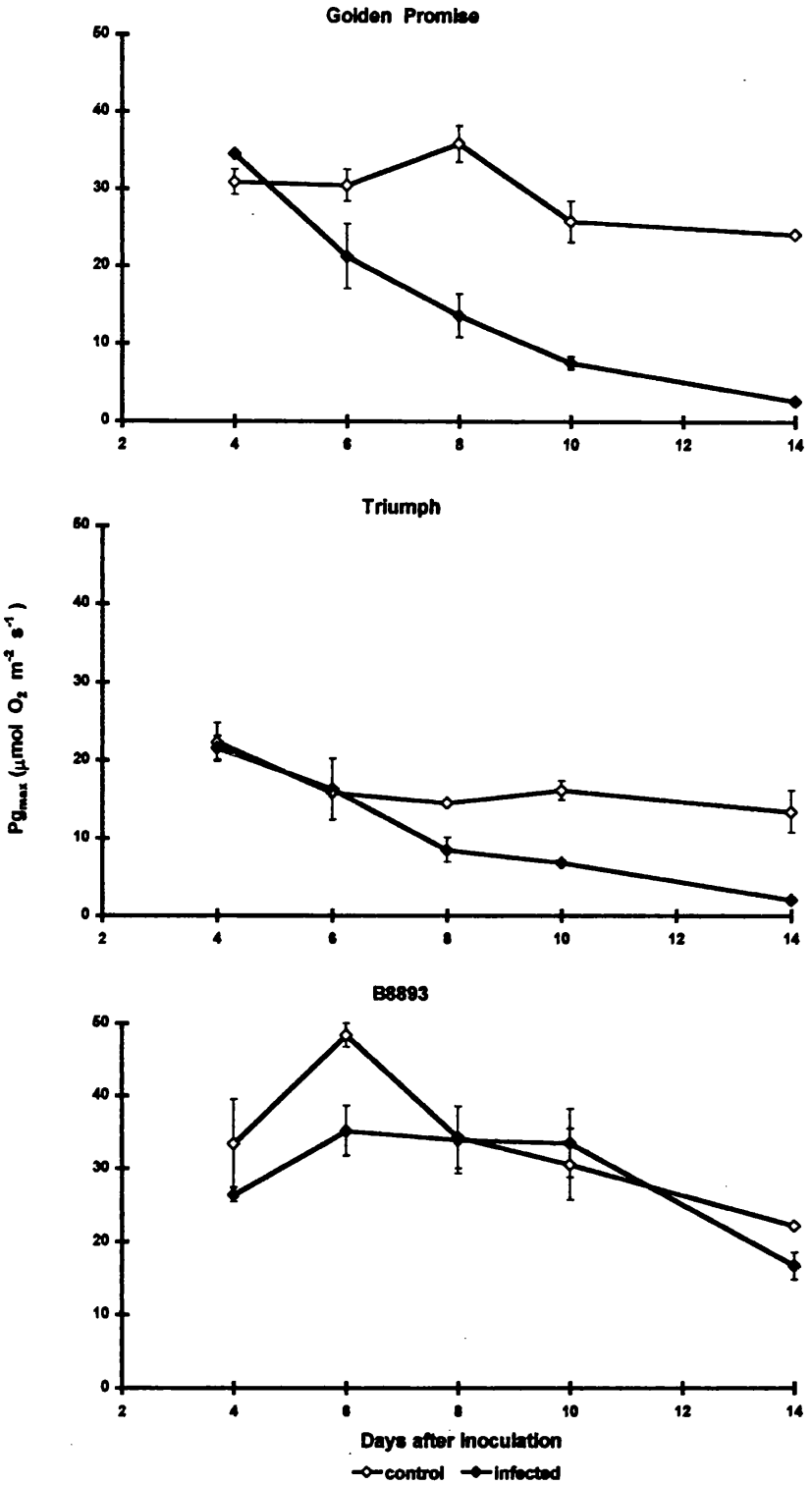


Fig. 64A. The effects of mildew infection on the rates of maximum gross photosynthesis (Pg_{max}) per unit area in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

Golden Promise and Triumph compared to the controls. As infection progressed, this reduction became greater falling to the same level in both cultivars. An analysis of variance showed that the differences in Pg_{max} between infected and uninfected leaves became significant ($p < 0.05$) in both lines from around eight days after inoculation. By the end of the experiment (14 days after inoculation), it was reduced by about 89% in cv. Golden Promise and by 85% in cv. Triumph. An analysis of variance showed significant differences ($p < 0.01$) between the degrees of reductions caused by infection in the two cultivated lines.

In contrast, infection did not alter P_{gmax} significantly in line B8893 except perhaps at six days after inoculation. However, this difference was probably due to random variation.

Pg_{max} per unit of chlorophyll (Fig. 54B) appeared to increase in infected leaves of both cv. Golden Promise and line B8893, but the increase was only significant ($p < 0.01$) in B8893. By the last harvest, Pg_{max} per unit of chlorophyll had decreased significantly ($p < 0.02$) in infected leaves of cv. Golden Promise. In cv. Triumph, Pg_{max} per unit of chlorophyll fluctuated between harvests but no significant changes occurred at any stage in response to infection.

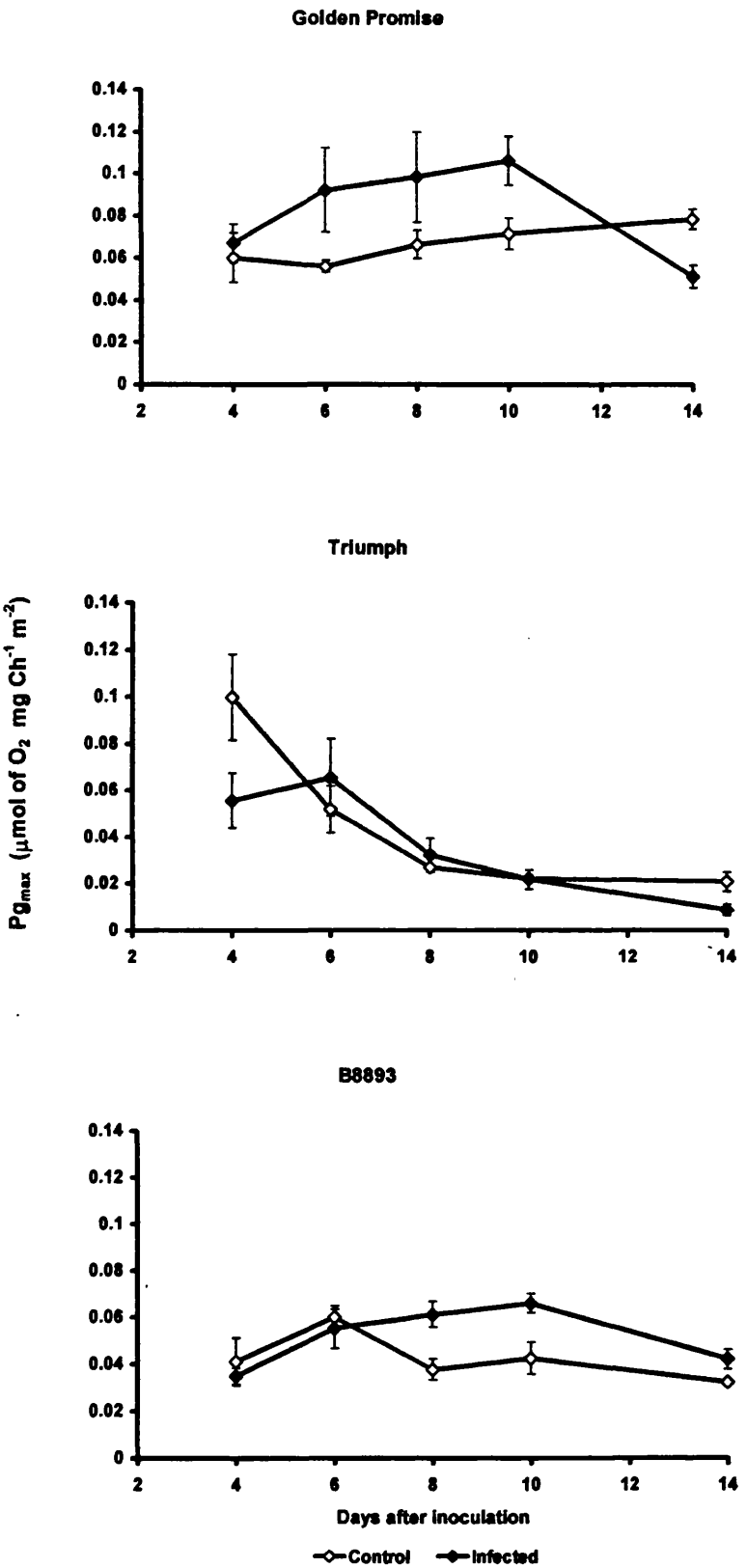


Fig. 54B. The effects of mildew infection on Pg_{max} per milligram of chlorophyll in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and the wild line B8893 (very resistant).

6.2.7.2. Maximum rates of net photosynthesis (Pn_{max})

Fig. 55A shows that infection significantly ($p < 0.03$) reduced Pn_{max} in infected leaves of both cv. Golden Promise and cv. Triumph from the eighth day after inoculation. Pn_{max} in infected leaves of the wild line B8893 fluctuated between sampling times but revealed no significant differences between infected and uninfected leaves.

Pn_{max} when expressed per milligram of chlorophyll (Fig. 55B) followed the same pattern as Pg_{max} per milligram of chlorophyll (Fig. 54B).

6.2.7.3. Relationship between Pg_{max} and fungal biomass

The regression lines are plotted in Fig. 55C and the regression parameters are presented in Table 7.

The reduction in the rate of maximum gross photosynthesis during infection in each of the two cultivars was found to be linearly correlated with fungal biomass, measured as conidial production, but not in the wild line. This correlation was higher in cv. Golden Promise than in cv. Triumph, probably due the fact that factors other than fungal development, such as tissue necrosis, were affecting photosynthesis in leaves of cv. Triumph.

The slope of the regression line of cv. Golden Promise was significantly different from that of cv. Triumph ($p < 0.05$) due to the high correlation between Pg_{max} and fungal production in the former.

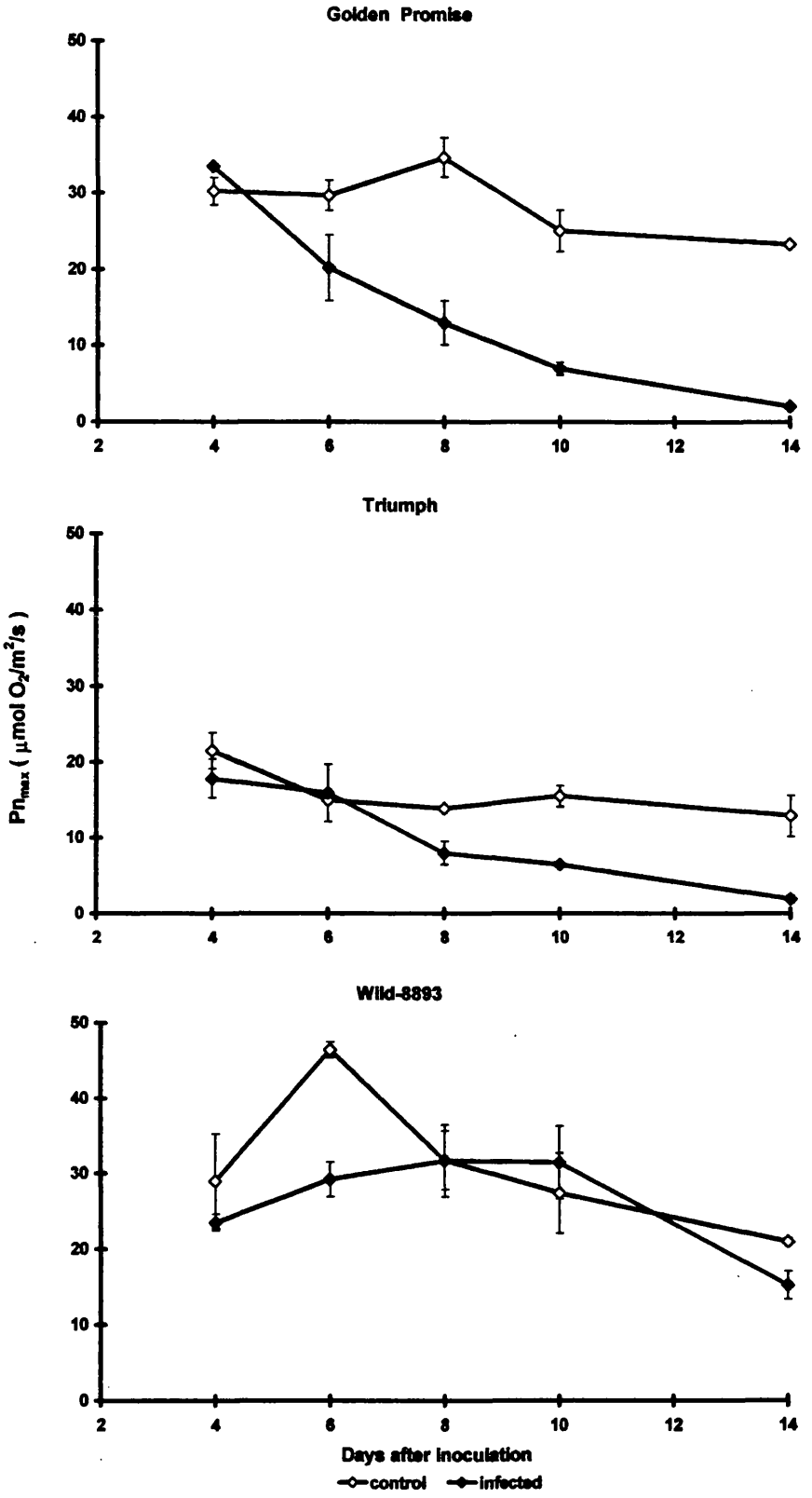


Fig. 55A: The effects of mildew infection on the rates of maximum net photosynthesis (Pg_{max}) per unit area in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

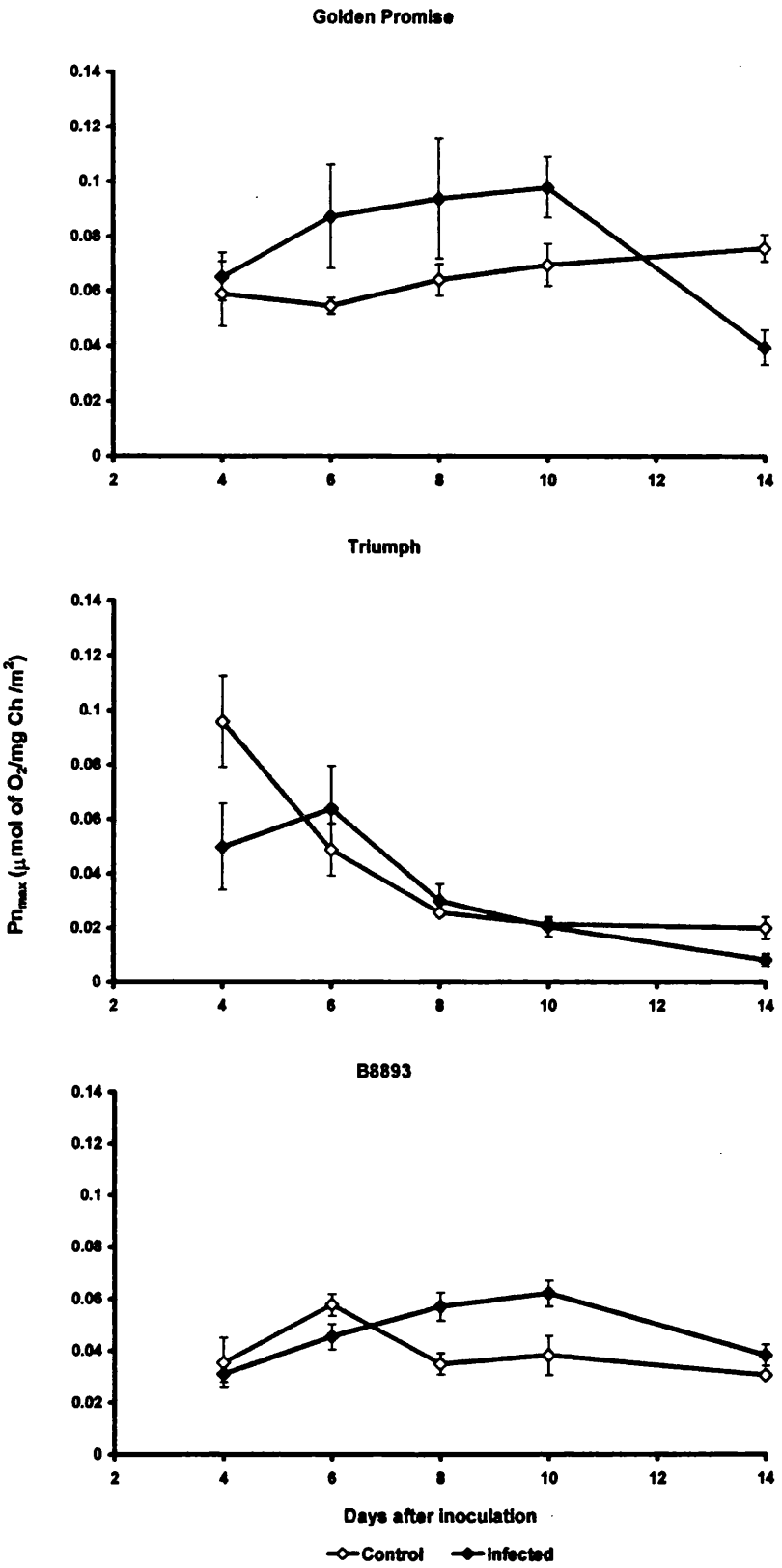


Fig. 55B. The effects of mildew infection on Pn_{max} per milligram of chlorophyll in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and the wild line B8893 (very resistant).

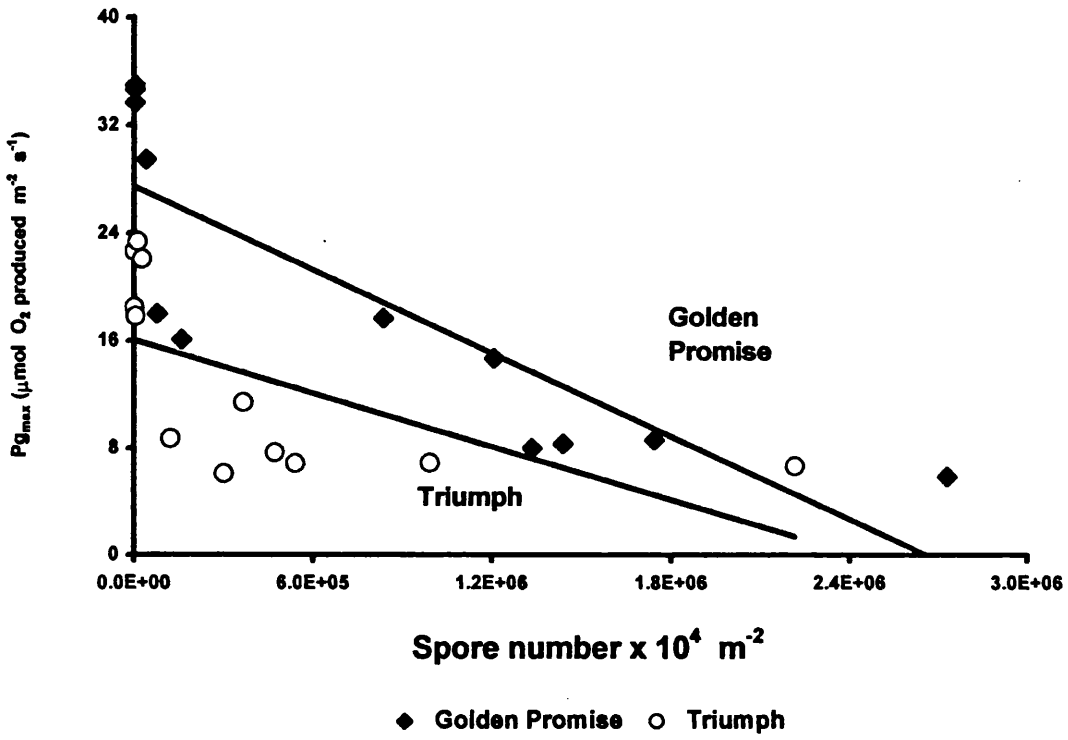


Fig. 55C : Relationship between gross photosynthesis and conidial production on the infected fourth leaf of the two lines.

Table 7.

Regression analyses of maximum gross photosynthesis (Pg_{max}) and fungal spore production in cvs. Golden Promise and Triumph and the wild line B8893.

Regression Parameters	**Golden Promise**	**Triumph**	**B8893**
Intercept (a)	27.455	31.043	27.655
Slope (b)	-1×10^{-5}	-3×10^{-5}	-2×10^{-5}
Coefficient of Correlation (R^2)	0.6934	0.3607	0.1125
Degrees of Freedom	11	11	11
F ratio	22.61	5.64	1.27
p	< 0.001	< 0.05	NS

NS : not significant

6.2.7.4. Quantum efficiency of photosynthesis (α)

Infection had no significant effect on α in either cv. Triumph or line B8893, the two lines which reacted necrotically to infection (Fig. 56). However, cv. Triumph showed a significant ($p < 0.05$) decrease fourteen days after inoculation when infected leaves showed large areas of necrosis. In cv. Golden Promise, α decreased significantly ($p < 0.001$) from the sixth day after inoculation. Thus quantum efficiency was most affected in cv. Golden Promise.

6.2.7.5. The ratio of physical to total resistance to CO_2 diffusion (θ)

Infection increased θ in cv. Triumph from the sixth day after inoculation and in cv. Golden Promise from the eighth day after inoculation (Fig. 57). An analysis of variance showed that the increases in θ in infected leaves were significant ($p < 0.005$) despite the high level of variation between replicates in cv. Triumph. In the highly resistant line B8893, θ was generally reduced by attempted infection, but none of the differences between inoculated and uninoculated plants was significant.

6.2.8. Effects of infection on chlorophyll content

6.2.8.1. Total chlorophyll content

Fig. 58A shows that infection significantly reduced total chlorophyll content from six days after inoculation in cv. Golden Promise ($p < 0.001$) and

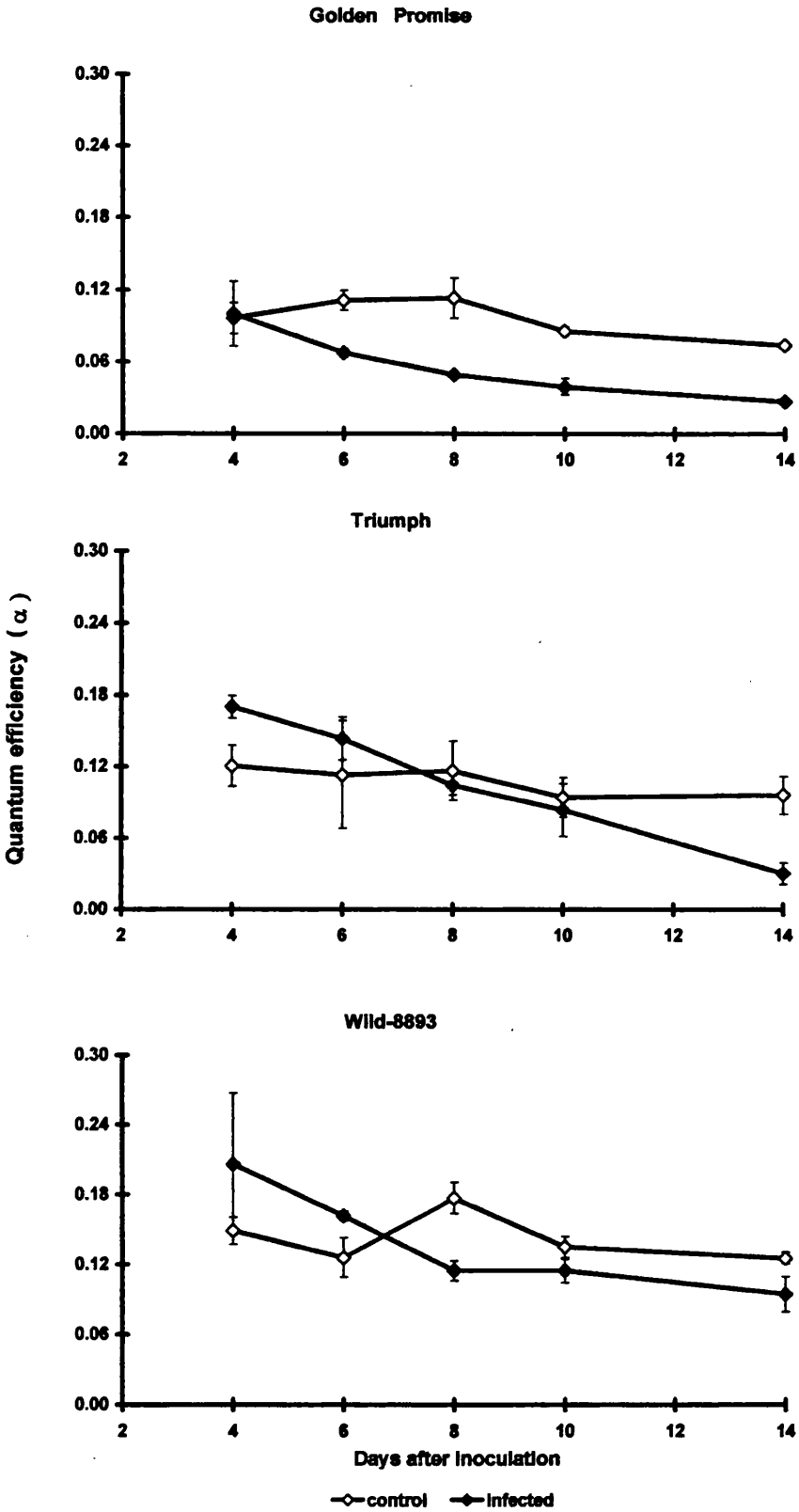


Fig. 58: The effects of mildew infection on the quantum efficiency in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

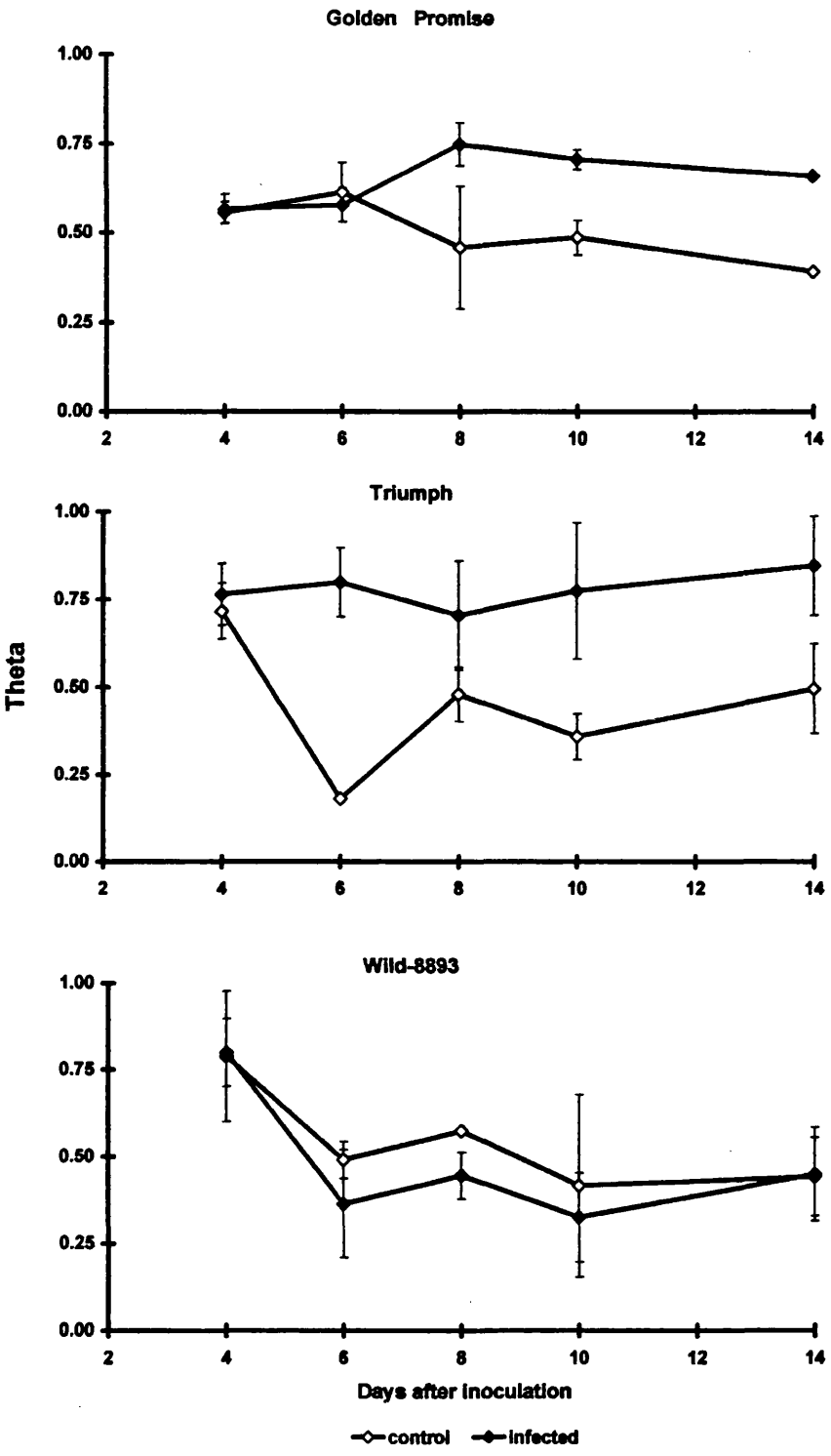


Fig. 57: The effects of mildew infection on theta in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

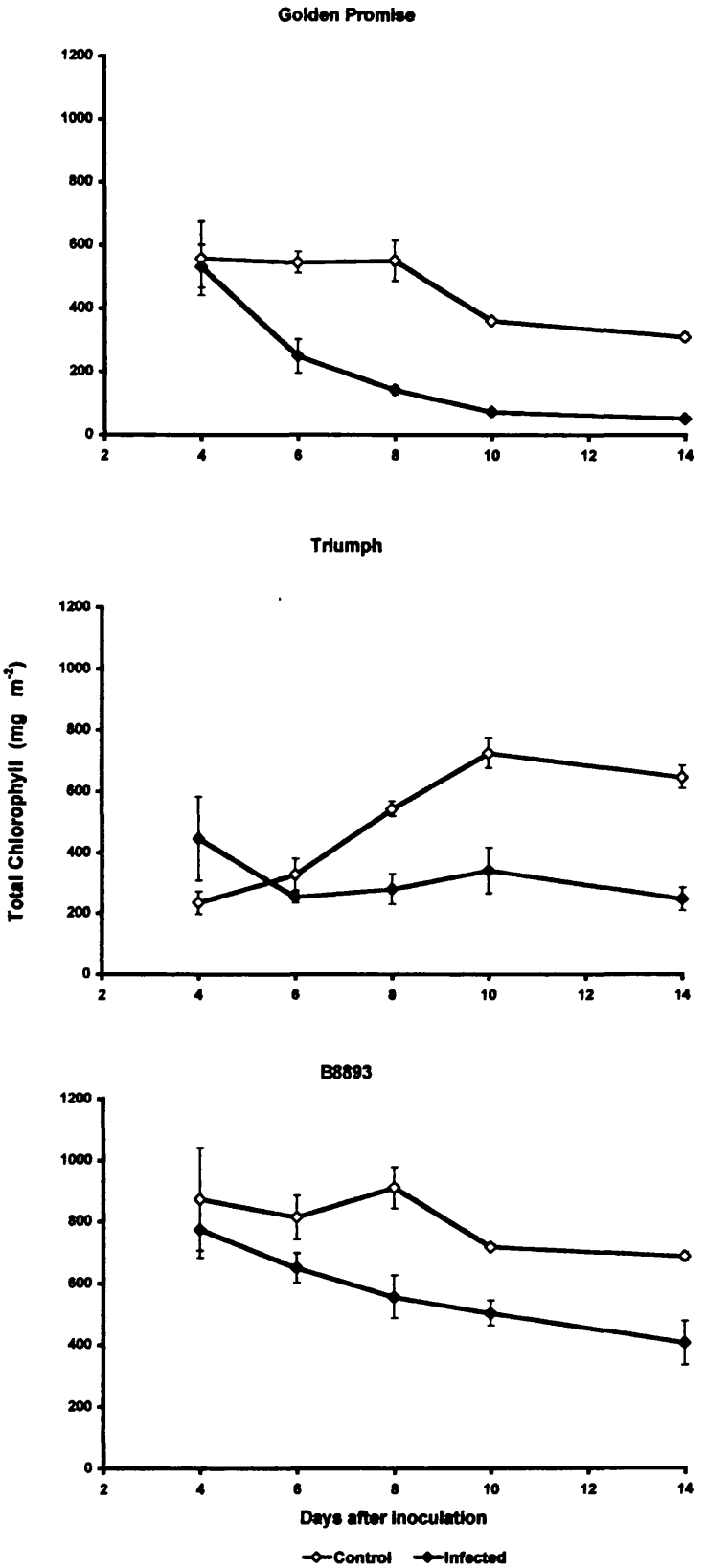


Fig. 58A: The effects of mildew infection on total chlorophyll in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

from eight days after inoculation in line B8893 and cv. Triumph ($p < 0.01$). The overall effect of infection, at fourteen days after inoculation, was to reduce total chlorophyll content of the infected leaves of wild line B8893 by about 41%, of cv. Triumph by about 62% and of cv. Golden Promise by about 83%.

6.2.8.2. Chlorophylls a and b

Fig. 58B and Fig. 58C show that both chlorophylls a and b were reduced in infected leaf tissues of all three lines and an analysis of variance showed that the reductions were significant.

For chlorophyll a (Fig. 58B), the differences became significant from four days after inoculation in cv. Golden Promise ($p < 0.001$) and from six days after inoculation in cv. Triumph and the wild line B8893 ($p < 0.001$). By the end of the experiment, fourteen days after inoculation, the percentage reductions in chlorophyll a were around 44% in line B8893, 67% in cv. Triumph and 85% in cv. Golden Promise.

For chlorophyll b (Fig. 58C), the reductions also became significant from four days after inoculation in cv. Golden Promise ($p < 0.005$) and from eight days after inoculation in cv. Triumph ($p < 0.03$) and the wild line B8893 ($p < 0.01$). By the fourteenth day after inoculation, infection had reduced chlorophyll b by about 33% in line B8893, 51% in cv. Triumph and 79% in cv. Golden Promise.

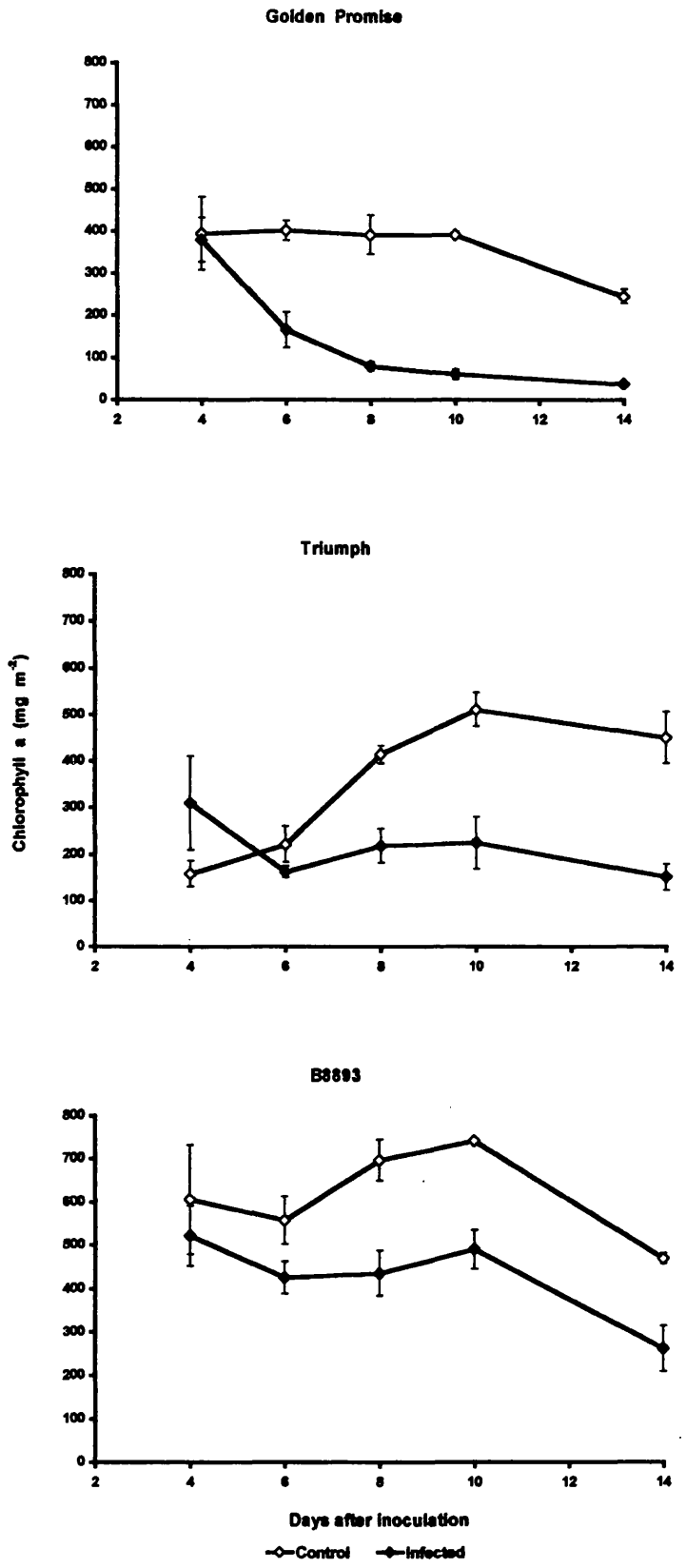


Fig. 58B: The effects of mildew infection on chlorophyll a in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

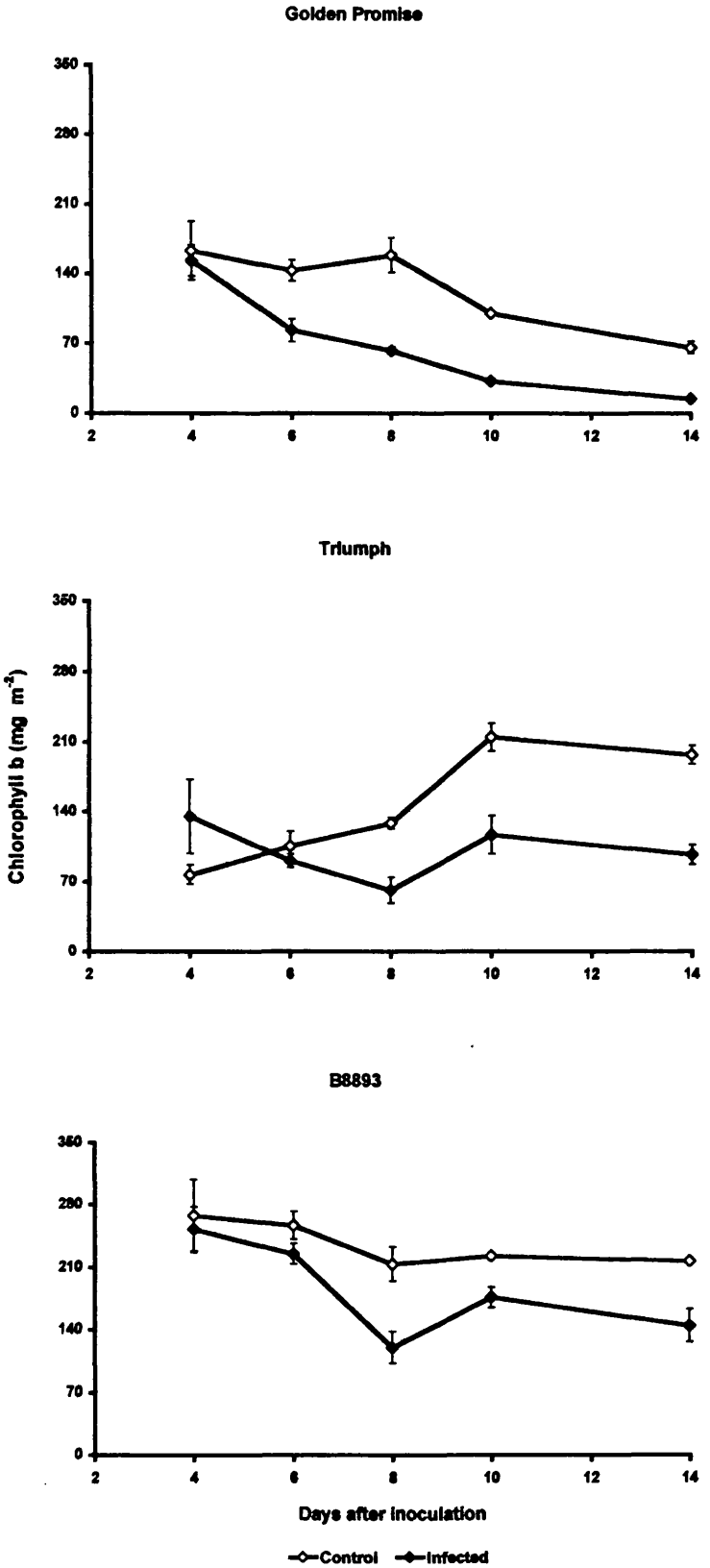


Fig. 58C: The effects of mildew infection on chlorophyll b in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

6.2.8.3. Ratio of chlorophyll a : b

Infection significantly reduced the chlorophyll a:b ratio in infected leaves of cv. Golden Promise from four days after inoculation (Fig. 58D), but the reductions in the other two lines were not significant until around ten days after inoculation ($p < 0.005$).

6.2.9. Effects of infection on dark Respiration (Rd)

Infection increased **Rd** slightly but significantly ($p < 0.05$) in cv. Golden Promise, and this increase was maintained during the whole period of measurements (Fig. 59). In infected leaves of cv. Triumph, **Rd** fluctuated between harvests, with a slight transient increase between the sixth and the tenth day after inoculation, but the increase was only significant ($p < 0.05$) at the sixth day after inoculation. In line B8893, no significant differences were found.

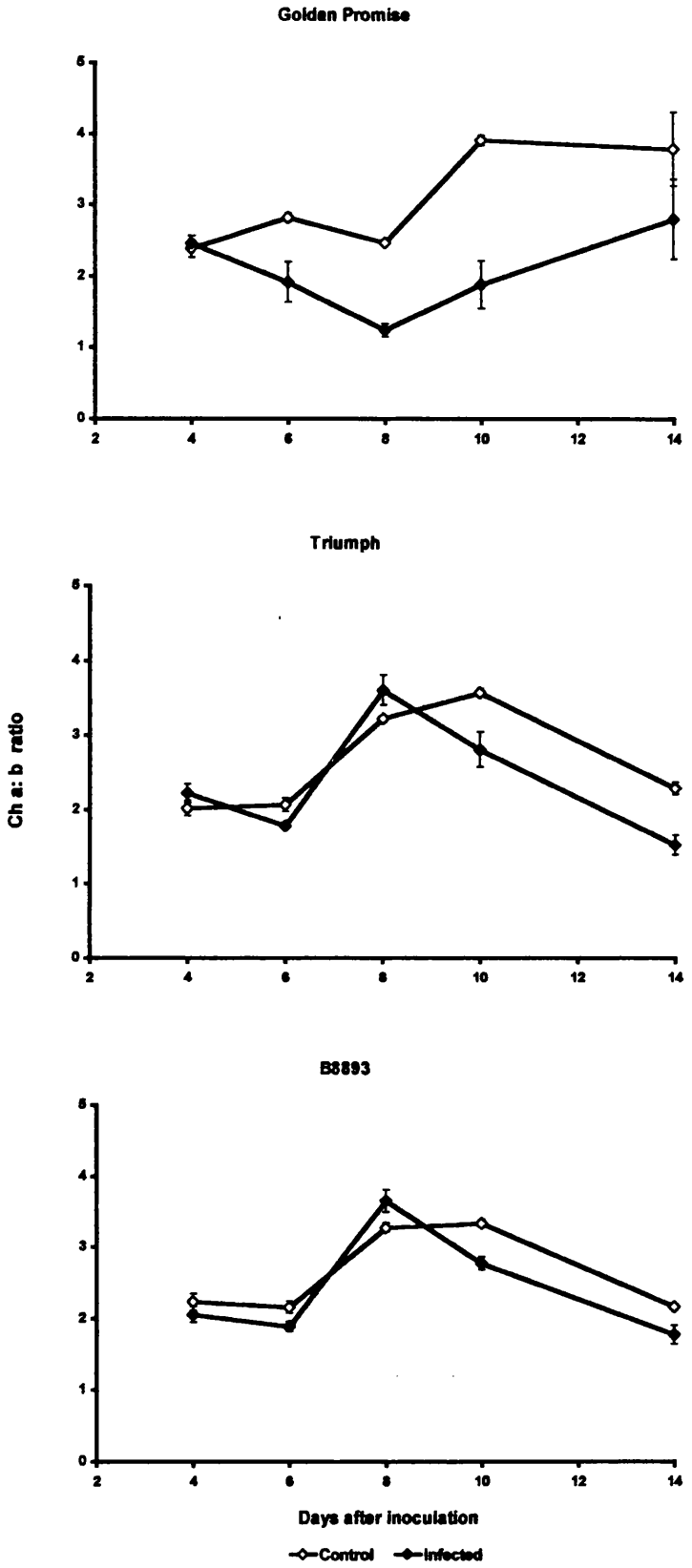


Fig. 58 D: The effects of mildew infection on the ratio of chlorophylls a and b in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

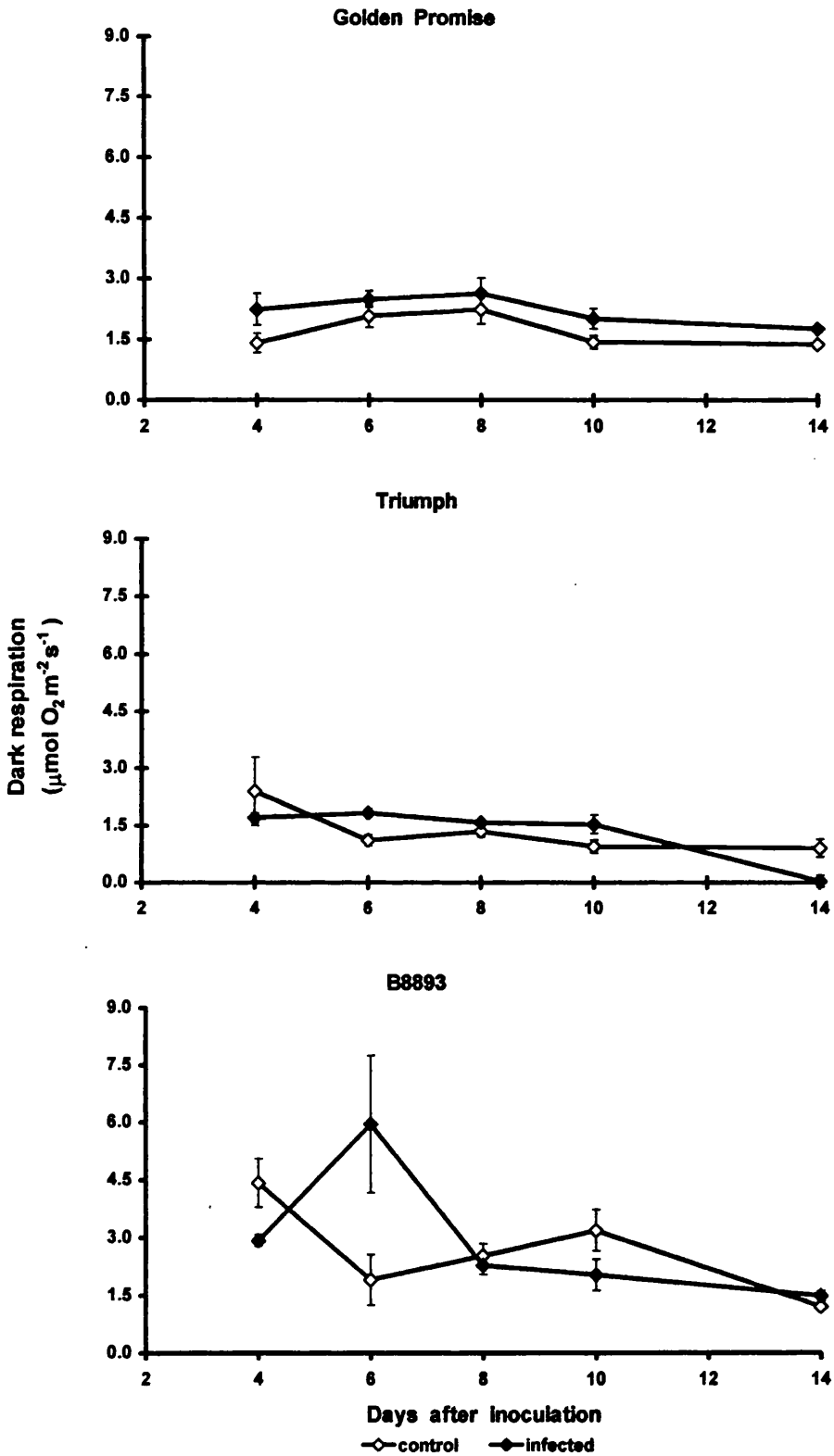


Fig. 59. The effects of mildew infection on dark respiration per unit area in the fourth leaf of 3 barley lines: Cv. Golden Promise (very susceptible), cv. Triumph (moderately resistant) and wild line B8893 (very resistant).

Chapter 7

THE EFFECTS OF INFECTION ON STOMATAL RESISTANCE IN THREE BARLEY LINES, CV. PRISMA AND WILD LINES B19909 AND I-17-40.

7.1. Introduction

The object of these experiments was to examine how stomatal resistance changed in infected leaves compared to uninfected leaves in both light and dark, and to determine how much changes in stomatal behaviour following infection could be involved in the reductions in photosynthesis reported in Chapter 6 and consequently in reduced dry matter production.

The effects of infection on stomatal resistance were determined in the two wild barley lines I-17-40 and B19909, and in the cultivated barley cv. Prisma. The experiment was carried out twice, with similar results being obtained on each occasion. Only the results of the second experiment are presented.

7.2. Results

Stomatal resistance was measured in the middle and tip regions of infected and uninfected leaves of the three lines. The results are given in Appendix Tables 11A, 11B and 15, and plotted graphically in Figs 60A and 60B.

7.2.1. Ontogenetic changes in stomatal function in the uninfected third leaf

7.2.1.1. Changes in the light

Stomatal resistance in the light in both the middle and tip regions of uninfected leaves remained relatively constant throughout the period of measurement, in all three lines (Figs. 60A and 60B).

7.2.1.2. Changes in the dark

In contrast, in the dark, marked changes occurred as the leaves aged in line I-17-40 and cv. Prisma at both the middle and the tip regions (Figs. 60A and 60B). For cv. Prisma and line I-17-40, at the beginning of the experiment, the stomatal resistances increased reaching a maximum two days later, but from then on, resistances declined and then an approximately steady state was reached and maintained to the end of the experiment.

In line B19909, stomatal resistance increased rapidly in the middle region from the second to the seventh day after inoculation and then stayed at a relatively constant state. In contrast to the other two lines, stomatal resistance in the tips of the leaves of line B19909 remained at an almost constant level during the whole period of the experiment.

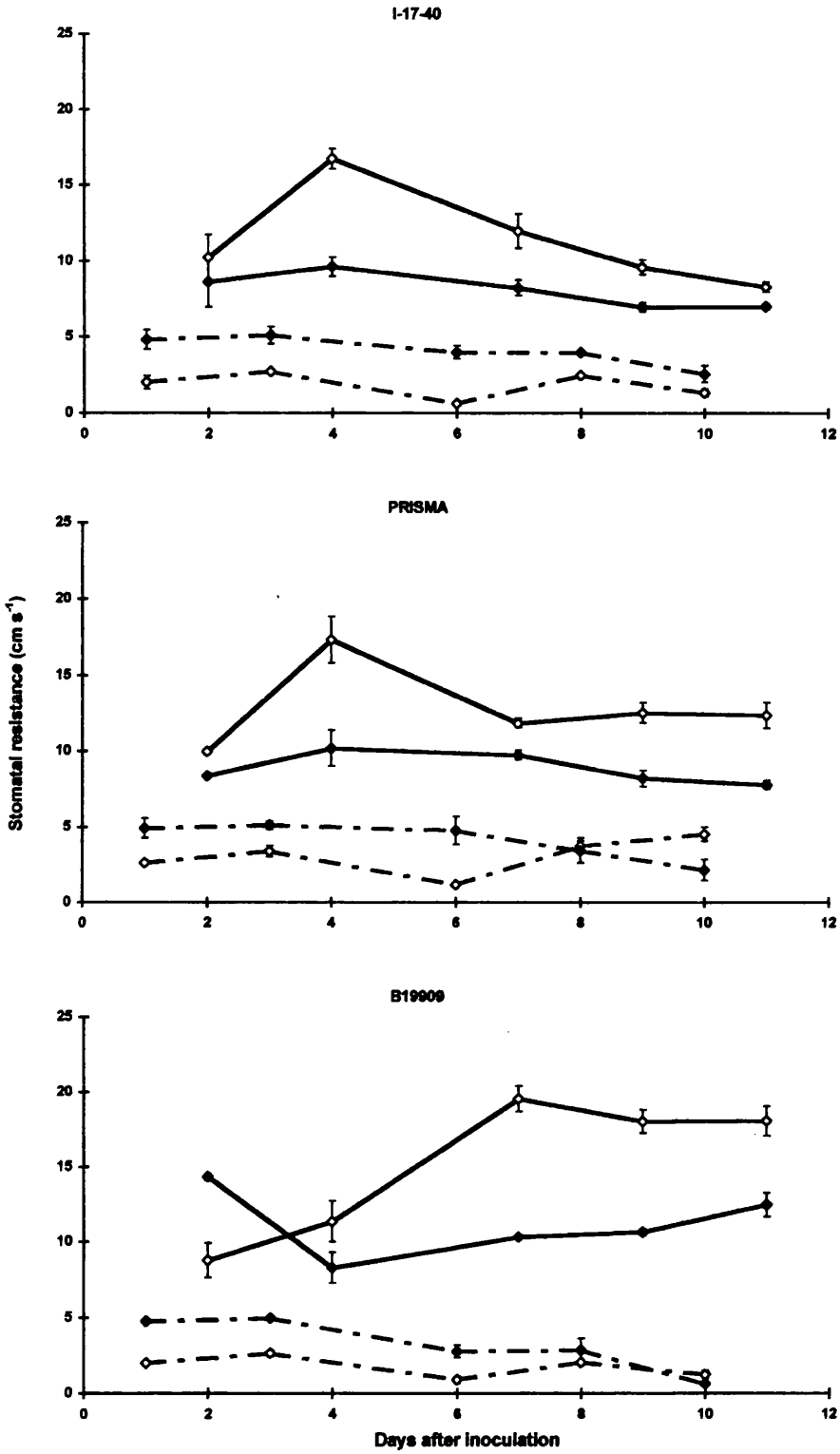


Fig. 60A: Stomatal resistance in the third leaves (middle region) of the three barley lines in light (·····) or in darkness (——) following inoculation with *B. graminis* f.sp *hordei*. Each datum is the mean of four replicates, with standard error. (◇) uninfected, (♦) infected.

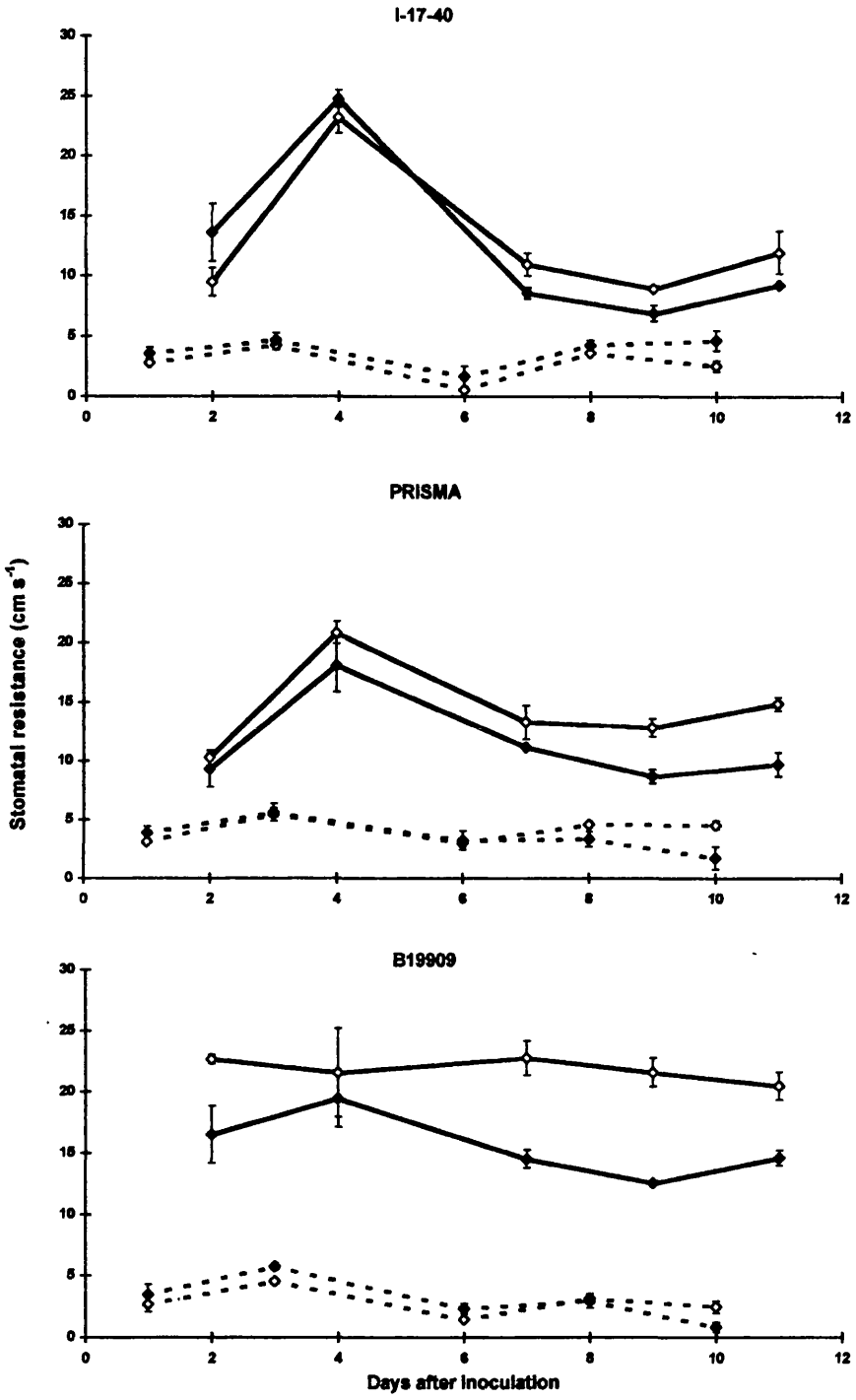


Fig. 60B: Stomatal resistance in the third leaves (tip region) of the three barley lines in light (·····) or in darkness (———) following inoculation with *B. graminis* f. sp. *hordei*. Each datum is the mean of four replicates, with standard error.

(◊) uninfected, (♦) infected.

7.2.2. Effects of infection on stomatal function in the light

7.2.2.1. Stomatal resistance in the middle, inoculated region, of the leaf

Within 24 hours of inoculation, stomatal resistance had significantly ($p < 0.01$) increased in infected leaves when compared to uninfected leaves in all three lines, but then as infection progressed it began to decrease. By the eighth day after inoculation it was similar to that of uninfected leaves in both cv. Prisma and line B19909, but in line I-17-40, it was still slightly higher ten days after inoculation. From the eighth day, the stomatal resistance of cv. Prisma continued to decrease to levels significantly ($p < 0.05$) below uninfected leaves. In contrast, stomatal resistance in line B19909 remained at control levels.

7.2.2.2. Stomatal resistance at the leaf tip

Infection in the centre of the leaf increased stomatal resistance in the uninfected tip of that leaf in all three lines (Fig. 60B) although the increase did not become significant, until around 10 days after inoculation ($p < 0.05$).

7.2.3. Effects of infection on stomatal function in the dark

7.2.3.1. Stomatal resistance in the middle inoculated region of the leaf

The high stomatal resistances of uninfected leaves in the dark indicate that the stomata were probably closed (Fig. 60A). Infection decreased the stomatal resistance of infected leaves significantly ($p < 0.02$) in both the wild line I-17-40 and cv. Prisma. As infection progressed the differences became smaller but still remained significant. In contrast, by two days after inoculation, stomatal

resistance in leaves of line B19909 had increased significantly above control levels ($p < 0.001$), but as infection progressed, it decreased again and followed the same pattern as in the other two lines.

The results suggest that infection by powdery mildew prevented the stomata from closing in the dark as fully as those in uninfected leaves.

7.2.3.2. Stomatal resistance at the leaf tip

Infection had little effect on stomatal resistance in the uninfected tip regions of inoculated leaves of the wild line I-17-40. In contrast, infection decreased stomatal resistance, in uninfected tips of infected leaves, both of cv. Prisma and line B19909. These decreases became significant ($p < 0.05$) between seven and nine days after inoculation.

Chapter 8

COMPENSATORY PHOTOSYNTHESIS AND DARK RESPIRATION IN THE THIRD LEAF OF CV. PRISMA AND THE WILD LINES B19909 AND I-17-40

8.1. Introduction

Livne (1964) reported that photosynthesis was stimulated in uninfected leaves of heavily infected rusted bean plants. Similarly, photosynthesis in the upper, uninfected leaves of powdery mildew infected barley and pea plants, was also reported to be stimulated significantly (Ayres, 1981b; Williams & Ayres, 1981; Walters & Ayres, 1983). Recent work on wild and cultivated oat lines, showed that the rate of photosynthesis in adjacent uninfected parts of infected leaves was reduced by powdery mildew infection but not to the same extent as photosynthesis in the infected tissues (Sabri, 1993).

These increases, referred to as compensatory photosynthesis, are possible mechanisms whereby the plant can compensate for photosynthates lost to the parasites in infected tissues. Compensatory photosynthesis could thus be a component of tolerance.

In the present investigation, photosynthesis and dark respiration were measured in the uninfected fourth leaves on plants whose lower three leaves had been inoculated and in plants in which the lower leaves were not inoculated of wild barley lines I-17-40 and B19909 and cv. Prisma. A summary of the results is presented in Appendix Table 16.

8.2. RESULTS

8.2.1. Effects of infection on photosynthesis

8.2.1.1. Maximum gross and net photosynthesis

Maximum rates of gross (Pg_{max}) and net (Pn_{max}) photosynthesis per unit leaf area or per milligram of chlorophyll, in the uninfected fourth leaf of inoculated and uninoculated plants are plotted in Figs. 61A-C. There were no significant differences in Pg_{max} per unit leaf area (Fig. 61A) between infected and uninfected plants up to eight days after inoculation. By the eleventh day after inoculation, the rates of maximum gross photosynthesis were higher in the uninfected fourth leaves of infected than in the same leaf on uninfected plants of both cv. Prisma and line B19909, but the difference was only significant ($p < 0.05$) in line B19909. In contrast, infection of the lower three leaves did not affect photosynthesis in the uninfected fourth leaf of line I-17-40 at any time during the experiment.

The effects on Pn_{max} per unit leaf area followed essentially the same pattern as on Pg_{max} in all three lines (Fig. 61B).

These results suggest that in line B19909, infection of the first three leaves stimulated photosynthesis in uninfected leaves to compensate for the photosynthetic losses which occurred in infected tissues.

When related to chlorophyll levels, the maximum rates of gross photosynthesis in the uninfected fourth leaf of infected plants were not significantly different from those in the fourth leaf of the uninfected plants (Fig. 61C).

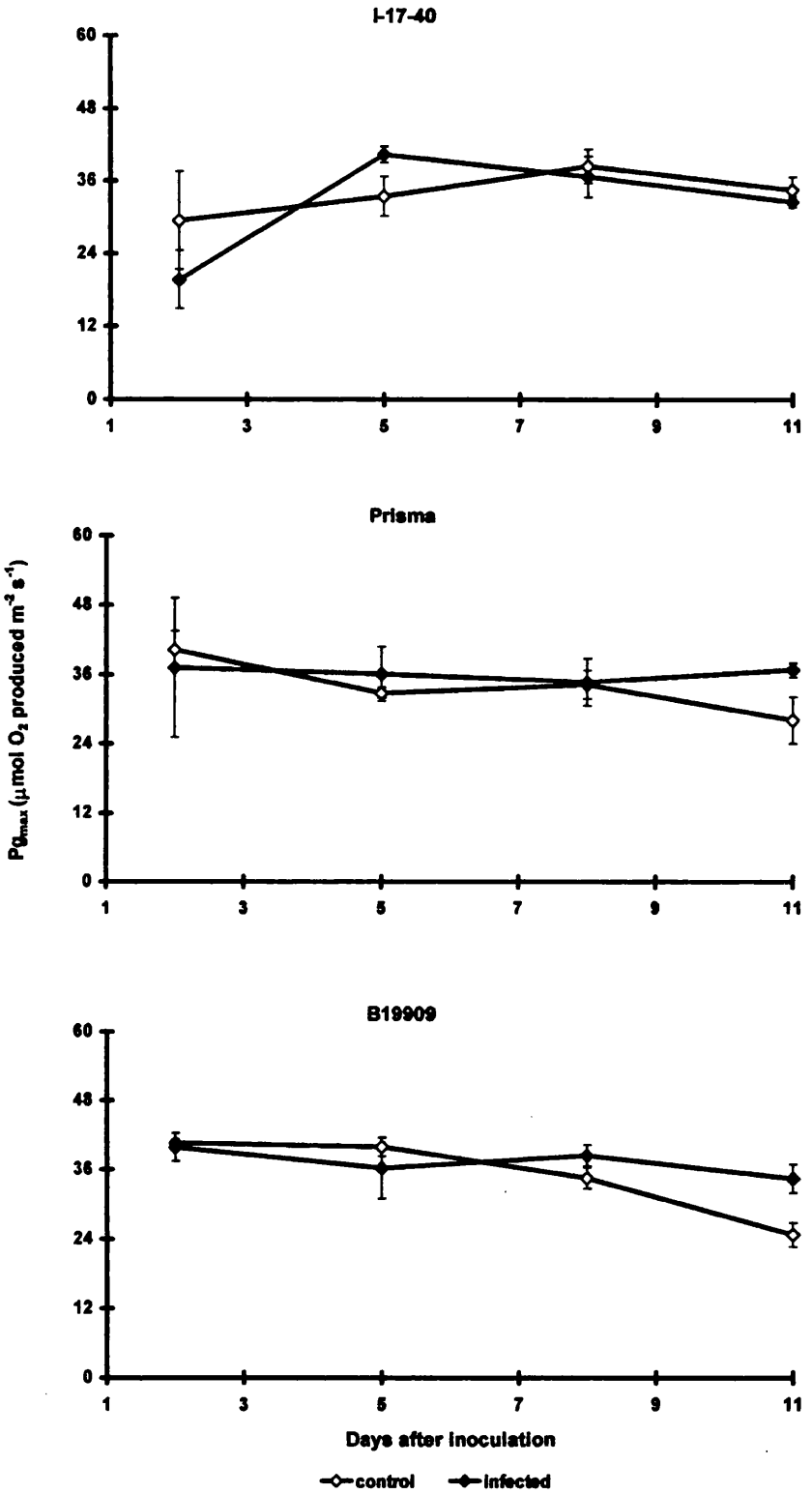


Fig. 61A: The rates of gross photosynthesis per unit area in fourth leaves of three barley lines with lower three leaves infected by mildew. Each datum point is the mean of three replicates.

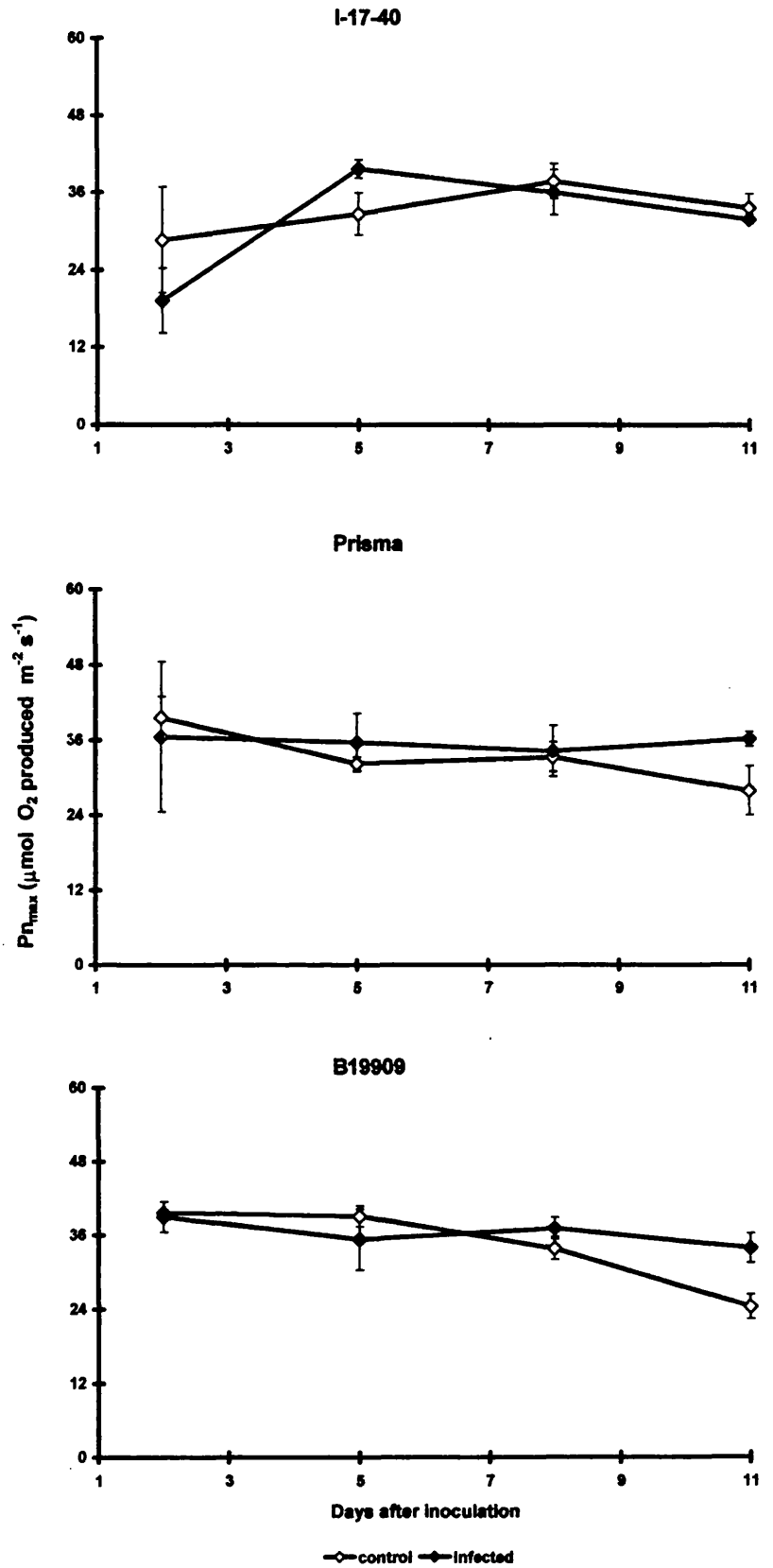


Fig. 61B: The rates of net photosynthesis per unit area in fourth leaves of three barley lines with lower three leaves infected by mildew. Each datum point is the mean of three replicates.

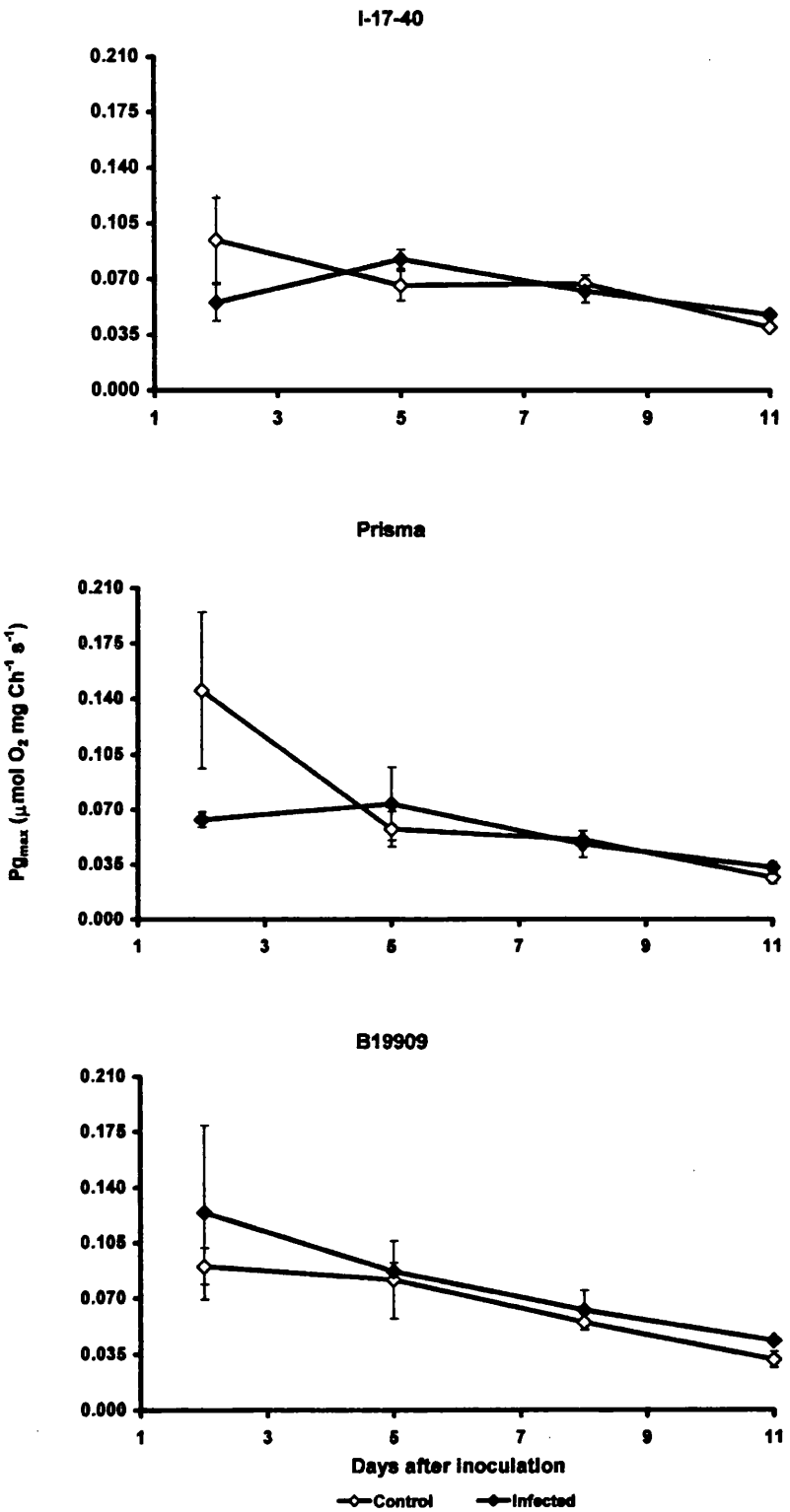


Fig. 61C: The rate of gross photosynthesis per milligram chlorophyll in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates.

8.2.1.2. Quantum efficiency or photochemical efficiency of photosynthesis (α).

The quantum efficiency of photosynthesis α of the three lines at each sampling time is plotted in Fig. 62. An analysis of variance showed that only in B19909, had α increased significantly ($p < 0.05$). In all other lines none of the differences was significant.

8.2.1.3. The physical and biochemical resistance to CO_2 diffusion (θ)

Changes in θ (a measure of the ratio of physical to total resistance to CO_2 diffusion into the leaf) in the uninfected fourth leaf of infected and uninfected plants are plotted in Fig. 63.

Infection of the lower three leaves increased θ in the uninfected fourth leaf of plants of both line I-17-40 and cv. Prisma up to the fifth and the eighth day after inoculation, respectively. Line B19909 showed fluctuating changes in θ to infection of lower leaves between the second and the eighth day after inoculation, but none of the differences were significant. Although the analyses of variance showed that the differences were significant ($p < 0.05$) in both line I-17-40 and cv. Prisma but not in the wild line B19909, it is difficult to draw a conclusion because of the range in values found.

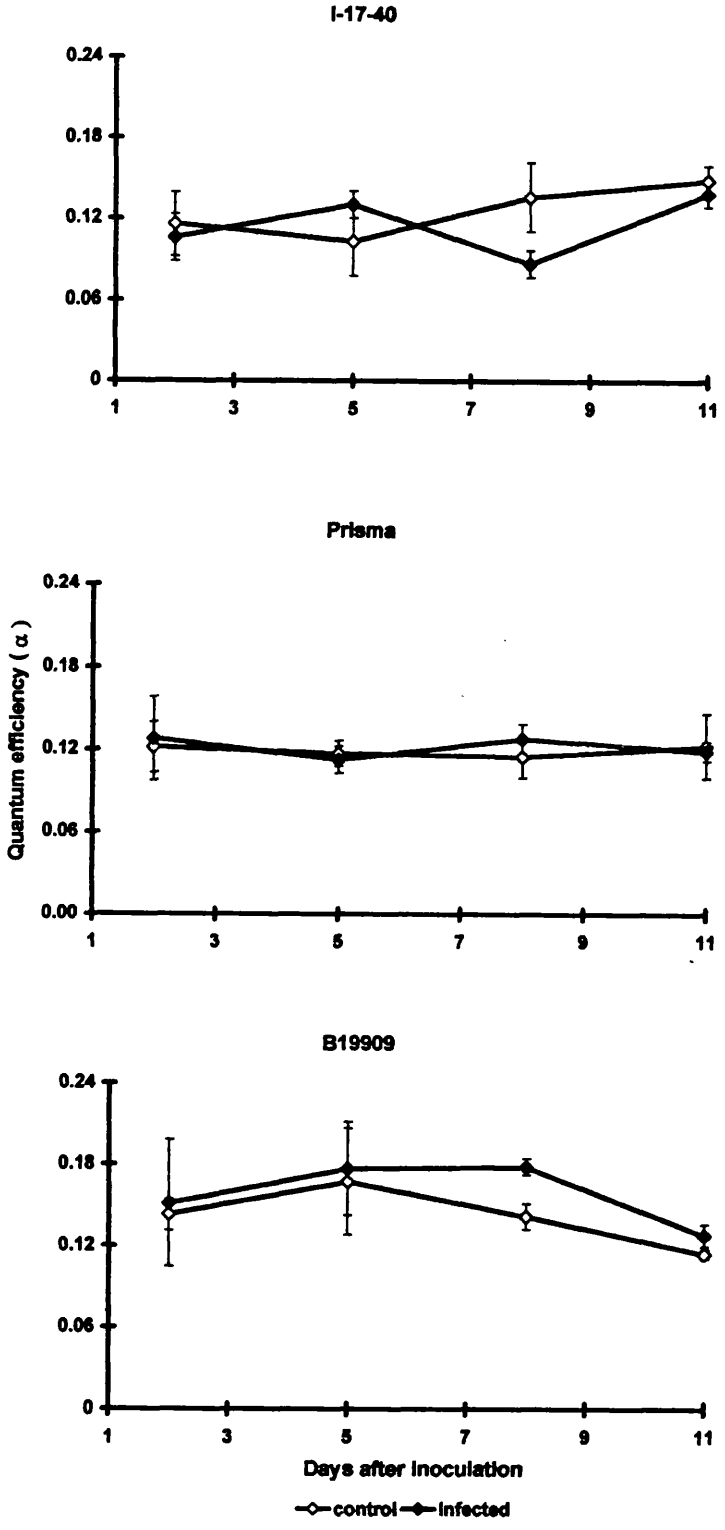


Fig. 62: The Quantum efficiency of photosynthesis in the fourth leaf of three barley lines whose lower three leaves were infected by mildew. Each datum point is the mean of three replicates.

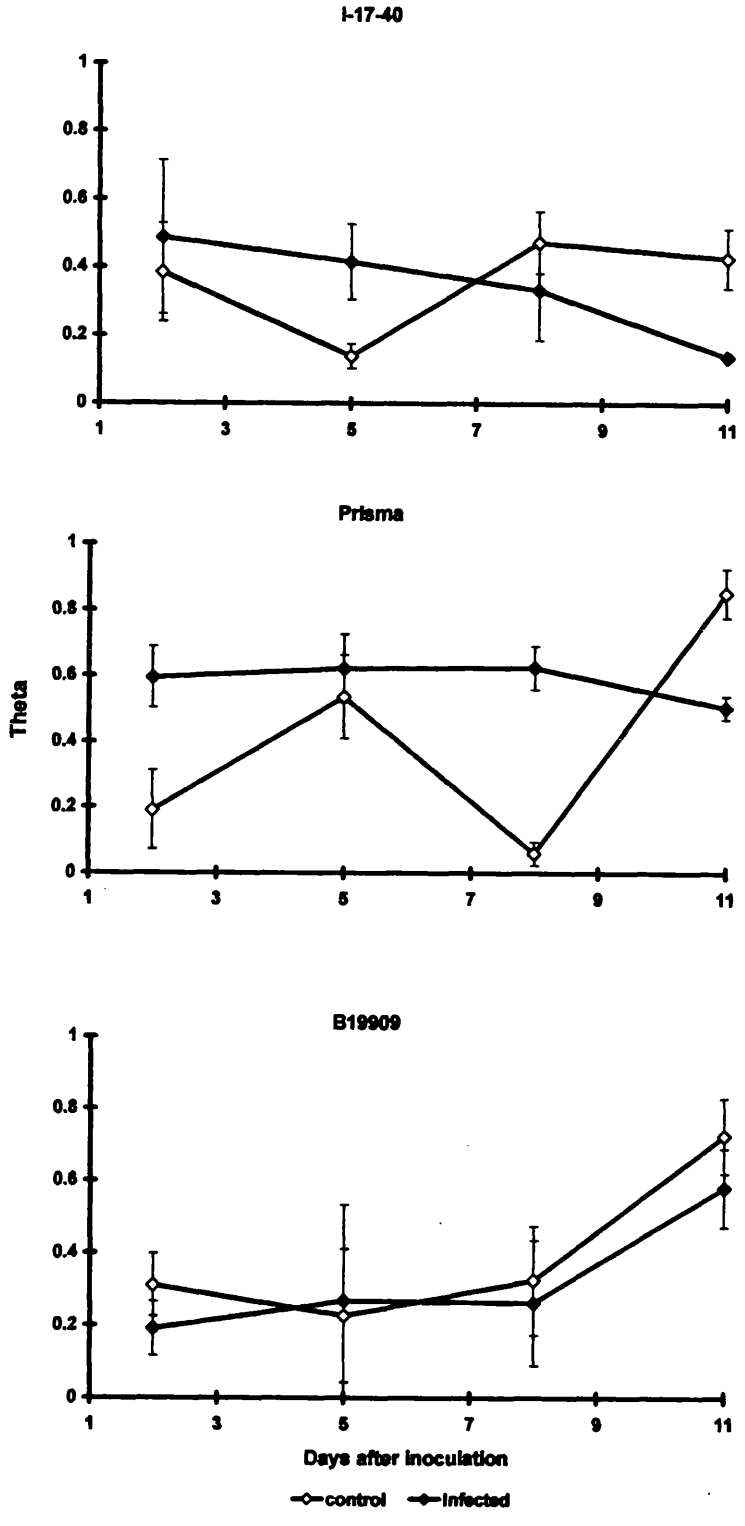


Fig. 63: The ratio of physical to total resistance to CO_2 diffusion (θ) in fourth leaves of three barley lines whose lower leaves were infected by mildew. Each datum point is the mean of three replicates.

8.2.1.4. Chlorophyll content

Changes in chlorophyll content of the uninfected fourth leaves of infected and uninfected plants of each line, at each sampling time, are plotted graphically in Figs. 64A to 64D.

Fig. 64A shows that during the course of the experiment, chlorophyll a levels per unit leaf area in the uninfected fourth leaf of both infected and uninfected plants of all three lines increased. Infection did not significantly affect this increase except in the wild line I-17-40, which showed a significant reduction ($p < 0.05$) by the eleventh day following inoculation of the lower leaves.

The effects of infection on chlorophyll b levels (Fig. 64B) in the uninfected fourth leaf was generally similar to that of chlorophyll a (Fig. 64A). However, the slight increase in chlorophyll b observed in the fourth leaf of line I-17-40 eleven days after inoculation was not significant ($p > 0.05$).

It follows that infection had no effects on total chlorophyll content (Fig. 64C) or on the chlorophyll a : b ratio (Fig. 64D).

8.2.2. Effects of infection on dark respiration

The rates of dark respiration in the uninfected fourth leaves of both infected and control plants of each line, are plotted in Fig. 65. Infection had no significant effect on dark respiration in the uninfected fourth leaf of infected plants compared to the controls in any of the three lines.

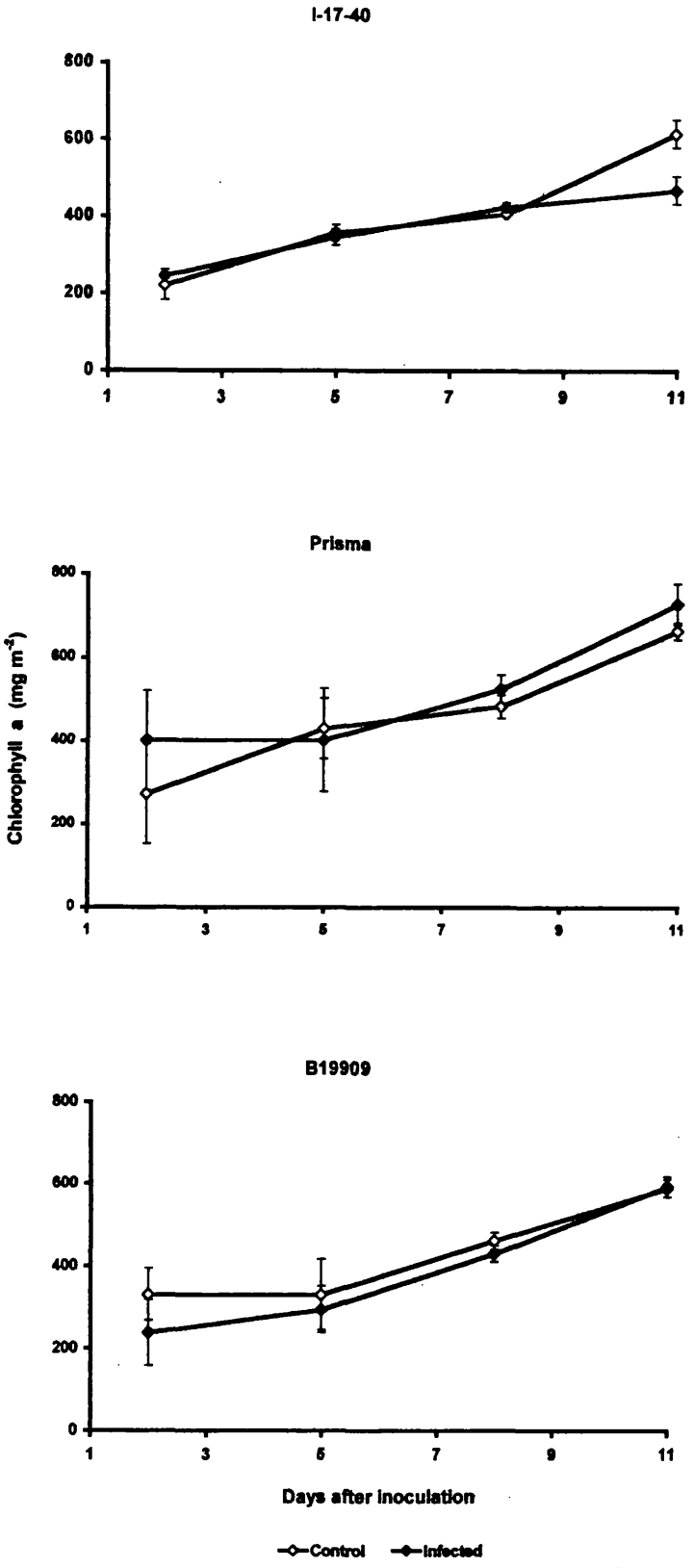


Fig. 64A: The chlorophyll a in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates.

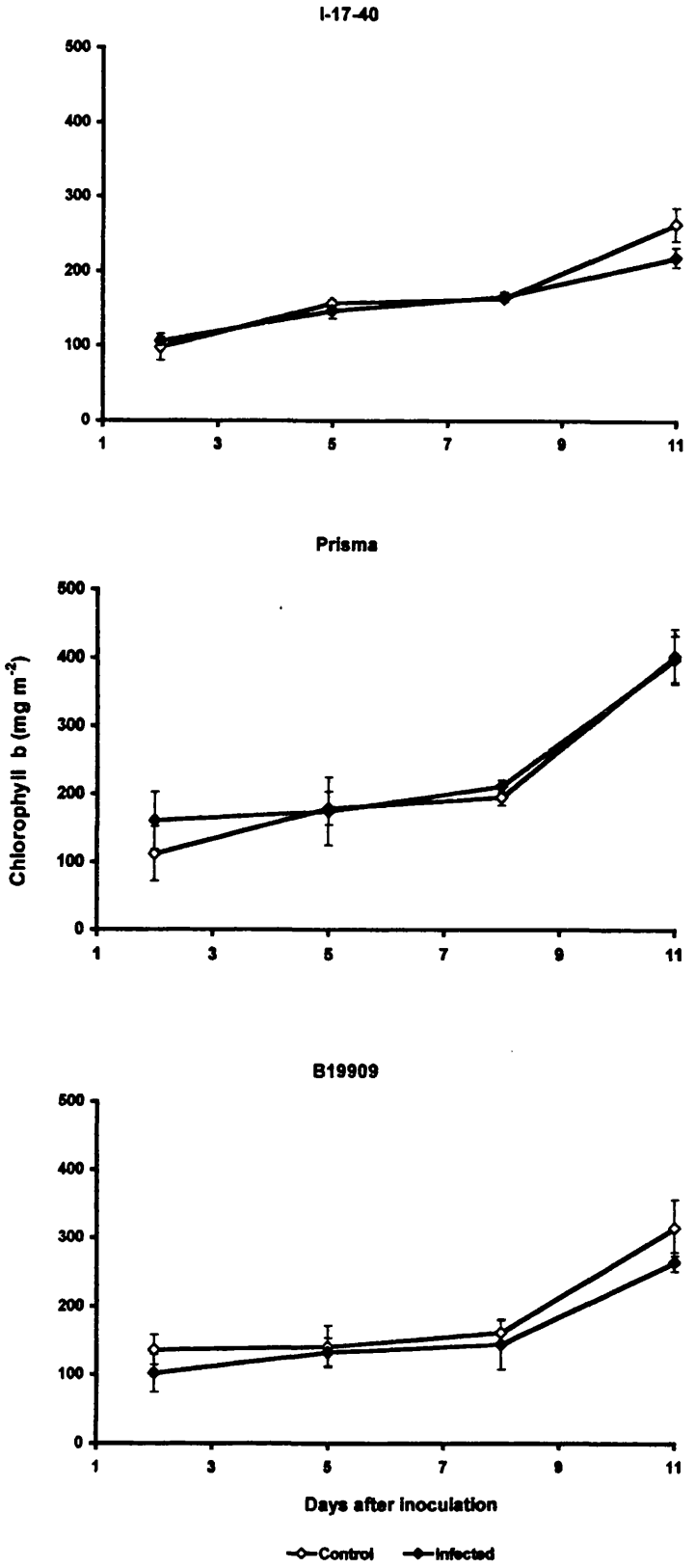


Fig. 64B: The chlorophyll b in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates.

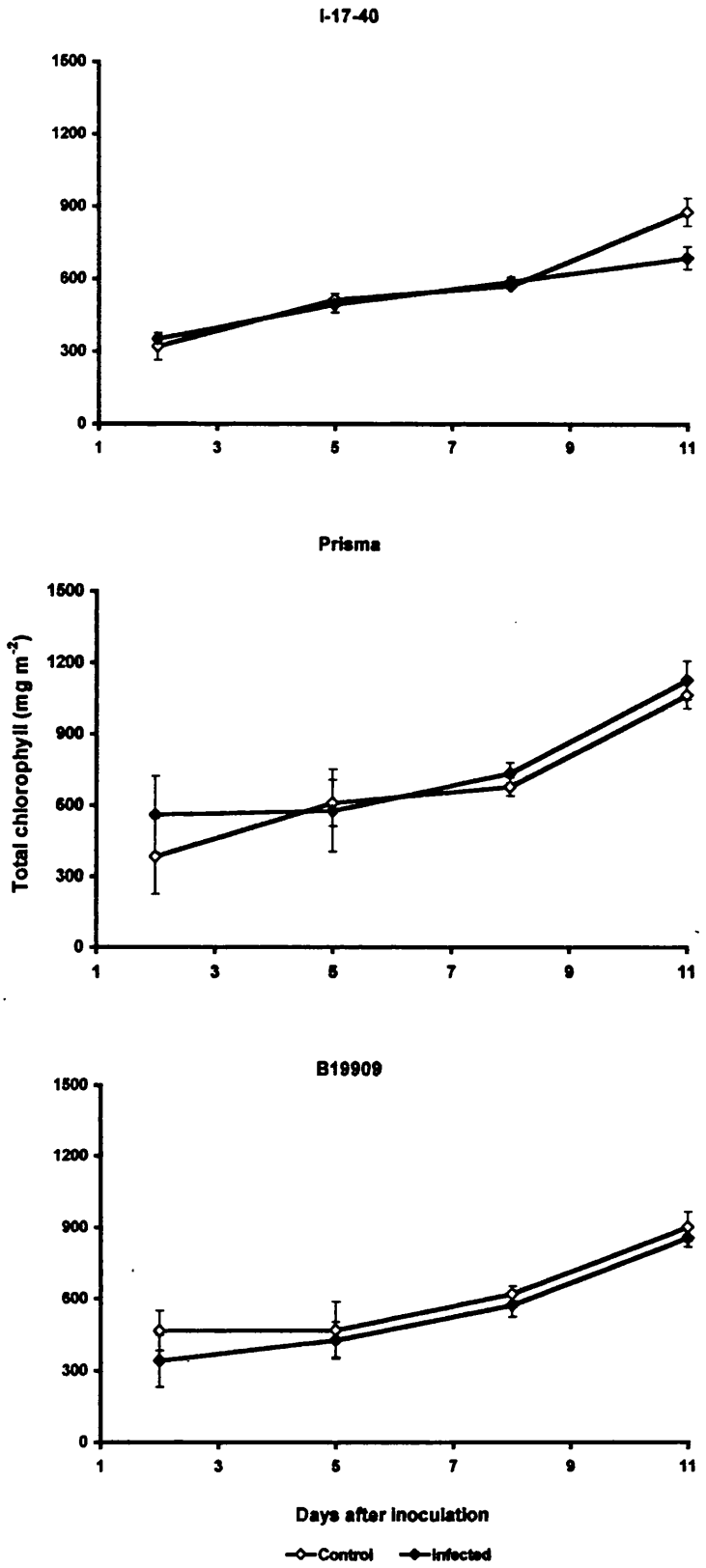


Fig. 64C: Total chlorophyll in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates.

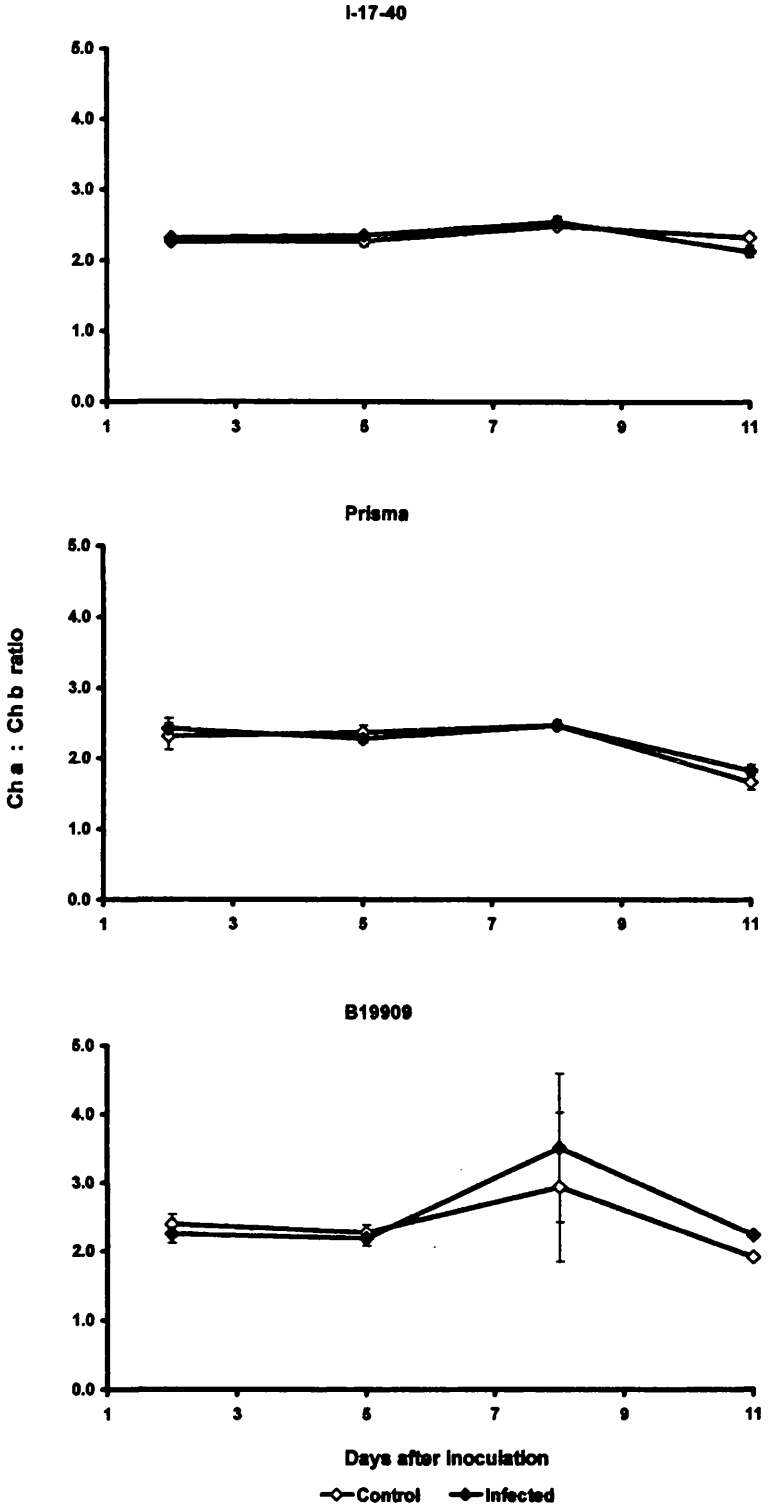


Fig. 64D: The chlorophyll a:b ratio in fourth leaves of three barley lines with the lower three leaves infected by mildew. Each datum point is the mean of three replicates.

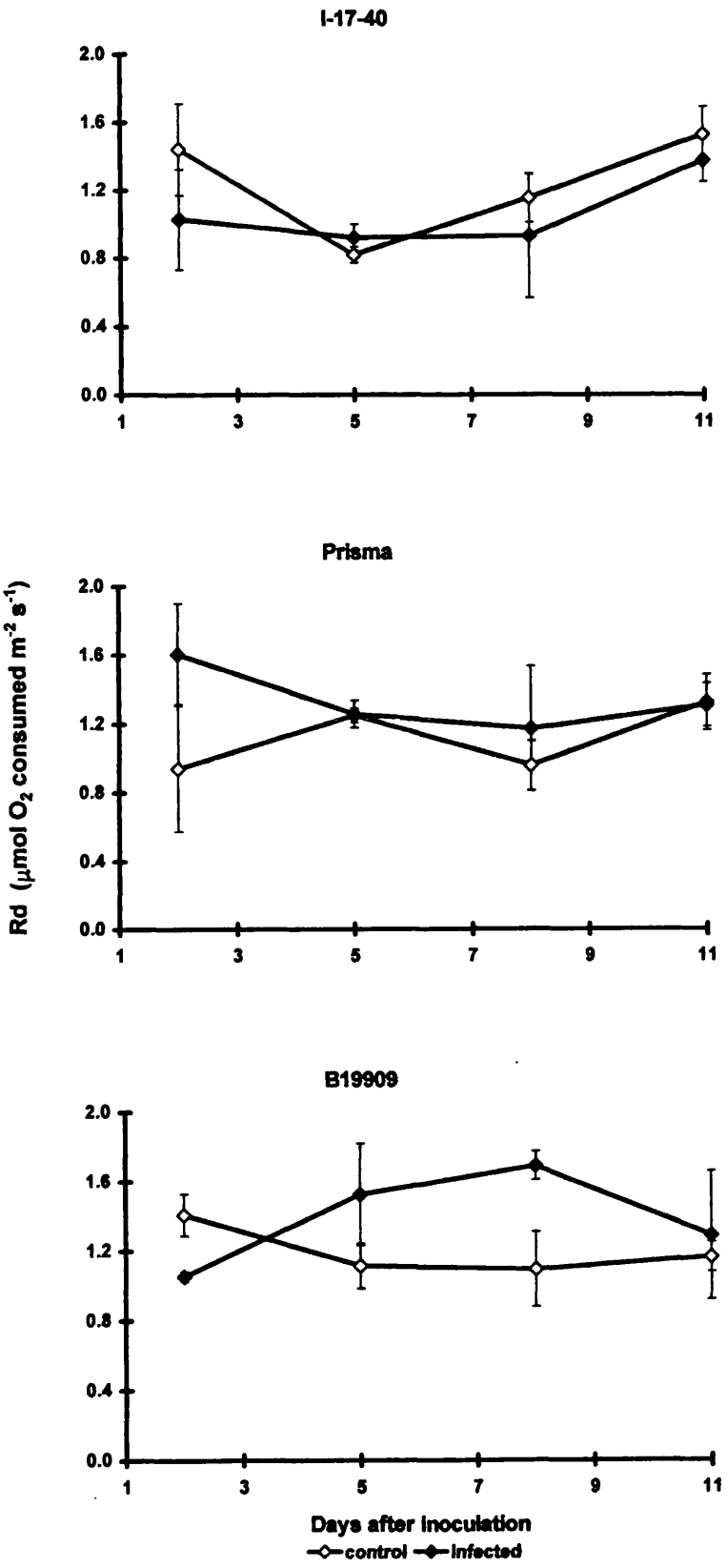


Fig. 65: Dark respiration per unit area in fourth leaves of three barley lines with lower three leaves infected by mildew. Each datum point is the mean of three replicates.

Chapter 9

DISCUSSION

9.1. Levels of tolerance

The relative levels of tolerance of the two wild barleys and one cultivated barley to *B. graminis* f.sp. *hordei* were determined by comparing the effects of different levels of infection on the growth and development of the lines.

9.1.1. Mildew development

Generally, mildew infection followed similar progressions on both the wild and the cultivated barleys, developing heavy infections on the lower leaves but becoming lighter on the upper leaves. However, the way the leaf blade area was colonised by the mildew differed between the lines. By four weeks after inoculation, 34% of the leaf blade area of line B19909 was covered by mildew and about 27% of both cv. Prisma and line I-17-40. The mildew continued to increase on both cv. Prisma and line B19909 to cover about 40% of the leaf blade area five weeks after inoculation, but as infection progressed further, the percentage GLA area colonised fell on both lines partly due to the loss of the heavily infected lower leaves through enhanced senescence but also to slower and lower levels of infection on the upper leaves. On line I-17-40, after reaching 27% leaf blade area cover, the reduction in GLA colonised was more dramatic, since much less infection developed on its upper leaves compared to cv. Prisma and line B19909 and the plants also lost their heavily infected lower leaves due to premature senescence. Thus, overall, line B19909 supported a higher percentage of leaf area colonised than line I-17-40 at all

stages and slightly more than cv. Prisma.

In greenhouse experiments using three cultivated barleys Proctor, Plumage Archer and Haisa II, Last (1962) observed that *B. graminis* f.sp. *hordei* infections, measured as percentage mildew cover, increased rapidly after inoculation so that within four weeks 30% of their leaf surfaces were colonised. After this stage, many of the heavily infected lower leaves died prematurely, leaving the upper comparatively lightly infected young leaves, and so as in this study the percentage of GLA covered by mildew decreased. The percentage of GLA colonised by mildew was also found to follow a similar pattern in greenhouse grown plants of the barley cvs Proctor, Deba Abed, Sultan, Zephyr, Midas and Julia (Scott *et al.*, 1980; Carver *et al.*, 1981; 1982).

Observations on one wild oat (*A. fatua*) and two cultivated oats (*A. sativa*) infected with *B. graminis* f.sp. *avenae* indicated that similar levels of infection, measured as percentage GLA covered by mildew, were achieved on both species (Sabri, 1993; Sabri *et al.*, 1995). When the oat plants were ten weeks old, the fungus had colonised approximately 40% of the leaf blade area of the wild oat and of one of the cultivated oats, cv. Lustre, although only about 30% of the leaf surfaces of the other cultivated oat cv. Peniarth. However, the lower leaves of cv. Peniarth were at least as susceptible, if not more so, than those of the other two lines but the upper leaves, including the flag leaf, developed much lower levels of infection.

Other studies carried out by Ben-Kalio *et al.* (1979) and Harry *et al.* (1992), on the wild plant *Senecio vulgaris* revealed that the powdery mildew,

Erysiphe fischeri, developed much higher levels of mildew than have been recorded for cereals. Thus, eleven weeks after inoculation between 75 and 100% of the leaf area had become colonised with the upper leaves developing similar levels of infection to the lower leaves.

In addition to following the percentage GLA colonised, mildew development in this study was measured in terms of the number of conidia produced during the course of the infection. This is a far better measure of fungal biomass production than percentage GLA colonised. The results showed that conidial production on the primary shoot leaves of the wild line B19909 (9×10^7 conidia) was higher than on cv. Prisma (8×10^7 conidia) and much higher than on line I-17-40 (1.8×10^7 conidia). Conidial production on the whole plant showed approximately the same pattern as on the primary shoot.

Sabri (Sabri, 1993; Sabri *et al.*, 1995) also determined conidial production on the two oat cultivars, Lustre and Peniarth, and on one wild oat line. Conidial production on cv. Peniarth was little more than half that on cv. Lustre while the wild oat produced the highest number although only slightly more than cv. Lustre.

9.1.2. The effects of mildew infection on the growth of the three lines

9.1.2.1. Shoot production and the development of leaf tissue

Mildew infection caused significant reductions in shoot development and these effects were evident in reduced primary shoot height, fewer tillers, and reductions in leaf area although these reductions were not entirely reflected in yield components and in final grain yield. Infection reduced primary shoot height more in cv. Prisma than in the other two lines, with line I-17-40 being the least affected. The number of tillers per plant was also reduced in all three lines but strangely to the greatest extent in the least infected wild line I-17-40. The overall reductions in total plant growth were greater in cv. Prisma than in line B19909 although the former, while developing similar levels of leaf area infected, supported lower conidial production than the latter. The lower growth reductions in line I-17-40, except in relation to tiller development, could be expected because this line supported the lowest levels of mildew biomass production.

Similar reductions were observed by Last (1962) who reported that *B. graminis* f.sp. *hordei* reduced the primary shoot height of barley cvs Proctor and Plumage Archer by about 34% and tiller production by about 27% when the percentage leaf area covered by mildew was over 20%. Sabri *et al.* (1995) also reported that *B. graminis* f.sp. *avenae* reduced primary shoot height and tiller production in two cultivated oats and a wild oat line with the latter being the least affected although it supported the greatest production of conidia. This suggests some similarities between the wild oat line and the wild barley line B19909 used in the present study. Similarly, Ben-Kalio *et al.* (1976)

observed that high levels of infection by *E. fischeri* were required before the mean stem height and the number of branches produced by *Senecio vulgaris* were reduced.

The production of leaves on the primary shoot of both barley cv. Prisma and line B19909, but not of line I-17-40, occurred at a slower rate on infected plants than on the controls. By six weeks after inoculation, the infected plants of both cv. Prisma and line B19909 had expanded 1 or 2 leaves fewer than the uninoculated controls, but by the end of the experiment, both infected and uninfected plants of line B19909 had expanded the same number of leaves. The infected plants of cv. Prisma never caught up with the controls because the primary shoot died after the tenth leaf had expanded, presumably because of the very heavy infections developing on the plants in the growth cabinet. Infected and uninfected plants of the more resistant line I-17-40 produced about the same number of leaves on the primary shoot with no significant delay in leaf production. It is possible that lines had developed all leaf primordia by the time of inoculation and the infections on line B19909 and cv. Prisma simply delayed leaf expansion compared to the controls. However, the growing points were not dissected to investigate this possibility.

Total leaf area on the primary shoot, by the end of the experiment, was reduced by infection in all three lines but again to different extents. When conidial production had reached about 55 x 10^7 conidia, the leaf area was reduced by about 42% in line B19909 but lower conidial production of about 40 x 10^7 reduced leaf area by about 60% in cv. Prisma. An even lower level of conidial production of about 16 x 10^7 on line I-17-40 reduced leaf area on the

primary shoot by about 69%. The effect of infection on the total GLA produced by the tillers followed the same pattern as on the primary shoot, except that, strangely, line I-17-40 showed the greatest reduction. The reduced leaf area was due mainly to premature senescence of the lower heavily infected leaves. Cv. Prisma and line I-17-40 showed slightly higher levels of leaf senescence than line B19909 and the delayed senescence in some cultivars has been suggested to be a possible basis for the breeding of tolerant cultivars (Finney, 1973).

The reductions in leaf size in the three barley lines were found to be due to reductions in both cell expansion and cell division. Cell size was reduced between 13 and 25% in line I-17-40, between 16 and 26% in cv. Prisma and between 10 and 22% in line B19909. It was also calculated from numbers of epidermal cells per leaf, that cell division was reduced by about 6% in line I-17-40, by about 7% in cv. Prisma but about 10% in line B19909. These results are not in agreement with the early observations that growth components were less affected in line B19909 than in the other lines and particularly cv. Prisma.

Sabri (1993) showed that infection of oats by *B. graminis* f.sp. *avenae* also caused the leaves to senesce more rapidly, particularly the lower leaves, on both the primary shoot and the tillers, with the senescence being greater in the two cultivated oats Lustre and Peniarth than in the wild oat, although the latter supported the highest level of conidial production. High levels of infection were also found to occur on *Senecio vulgaris* before reductions in leaf area were evident in response to infection with *E. fischeri* (Ben-Kalio *et*

al., 1979 and Harry *et al.*, 1992).

There has been a conflict between researchers concerning the effects of mildew infection on leaf size and green leaf area of the crop. For example, Last (1962) reported a reduction in individual leaf size in barley due to mildew infection. However, Carver & Griffiths (1981) showed that 30% mildew cover on barley cv. Julia affected neither the number nor the size of leaves. The only obvious effect being a reduction in green leaf area due to the loss of the lower leaves due to premature senescence.

Sabri (Sabri, 1993; Sabri *et al.*, 1995) observed reductions in leaf blade area in cvs Peniarth and Lustre and in wild oat in response to infection with *B. graminis* f.sp. *avenae*. She also showed that in the wild oat and cv. Peniarth, as in this study, the reductions in individual leaf blade area were due to reductions in both cell division and cell expansion. Cell division was reduced by about 13% in the wild oat and about 17% in cv. Peniarth, when about 75% of the leaf area was covered by mildew. Studies with *S. vulgaris* infected with *E. fischeri* also revealed that the size of epidermal cells in infected leaves was reduced between 21 and 33% and cell division between 10 and 15% with the topmost, youngest, leaves being affected the most (Harry *et al.*, 1992).

The process of cell division and cell expansion are both very sensitive to changes in water potential (Ayres, 1981a) and changes in water potential are frequently observed to be caused by infection (Ayres, 1972; Ayres, 1978). In the present study, powdery mildew infection certainly affected stomatal function and this may well, through increased rates of transpiration, have lowered leaf water potentials. This could have inhibited leaf expansion by

inhibiting cell expansion, which depends on the attainment of high turgor, and also cell division. However, all the plants were watered regularly and sufficiently and so some of the increased loss of water should have been compensated for by increased uptake unless root function was affected and several workers have obtained evidence for this (e.g. Walters, 1981).

Mildew infection not only reduced leaf size but it also affected the photosynthetic efficiency of the remaining leaf tissue in all three lines. Thus, ULR was reduced by 99% in line I-17-40, 57% in cv. Prisma and 60% in line B19909 in the period between the third and the fourth week after inoculation. At this stage the fungal biomass produced had reached about 9×10^7 conidia on line I-17-40, 11×10^7 in cv. Prisma and about 16×10^7 conidia on line B19909. As infection progressed, infected leaves on both lines became transiently as efficient as those of the controls and then the rates of ULR decreased again. However, as conidial production decreased dramatically on the more resistant upper leaves of line I-17-40, ULR increased to a level higher than that of the controls probably reflecting compensatory photosynthesis.

Last (1962) also found that infection of barley by *B. graminis* f.sp. *hordei* reduced the mean ULR by about 27% compared to the controls. The lower effect of infection on the ULR in Last's experiment compared to the present study is probably due to lower levels of infection achieved in last's study, which did not exceed 30% of the leaf area colonised at any time. In studies (Cameron, 1993) involving two barley, cvs Proctor and Triumph, infected with *B. graminis* f.sp. *hordei*, the ULR was found to be reduced in both

cultivars but to different extents. Although cv. Triumph supported more mildew than cv. Proctor, its ULR was reduced by about 5% but that of cv. Proctor was reduced by about 48%. Similarly, Sabri *et al.* (1995) observed that infection of oat cvs Peniarth and Lustre and a wild oat by *B. graminis* f.sp. *avenae* reduced ULR in all three lines, with the latter being less affected than the former two cultivars although it supported the production of the most mildew biomass. In contrast, studies on the effects of *E. fischeri* infection on *Senecio vulgaris* showed that even when 90% of the host plants leaf area was colonised by mildew the ULR was not different from control uninfected plants.

Although the leaves were smaller, the dry matter content of the infected leaves per unit plant dry weight (LWR) increased in all lines and particularly in line B19909. These increases could be attributed partly to the amount of mildew, which had developed, and partly to the accumulation of soluble or insoluble carbohydrates within the leaves. The ratio of leaf area to leaf dry weight, or SLA, which is a measure of leaf thickness, was also found to decrease in all lines, but line B19909 was the least affected while cv. Prisma was the most affected. The reduction in SLA in the infected plants was mainly due to the greater loss of the lower leaves by senescence but also to the accumulation of more dry matter in the relatively small leaves. How much the relative increases in dry matter per unit area of leaf was due to additional structural materials, such as cell walls and vascular tissues, or to non-structural, storage carbohydrates or to fungal development was not determined. Infection reduced the leaf area ratio (LAR) in cv. Prisma and line

I-17-40, suggesting that leaf area was smaller compared to the size of the plants relative to the controls. In contrast, LAR was not affected in line B19909, indicating that the reduction in leaf area was proportional to the reduction in the size of the plant in this line.

Last (1962) observed that the LARs of barley cvs Proctor and Plumage Archer infected with *B. graminis* f.sp. *hordei*, were reduced by about 10% when the percentage leaf area covered by mildew was between 20 and 30%. Sabri (Sabri, 1993; Sabri *et al.*, 1995) also found that *B. graminis* f.sp. *avenae* infections reduced the LWR of infected plants of cvs Lustre and Peniarth beginning from the sixth week after inoculation particularly in cv. Peniarth. These reductions in LWR in the cultivated oats were attributed to the greater loss of leaves through senescence rather than to changes in the proportions of dry matter allocated to the leaves. In contrast, infection did not affect the LWR of the wild oat and no significant differences in LAR or SLA between infected and uninfected plants of cultivated or wild oats were found. These results contrast with the present study, which revealed significant changes in LWR and SLA in all lines including the wild barley B19909. In *S. vulgaris*, infections with *E. fischeri* affected LWR, LAR and SLA slightly but only when over 75% of the leaf surfaces had been colonised (Ben-Kalio, 1976). Harry (1980) however, later reported that *E. fischeri* had no effect on these growth indices in *S. vulgaris*, but this was probably because the experiment was terminated a week earlier than Ben-Kalio's experiment. Thus although infection by *E. fischeri* reduced the total leaf area of groundsel, the size of the leaves remained proportional to the size of the plant, and infection

did not appear to alter the allocation of photosynthates to the leaf relative to the rest of the plant.

9.1.2.2. Root production

Infection caused significant reductions in root growth in all three barley lines. Although the total number of seminal roots was not affected by infection, as the number in each line was determined before the seedlings were inoculated, the number of lateral roots produced on the seminal roots was reduced. Both cv. Prisma and line B19909 produced 51% fewer lateral roots on seminal roots than uninfected plants, but infection had no effect on the number produced on the more lightly infected line I-17-40. The total length of the seminal roots was reduced by about 65% in cv. Prisma and 47% in line B19909 although again no significant reduction was observed in infected plants of line I-17-40. Mean seminal root diameter did not appear to be affected by infection in any line, probably because any differences were masked by the large number of thin laterals with relatively uniform diameters, which were produced. Because infection reduced the total length of the seminal roots and their laterals, the surface area of the seminal root system was reduced with cv. Prisma being more affected than the other two lines.

Infection affected all morphological components of the nodal roots. Infected plants of cv. Prisma produced about 74% fewer nodal roots and line B19909 about 31% fewer nodal roots than the uninfected controls. However, line I-17-40, despite the light infection, showed a 46% reduction. Numbers of nodal lateral roots were reduced by about 71% by infection in cv. Prisma, but

no significant reductions occurred in the two wild lines. Infection however did reduce the total lengths of nodal roots in all lines, but to different extents. The length of the nodal roots was reduced by about 78% in cv. Prisma but only about 27% in line B19909 when fungal biomass had reached about 40 x 10^7 conidia per plant on cv. Prisma and about 55 x 10^7 conidia per plant on line B19909. At this stage, line I-17-40 had supported the production of only about 16 x 10^7 conidia but the total length of its nodal root system was reduced by 39%, a greater reduction than line B19909. Similarly, infection reduced the surface areas of nodal roots in all lines, but to the least extent in line B19909. However, as with the seminal roots, infection had no significant effect on overall nodal root diameter in any line.

Unfortunately, no other study on the effects of powdery mildew infections on the development of the host's root system has involved measurements of the amount of parasite present, and so a direct comparison with other studies is not possible. In those few studies where the level of infection or of fungal biomass production was measured only root dry weights were determined.

However, some less detailed studies have revealed similar effects on the branching pattern of roots in response to mildew infection to those noted in this study. Thus, Vizárová & Minarčic (1974) observed that mildew infections inhibited the elongation of seminal roots together with the growth and formation of lateral roots in barley plants. A decrease in the diameter of seminal roots was also noted, a result which contrasts with that of the present study. However, the failure to detect effects on root diameter in this study could be because the diameters of the larger seminal or nodal roots, which

might have changed, were measured together with a large number of thinner lateral roots with uniform diameters, so that any reductions in diameter in the larger roots may have been masked. A more detailed study of the effects of mildew infections on the growth of barley roots was carried out by Walters (1981) and Walters & Ayres (1981a). They observed that total root dry weight, total root length, as well as the length of nodal and seminal roots individually were significantly decreased by infection. Their results were in agreement with the present study. However, the numbers of nodal roots were found to be unaffected by infection in contrast to the present study where the number of nodal roots was found to be reduced in all lines but to different extents.

The amount of dry matter allocated to roots per unit leaf area was also reduced by mildew in all three lines but to the greatest extent in cv. Prisma. Similarly, root surface area per unit of total plant dry weight was affected by infection in the same way as root dry weight per unit of total plant dry weight. Infection had increased root length per unit of root dry weight in cv. Prisma when 40% of the leaf area was colonised by mildew, but it had no effect on the two wild lines. This increase in specific root length suggests that a relatively lower proportion of photoassimilates was allocated to the relatively longer thinner root systems.

Last (1962) also reported that the RWR of barley infected with *B. graminis* f.sp. *hordei* had been reduced by about 33% by the time the percentage leaf area colonised by mildew had reached about 23%. This indicates that as plants grew older and as total plant dry weight increased, the

amount of dry matter accumulating in the roots diminished. The ratio of root dry weight to leaf area was also found to have decreased by about 46% when the percentage leaf area colonised by mildew was about 23%.

The reductions in root length could be due to reductions in cell expansion and or in cell division. In the present study, even when 50% of the leaf area was colonised by mildew, the rates of mitotic cell division in the apical meristems of the nodal root tips were not reduced either in cv. Prisma or line I-17-40, although a slight but significant reduction was observed in line B19909. The only other detailed investigations on the effects of infection by *B. graminis* f.sp. *hordei* on cell division in the apical meristems of barley roots are those of Minarčic & Paulech (1975) and Walters (1981) although, neither related the effect of different levels of infection on root cell division. Both studies revealed decreases in the rates of cell division in the root tips and this contrasts with this study. These discrepancies may be due simply to the fact that in this study only one set of measurements were taken eight days after inoculation. The reductions found by Minarčic & Paulech (1975) and Walters (1981) were attributed to the reduction in the quantity of photoassimilates reaching the root system (Fríc, 1975). A reduced supply of ^{14}C photoassimilates reaching the root tips of mildewed barley plants was indicated to be the major cause of the reduced meristematic activity of the root tips (Minarčic & Paulech, 1975). In contrast, Vizárová & Minarčic (1974) attributed the morphological changes in the roots of barley plants in response to mildew infection to increases in cytokinins brought about by infection.

Undoubtedly, the altered root morphology and anatomy in mildewed barley would depress root physiology. Walters (1981) found that roots of mildewed barley plants took up more ^{32}P-labelled phosphate, more potassium and more chloride, and consequently the ionic content of the tissues was higher than that of uninfected plants. A decrease in nitrate uptake, thereby lowering the nitrate content of the roots, was also found. These changes were explained by the lack of photoassimilates received by the roots from the infected leaves (Walters & Ayres, 1980). In contrast, sodium uptake and accumulation in the roots were unaltered by mildew infection. Other physiological processes such as root tissue respiration, were observed to increase in response to mildew infection in barley plants (Walters, 1981). However, none of these physiological processes were investigated in this study.

The alterations in root morphology, anatomy and physiology resulting from infection are likely to play a role in host growth and yielding capacity. Last (1962) suggested that as reductions in the root system developed, a stage could be reached when the reduction could affect leaf efficiency.

9.1.2.3. Dry matter production

As expected from the reductions in the shoot and root systems, infection substantially reduced total plant dry matter accumulation in both the cultivated and the wild barley compared to the controls although to different extents in the three lines. Six weeks after inoculation, when conidial production had reached about 40 x 10^7 conidia on cv. Prisma and about 55 x

10^7 conidia on line B19909, the percentage reduction in total dry matter was about 63% in cv. Prisma and about 58% in line B19909. In the case of line I-17-40, total plant dry matter was reduced four weeks after inoculation by about 55% when conidial production had reached its maximum of 9 x 10^7 conidia per plant; after this total plant dry matter caught up with the controls presumably due to compensatory photosynthesis in the upper leaves, which supported little infection. Thus although line B19909 supported more conidial production than cv. Prisma, its dry matter content was reduced less indicating the presence of a higher level of tolerance.

The reductions in total plant dry matter accumulation affected both shoot and root development in the three lines but to different extents in each. A low total conidial production of 40 x 10^7 conidia reduced shoot dry weight in cv. Prisma by about 60%, but the higher levels of conidial production of 55 x 10^7 conidia on line B19909 had no significant effect on its shoot dry weight. Line I-17-40 was also affected and when conidial production reached a maximum of about 9 x 10^7 conidia, shoot dry weight was reduced by about 54%.

Total root dry weight was reduced by infection in all the three lines but to different extents in each line. The root dry weight of line I-17-40 was reduced by about 59%, while that of cv. Prisma was reduced by about 83% in cv. Prisma. However, line B19909, which supported the development of the highest fungal biomass, was hardly affected. Clearly, the proportion of dry matter partitioned between the roots and shoots (expressed as root : shoot ratio) was decreased significantly by infection in both cv. Prisma and line I-17-40, but in comparison, line B19909, despite supporting the highest level of

mildew infection showed no significant changes in its root : shoot ratio indicating the extent to which this line can tolerate the presence of high levels of infection without the distribution of dry matter between root and shoot being affected.

Infection began to reduce the rates of dry matter production soon after inoculation. Thus, the relative growth rates (RGR) relative to the controls began to fall between the second and the fourth week after inoculation in all three lines, concomitantly with a reduction in tiller formation as well as with the increase in the proportion of non-photosynthetic to photosynthetic tissue. During this period, the RGRs were reduced by about 60% in line I-17-40, about 57% in cv. Prisma and about 54% in line B19909. By this time, fungal biomass production on line I-17-40 had attained its maximum level of about 9 x 10^7, but on cv. Prisma, it was about 11 x 10^7 conidia and rising on line B19909 to about 16 x 10^7 conidia. When stem elongation began, RGR began to increase again in both the infected as well as the uninfected plants of the three lines. A later reduction occurred in cv. Prisma and line B19909 associated with the loss of the infected leaves due to enhanced senescence and to reduced tiller development.

Last (1962) reported similar effects of *B. graminis* f.sp. *hordei* infection on the susceptible spring barley cultivars Proctor and Plumage Archer. When 25% of the leaf areas were colonised by mildew, total plant dry weight had been reduced by about 59% and shoot dry weight by about 50% compared to the controls. Sabri (1995) also reported that *B. graminis* f.sp. *avenae* reduced total plant dry weight and shoot dry weight of wild and cultivated oats but to

different extents. Thus, high conidial production of about 18 x 10^9 conidia reduced total plant dry weight by about 45% in the wild oat but lower conidial production of about 16 x 10^9 and 8 x 10^9 conidia reduced total plant dry weight by about 61% in cv. Lustre and about 55% in cv. Peniarth respectively. These results showed clearly that the wild oat was more tolerant of infection than the oat cultivars.

As in this study, infection of barley with *B. graminis* f.sp. *hordei* has been reported to reduce root dry weight by several workers (e.g. Last, 1962; Paulech, 1969; Brooks, 1972; Walters & Ayres, 1981a). Last (1962) found that mildew infection of barley cvs Proctor and Plumage Archer reduced root dry weights by about 70% when the percentage leaf area colonised was only between 20 and 25%. Sabri (1993) also showed that five weeks after inoculation with *B. graminis* f.sp. *avenae*, root dry weight was reduced significantly compared to controls in oat cvs Lustre and Peniarth when the percentage mildew cover was about 20% in cv. Peniarth and about 23% in cv. Lustre but not until the sixth week after inoculation in a wild oat line when the percentage mildew cover was about 30%. The reductions became more pronounced as infection progressed, especially in cvs Lustre and Peniarth. Root growth was signficantly inhibited in cv. Peniarth when mildew cover was 20% or more but it was not affected in either cv. Lustre or the wild oat. Studies on the reactions of *S.vulgaris* to infection by *E. fischeri* also showed that the distribution of dry matter in relation to the development of roots or other organs was not affected by infection even when 90% of the leaf area was infected (Ben-Kalio *et al.*, 1979; Harry *et al.*, 1992). The reactions of the

wild barley line B19909, the wild oat and the groundsel contrast with those of many crop plants where even low levels of infection reduce the proportion of dry matter allocated to the roots e.g. the barley cultivars studied by Last (1962), and the oat cv. Peniarth studied by Sabri (1993) and the barley cv. Prisma used in the present study.

The RGR was also reported to reduce in wild and cultivated oats infected with *B. graminis* f.sp. *avenae*, but the wild oat was least affected although it supported the highest conidial production (Sabri, 1993). In another study (Cameron, 1993) on two cultivated barleys, cv. Proctor and cv. Triumph, infection with *B. graminis* f.sp. *hordei* was also found to reduce the RGR, with cv. Proctor being affected more than cv. Triumph despite the latter supporting more mildew growth.

Infections in other wild plants have also been found to reduce the RGR but the reduction relative to the uninfected plants occurred at a late stage of infection. Thus, Harry (1980) reported that infection of *Senecio vulgaris* by *E. fischeri* did not affect RGR until the percentage leaf area colonised by the mildew was over 73%. This was taken to indicate the ability of the host to tolerate the high levels of infection. Other workers have reported reductions in the RGR by powdery mildew infections on barley plants similar to those found in this study, but close comparisons are not possible because the levels of mildew development were not reported (Walters & Ayres, 1981a and Hibberd *et al.*, 1996).

9.1.2.4. Production of yielding structures

The effects of infection on all yield components were also determined in this study. Infection reduced the number of grains produced by the primary shoot by about 36% in cv. Prisma, but had no significant effect on this yield component in either of the wild lines, even though fungal biomass production was greater on line B19909 than on cv. Prisma. The total dry weight of grains was also decreased by about 41% in cv. Prisma. Surprisingly, total dry weight of grains per primary shoot was increased significantly in infected plants of line B19909. Thousand-grain weight, a measure of grain size, was also slightly increased by infection in line B19909, but not in the other two lines.

The number of fertile tillers was not significantly reduced by infection in any line, probably due to the large variation in numbers of fertile tillers produced between plants. The total number of grains and total dry weight of grains produced by the tillers were both reduced by infection in cv. Prisma but not in the two wild lines. The percentage reduction in total dry weight of grains in cv. Prisma was about 55%. Thousand-grain weight was not affected by infection in any line indicating that infection had no effect on grain size. Although infection reduced the development of the vegetative structures e.g. green leaf area, shoot dry weight, root dry weight and total plant dry weight, the proportion of total biomass converted to grain, i.e., the harvest index, was significantly reduced by infection only in cv. Prisma and not in the two wild lines. It appears that infection had no effect on any of the yield components of the two wild lines even though line B19909 supported the development of the highest levels of fungal biomass. Clearly, in relation to reproductive output

line B19909 is much more tolerant of mildew infection than cv. Prisma. It is also interesting that line I-17-40 whose vegetative growth was significantly affected by infection suffered no effects on yield. This is not in agreement with what was reported by several workers that an early mildew attack reduced grain size and number of grains per tiller (Griffiths *et al.*, 1975). These discrepancies could be explained only by the fact that the wild line I-17-40 has some level of tolerance, which enabled later growth to compensate for early vegetative loss.

The similar study carried out on wild and cultivated oats infected with *B. graminis* f.sp. *avenae* by Sabri *et al.* (1995) also found that the wild oat produced a significantly higher number of grain on the primary shoot and on the whole plant than either of the two cultivars, Lustre and Peniarth, although the former supported the greater fungal biomass. However, the individual grains were much smaller and total yield per plant, in terms of the weight of grain produced, was significantly lower. Furthermore, the harvest index of the wild oat was slightly lower than that of the two cultivars and it was not reduced significantly by infection in any line. However, the percentage reduction brought about by infection in all yield structures and in total dry weight of grain produced by infected plants was lower in the wild oat than in the two cultivars. Thus these results are similar to those of the present study in that they show that, in relation to the development of yielding structures, the wild lines may be more tolerant of infection than the cultivated lines.

9.2. Photosynthesis and respiration in infected leaves

The reductions in shoot and root development and in yield or reproductive output in response to infection are the consequences of underlying changes in physiological processes of the host including photosynthesis, respiration and stomatal function. An understanding of the ways in which infection affects these processes may lead to a better understanding of the causes of how some lines are able to tolerate infection better than others. For this reason, the effects of mildew infection on photosynthesis, respiration and stomatal function in barley were investigated in the third leaf of the same lines, cv. Prisma and the two wild lines B19909 and I-17-40, as were used in the growth analysis study. In addition, a further three lines, cv Golden Promise, cv. Triumph and the wild line B8893 were studied in the same way but in their case the fourth leaf was used.

9.2.1. Mildew development

Line B19909 and cv. Golden Promise were the most susceptible to mildew infection of all the lines with the leaf used for measurement producing about 42 x 10^5 conidia cm^{-2} leaf area by the end of the experiment. Cultivars Prisma and Triumph were also susceptible, but less so, producing about 24 x 10^5 conidia.cm^{-2} leaf area Line I-17-40 supported an even lower conidial production of about 10 x 10^5 conidia, while line B8893 was highly resistant and produced only about 1 x 10^5 conidia. Slight necrotic flecking was produced on the leaf of line B8893 in response to inoculation, but the leaf of cv. Triumph developed significant necrosis in association with the developing

mycelium. None of the other lines developed necrosis in response to infection.

9.2.2. Effects on photosynthesis

The effect of infection on the rate of photosynthesis in the third or the fourth leaf was different in the six lines. When the rate of photosynthesis was related to fungal biomass production, the rate of decline in Pg_{max} and Pn_{max} in line B19909 was slower than in cv. Prisma and line I-17-40 (Fig. 48). The mean effect of similar high levels of conidial production decreased the rates of Pg_{max} and Pn_{max} by about 63% in cv. Golden Promise but by only 41% in line B19909. Furthermore a low level of conidial production decreased the rates of Pg_{max} and Pn_{max} by about 61% in cv. Triumph, about 52% in cv. Prisma and an even lower level of conidial production than on Triumph and Prisma reduced the rates of Pg_{max} and Pn_{max} by about 50% in line I-17-40. In contrast, Pg_{max} and Pn_{max} were not affected in the most resistant line B8893 which supported a very low level of conidial production compared to the other lines and cultivars. These results show clearly that there are differences in the tolerance of the photosynthetic mechanisms of the lines to mildew development and that line B19909 was relatively more tolerant than the other lines since relative to fungal development its photosynthesis was least affected.

Many workers have reported that the rates of photosynthesis in many hosts are reduced by powdery mildew infections. However, these investigations have rarely related the degree of reduction to the level of infection supported. Last (1963) observed that when 30% of the leaf area of

susceptible barley cultivars was colonised by *B. graminis* f.sp. *hordei*, the rate of photosynthesis was decreased by about 43% of the levels in uninfected control plants. Habeshaw (1979) investigated the effects of different percentages of leaf colonisation by *B. graminis* f.sp. *hordei* on the rate of net photosynthesis in the very susceptible cv. Golden Promise and the moderately resistant cv. Maris Mink, the later expressing a necrotic response to infection. He found that the percentage reduction in net photosynthesis was equal to the percentage of leaf area colonised in cv. Golden Promise, indicating that the impairment in photosynthetic capacity was confined to the infected parts of the leaf, whereas in cv. Maris Mink, the reduction occurred in both colonised tissues and the necrotic tissues. Similar differences in the response of photosynthesis were found between cv. Prisma and cv. Triumph in this study. Although both of these cultivars supported similar levels of fungal biomass development, cv. Triumph showed the greater reduction in photosynthesis probably because of the additional loss due to the necrotic response to the infection. However, studies carried out by Scott & Smillie (1966), on a major gene resistant barley cultivar inoculated *with B. graminis* f.sp. *hordei* showed no effects on photosynthesis, probably because cell collapse in the infection courts appeared to be too small to cause a significant decrease in the photosynthetic rate of the leaf. The highly resistant wild barley B8893 used in this study reacted to inoculation by producing small necrotic lesions but no reduction in the rate of photosynthesis was measured. Haigh *et al.* (1991) observed that oat lines, cv. Mostyn and breeding lines 1621 and 1674, each developing similar levels of infection, between 240-245 haustoria mm^{-2} of the

second leaf, had their photosynthesis inhibited to different extents. Thus, that of cv. Mostyn was inhibited by about 60% within five days of inoculation, but that of line 1621 was inhibited by only 39% nine days after inoculation, while line 1674 showed no significant response. The fourth line, the more resistant cv. Maldwyn, which supported the development of 131 haustoria mm^{-2} of leaf, had its rate of photosynthesis inhibited by about 18% nine days after inoculation. It appears that these oat lines not only differed in their degree of partial resistance but also in their degree of tolerance of that level of mildew infection, which did develop.

The most detailed study, clearly relating the amount of fungal biomass produced to effects on photosynthesis, was that of Sabri (Sabri, 1993; Sabri *et al.*, 1997). She showed that infection of susceptible wild and cultivated oat lines with *B. graminis* f.sp. *avenae* reduced the rates of gross and net photosynthesis in all lines, but to different extents in each. Fifteen days after inoculation, Pg_{max} expressed per unit area of leaf was reduced by about 36% in cv. Peniarth, but only about 27% in cv. Lustre, and about 26% in the wild oat. At these stages, cv. Peniarth had supported the production of about 3131 conidia mm^{-2} leaf, cv. Lustre about 3388 conidia mm^{-2} leaf and the wild oat about 3568 conidia mm^{-2} leaf. Photosynthesis in the wild oat was reduced the least yet it supported the production of the highest mildew biomass indicating that it was more tolerant of mildew infection than were the two cultivated oats. Also, cv. Lustre showed a higher level of tolerance than cv. Peniarth.

The inhibitory effects on photosynthesis demonstrated for leaf segments in the O_2 electrode are clearly not directly related to the amounts of mildew

biomass produced on the leaves. This raises the question of what properties or mechanisms the more tolerant line, B19909, possesses that protects its photosynthetic system better than those of the less tolerant cultivars.

In the present study, all measurements of photosynthesis were carried out using leaf segments under conditions of saturating CO_2 (Delieu & Walker, 1981) and so any changes in rate were unlikely to be due to reductions in CO_2 supply or enhanced photorespiration, but to changes within the chloroplasts. Two models, the linear and the non-linear, have been developed to describe the photosynthetic light-response curves obtained using the O_2 electrode. The linear model, rectangular hyperbola, derived by Rabinowich (1951) from the hyperbolic relationship between the rate of an enzyme-catalysed reaction and the concentration of its substrate, assumes that resistance to CO_2 diffusion to the carboxylation sites, θ, is equal to zero. However, the alternative, non-linear model (non-rectangular hyperbola), derived by Thornley (1976) and Marshall and Bisco (1980), assumes that the resistance to CO_2 diffusion to the carboxylation sites, expressed as the ratio of physical to total resistance ($\theta = r_p / r_p + r_x$), is always greater than zero as explained in detail in Materials and Methods. Both models were applied to analyse the photosynthetic light response curves (PLRC) obtained in this study and since the results were more consistent with the second than the first only the results for the second model are presented here. The results showed that the values of θ in the infected tissues were not significantly different from those in the uninfected tissues at any stage in wild lines B19909, I-17-40 and B8893, indicating that

the decline in the rate of photosynthesis was unlikely to be due to decreasing amounts of CO_2 reaching the carboxylation sites. However, θ values for cvs. Golden Promise, Prisma and Triumph were significantly higher in infected tissues than in the controls indicating that either the physical resistance to CO_2 reaching the carboxylation sites or biochemical resistance or both were altered by infection. According to the predicted effects of changes in the physical resistance (r_p) and biochemical resistance (r_x) on the rate of photosynthesis, and the value of θ, given in Appendix Table 12, it can be concluded that both r_p and r_x were affected by infection, and further that r_p was equal to r_x in cvs Golden Promise, Prisma and Triumph. This suggests that although CO_2 was used at saturating levels, CO_2 supply to the chloroplasts of infected leaves was still limiting in these cultivars. This was not the case in the wild lines, which showed no changes in the values of θ, indicating that the CO_2 supply was not limiting.

The value of θ was found to increase in leaves of sugar beet infected by beet yellows virus (Hall & Loomis, 1972) and peach infected by prune dwarf virus (Smith & Neales, 1977), but leaves of tobacco plants infected with Sonchus yellow net virus showed no significant effects (Askeer, 1993). Other studies on a range of hosts have found that infection by different pathogens may alter the value of θ, but the results are contradictory. For example, studies on mildew infections of wild and cultivated oats (Sabri, 1993; Sabri *et al.*, 1997) demonstrated that θ was not affected in the most tolerant wild oat and nor in cv. Lustre, but it was decreased by infection in the less tolerant cv.

Peniarth, although not until the twelfth day after inoculation just prior to the leaf tissue becoming flaccid and chlorotic. This result for cv. Peniarth is at variance with the increases found in the cultivated barleys in this study. How infection affects θ in some lines and not in others is not known.

Reductions in the maximum photochemical efficiency of photosynthetic O_2 evolution (quantum efficiency or quantum yield) in response to infection were revealed in this study. Thus, it was reduced less in line B19909 (27%) and line I-17-40 (28%) than in cv. Golden Promise (53%), cv. Prisma (46%) and cv. Triumph (30%). Such reductions indicate that, the reductions in photosynthesis could be due to either a decrease in the light-harvesting capacity of the chloroplasts, a reduction in photosynthetic electron transport rates, or, to a lesser extent, a decrease in carboxylation efficiency, or to a combination of these processes. At low light intensity, the number of quanta of light reaching the reaction centres determines the rate of electron transport and this is dependent upon the amount of light-harvesting complex present. In the present study, the decreased quantum efficiency resulting from infection could be due to a loss of light-harvesting complex, either from those complexes that are proximal or distal to the reaction centres of PSI and PSII and consequently to a reduced light-harvesting capacity.

Sabri (Sabri, 1993; Sabri *et al.*, 1997) in her comparative study of wild and cultivated oats showed similar results in response to mildew. Fifteen days after inoculation, the quantum yield in cv. Peniarth was reduced by about 27%, but in cv. Lustre and the wild oat it was only reduced by about 18%. In cv. Lustre the quantum efficiency continued to fall, to about 26% less than the

uninfected controls by twenty days after inoculation, but no further decrease occurred in the wild oat although it had supported the highest level of fungal biomass production. Reductions in quantum efficiency have been noted in other host parasite interactions e.g. *Erysiphe polygoni* on sugar beet and so such reductions may be a common response to infection in crop plants, but none of the studies have related the degree of reduction to the amount of parasite developed.

As indicated above the reductions in quantum efficiency could be to differences in effects on the light-harvesting capacity or simply to the loss of the photosynthetic units (chloroplasts), since the maximum rate of gross photosynthesis expressed per unit of chlorophyll was not altered at any stage in any line, except in one line. This line, the highly resistant line B8893 showed an increase in the rate of photosynthesis per unit of chlorophyll. However, the amount of chlorophyll present in the infected leaves of all lines was reduced by infection. For example, fourteen days after inoculation, the percentage reduction in total chlorophylls was about 41% in line B8893, about 62% in cv. Triumph and about 85% in cvs. Prisma and Golden Promise and in line B19909. At the time of the last measurement in line I-17-40, ten days after inoculation, the percentage reduction was about 77% (measurements were not continued after ten days in this line because the leaf had by then senesced). These results show that chlorophyll loss from cv. Prisma was as high in response to infection as that from line B19909 and cv. Golden Promise although cv. Prisma supported the lowest level of fungal biomass production.

The loss of chlorophyll involved both chlorophylls a and b in all lines with the loss of chlorophyll a being relatively greater than chlorophyll b in cvs Golden Promise, Triumph and line I-17-40. Thus, the reduced photochemical efficiency of these cultivars could be due to both the loss of the light-harvesting complex, which contains chlorophylls a and b, as well as the loss of chlorophyll a from the light-harvest complex more proximal to the photosystem PSII. However, this could not be the case in lines B19909 and cv. Prisma since in these lines infection reduced chlorophylls a and b equally indicating that chlorophyll a was lost from the light-harvesting complex but not from the PSII antenna matrices. Although the rate of photosynthesis in line B8893 was not affected by infection, the levels of both chlorophyll a and b fell with the former being more reduced. This suggests that despite this loss, the light-harvesting capacity was still as efficient as that of the uninfected controls.

In contrast to the finding in the present study which showed that the reduction in chlorophylls occurred to similar extents in both the more tolerant line B19909 and the less tolerant cvs Golden Promise and Prisma, Sabri *et al.* (1997) observed that the chlorophyll content of the wild oat, which appeared to be more tolerant of *B. graminis* f.sp. *avenae* infection than the two cultivated oats, was reduced the least. Although infection reduced both chlorophylls a and b, in the three oat lines they were reduced to similar extents. This is in agreement with the findings with cv. Prisma and line B19909 to mildew infection in the present study. Similarly, a study of barley plants infected with *B. graminis* f.sp. *hordei*, Holloway *et al.* (1992) showed

that both chlorophylls a and b were reduced following infection, but both to similar extents. In contrast, Scholes & Farrar (1985) observed that although both chlorophylls a and b declined in bluebell leaves infected with *Uromyces muscari*, chlorophyll a declined to the greater extent.

It was mentioned earlier that the differences in the mildew-induced suppression of photosynthesis rates (expressed per unit leaf area) were not apparent when they were expressed on a chlorophyll basis. However, measurements of chlorophylls a and b in the infected plants showed that in some cases differences were reflected in significantly reduced chlorophyll a : b ratios. Taken together, these two findings are difficult to reconcile. Infection induced differences in the per unit leaf area photosynthesis rates, but not the per milligram chlorophyll photosynthesis rates, can be explained simply by suggesting a loss of complete photosynthetic units from the photosynthetic membranes, or, more likely, the loss of whole chloroplasts (P. Dominy, personnel communication). However, this should not result in changes in the chlorophyll a : b ratios. But this study has presented evidence that significant decreases in chlorophyll a : b ratio accompany infection in cvs Golden Promise and Triumph, and in the wild lines I-17-40 and B8893. In these plants an increased loss of chlorophyll a over chlorophyll b, but no changes in maximum photosynthesis rates expressed on a per milligram chlorophyll basis is perplexing. The reasons for these discrepancies are unclear and a more detailed analysis of the types of pigment proteins present may be required before this issue can be fully resolved (P. Dominy, personnel communication).

Reductions in photosynthesis in whole leaves have been attributed to

reduced rates of CO_2 diffusion into the leaf through the stomata (Gordon & Duniway, 1982b). However, redcutions in photosynthesis found in the O_2 electrode studies could not have resulted solely from changes in stomatal resistance since, in these studies leaf segments not whole leaves were used.

Measurements of stomatal function in whole leaves of the three lines cv. Prisma and the two wild lines, B19909 and I-17-40 following inoculation showed significant alterations as the result of infection. Stomatal resistance in the light in the inoculated middle region of infected leaves was initially increased by infection in all three lines, but as the leaves became more heavily infected, it began to decrease to a level similar to that of uninfected plants in line B19909 but lower than the uninfected plants in cv. Prisma. In contrast, stomatal resistance in the dark in the inoculated middle regions of infected leaves began to fall in both line I-17-40 and cv. Prisma from two and four days after inoculation respectively, but not until seven days after inoculation in line B19909. However, stomatal resistance eventually fell to lower levels in line B19909 than in the other two lines.

The initial increase in stomatal resistance in infected leaves in the light is likely to reduce the diffusion of CO_2 to the mesophyll cells and could thus be partly responsible for the decline in photosynthesis that occurs following inoculation. However, the subsequent reductions in stomatal resistance should allow increased CO_2 uptake. Altered stomatal behaviour following infection could also be expected to alter the rate of transpiration and the leaf water content, and reductions in leaf water content could affect rates of photosynthesis.

The transpiration rate from leaves usually follows the pattern of stomatal behaviour (Ayres, 1976). Thus in mildew infected barley leaves transpiration would be expected to initially decrease in the light because infection causes the stomata to close. It would also increase in the dark, when the stomata failed to close completely. Changes in transpiration in infected leaves could also result partly from the increase in the boundary layer resistance caused by the presence of the fungal mycelium over the leaf surface and partly from the mycelium itself which also provides an increased route for water loss. In the light, stomatal resistance increased significantly from 24 hours after inoculation when mildew development was very limited in all three lines, suggesting that the stomata provided the main control over water loss during the early stages of infection. However, in the dark, stomatal resistance decreased from 48 hours after inoculation in cv. Prisma, but not until 72 hours after inoculation in line I-17-40 and 168 hours after inoculation in line B19909. Thus stomatal function was impaired at a much earlier stage in cv. Prisma than in leaves of line B19909, the latter being able to control water loss up to quite a late stage of infection when 25% or more of the leaf area was colonised by mildew.

The increased stomatal closure in the light from 24 hours after inoculation of barley leaves is in agreement with the observations of Majernik (1965) who found that *B. graminis* f.sp. *hordei* decreased stomatal opening of barley leaves from 24 hours after inoculation. Martin *et al.* (1975) also observed that mildew infection decreased stomatal opening but from around six hours after inoculation slightly earlier than reported by Majernik (1965). However,

neither of these studies related changes in stomatal function to the amount of mildew present at the time of stomatal resistance change and also they used different cultivars to the ones used here.

Infection also decreased stomatal opening in the light in mildewed pea leaves from three days after inoculation (Ayres, 1976) and from five days after inoculation in mildewed oat plants (Sabri, 1993). However, an initial increase in stomatal apperture was observed in pea leaves 48 hours after inoculation with *Erysiphe pisi* (Ayres, 1976) and in oat plants 72 hours after inoculation (Sabri, 1993). Martin *et al.* (1975) and Majernik (1965) in studies of wheat infected with *B. graminis* f.sp. *tritici* suggested that a volatile product of the fungus could be involved in the alteration of stomatal behaviour. If this is so, it may be that *B. graminis* f.sp. *hordei* produces a similar substance. However, other causes have been suggested, such as infection induced changes in the turgor pressures of the guard cells and of other epidermal cells (Ayres, 1976).

Mechanisms other than reductions in the rates of CO_2 diffusion into the leaf and in chlorophyll content could be responsible for the decline in photosynthesis in infected leaves. For example, decreases in the turnover of the C-3 cycle, either by a direct effect on one or more of the key steps in the process, or by a reduction in the supply of NADPH and/or ATP. However, these possibilities were not investigated in this study.

9.2.3. Effects on dark respiration

In contrast to the reductions in photosynthesis, the rates of dark

respiration increased significantly in the infected tissues of all lines except the highly resistant line B8893, with cv. Prisma being most affected. The maximum percentage increase in the rate of respiration was reached six days after inoculation in cv. Triumph and cv. Prisma, the rates being 64% higher in cv. Triumph and about 178% in cv. Prisma. The fungal biomass at this stage was about 4.8×10^4 conidia cm^{-2} leaf area in cv. Triumph and about 5.6×10^4 conidia in cv. Prisma. However, in the other lines the increase was slower reaching a maximum of 70% two days later in line I-17-40, 106% in line B19909 and 40% in cv. Golden Promise. The fungal biomass at this stage was about 4.4×10^4 conidia in I-17-40, about 9.3×10^4 in cv. Golden Promise but about 20×10^4 conidia in line B19909.

Increased respiration in leaves, is a feature of most infections including powdery mildew infections. Such increases were suggested to be required to provide the carbon skeletons needed for biosynthesis by both the host and the pathogen, the host for defence and the fungus for growth (Farrar & Raynes, 1987). The greater increase in respiration shown by the more tolerant line B19909 than the less tolerant cv. Golden Promise, could have been to meet the energy demand for the higher levels of fungal development than on the latter. The moderately resistant cv. Triumph showed a high rise in dark respiration even more than the very susceptible cv. Golden Promise, but this higher level was transient. Line I-17-40 which supported a relatively low level of mildew development and so appears to have some level of partial resistance, also expressed a high level of respiration. This respiration could reflect resistance reactions or intolerance to the fungus. The highly resistant

line B8893 showed no increase at all in respiration. These results show that the effect of infection on dark respiration differs from cultivar to cultivar, probably depending on the level of fungal biomass developed, level of tolerance of the host to the parasite as well as the level of active resistance expressed by the host.

While workers agree generally about respiratory responses in susceptible plants, there is much less agreement for resistant plants even among authors working with the same host-pathogen system. Working with a major-gene resistant cultivar of barley inoculated with an avirulent race of *B. graminis* f.sp. *hordei*, Smedegaard-Petersen (1980) and his group (1981) found a rapid initial rise in dark respiration, which returned to control rates a few days later. Millerd & Scott (1956) found a major gene resistant barley cultivar which supported no mildew growth showed the same pattern. However, Millerd & Scott (1956) found that a cultivar with high partial resistance in which cell collapse takes place over a long period of time as mildew progresses, showed no difference between control and infected in respiration rates up to four days after inoculation, and a less resistant cultivar, which supported some fungal growth and very slow cell collapse, reacted the same way as a fairly susceptible cultivar with an increase in respiration after 48 hours. Similarly, Scott & Smillie (1966) observed that a resistant barley cultivar showed a small early increase in the respiratory rate in response to mildew inoculation compared to the control, but the magnitude of this increase was less than the eventual rise in respiratory rate of a susceptible cultivar. *B. graminis* f.sp. *avenae* was also found to increase the rate of dark respiration nine days after

inoculation in two cultivated oats but not in the wild oat (Sabri *et al.*, 1997).

Haigh *et al.* (1991), measured changes in respiration in the fifth leaf of several oat cultivars that supported different levels of *B. graminis* f.sp. *avenae* growth, measured as the number of haustoria per unit leaf area. Cv. Mostyn with 28 haustoria mm^{-2} leaf area and line 1621 with 12.5 haustoria mm^{-2} leaf area had respiration rates increased by about 33% of the controls nine and three days after inoculation respectively. Similarly, cv. Maldwyn with 12.3 haustoria mm^{-2} leaf area, had its respiration rates had increased by about 30% by five days after inoculation. However, no effects on the rate of respiration were measured in the oat line 1674 which supported 22.5 haustoria mm^{-2}. It appears that the increases in respiration are not related to the amount of mildew development and so the differences between cultivars could reflect difference in tolerance.

The decline in photosynthesis following infection has been proposed to be a possible cause of respiratory increases in response to mildew infection in barley (Scott & Smillie, 1963). Thus, using mildewed barley leaves, Scott & Smillie (1966) suggested that the reduced rate of photosynthesis in a susceptible barley cultivar could lead to a higher $NADP^{+}$/NADPH ratio and consequently an increase in the activity of the pentose phosphate pathway. The pentose phosphate pathway has been found to be involved in the respiratory increases in other hosts following infection (Daly, 1976). Scholes & Farrar (1986) suggested that the loss of chlorophyll from infected leaves could result in an increased respiratory activity similar to that reported to occur during the senescence process (Farkas *et al.*, 1964).

9.3. Compensatory photosynthesis in uninfected leaves of infected plants

Many authors have shown that the rates of photosynthesis in uninfected parts of infected plants are stimulated by infection and clearly the more capacity a plant has for compensatory photosynthesis the higher the level of tolerance of infection it might possess. In the present study, infection of the three lower leaves was found to increase the rate of photosynthesis in an uninfected upper fourth leaf. The rates of maximum net and gross photosynthesis, expressed per unit leaf area, in the uninfected leaf on the infected plants were higher than in the corresponding leaf of uninfected plants in line B19909 but not in cv. Prisma and line I-17-40. The Quantum efficiency of photosynthesis (α) was also found to be slightly increased in the uninfected leaf of the infected plants of the wild barley B19909 but not in the other two lines. These increases may be attributed to an increase in light-harvesting capacity, a stimulation of photosynthetic electron transport, an increase in carboxylation efficiency, or a combination of these processes. When compared with controls, the uninfected fourth leaf of infected plants had similar chlorophyll levels and similar values of Pg_{max} and Pn_{max} expressed per unit chlorophyll. The ratios of physical resistance to total resistance to CO_2 diffusion into the leaf (θ) varied steadily between replicates but no significant differences were found between infected and uninfected plants in any line. Thus, the amount of CO_2 reaching the carboxylation sites appears not to be affected. Other mechanisms must be involved in the stimulation of

photosynthesis in uninfected parts of infected plants in line B19909.

Although infection of the lower leaves gave a consistent slight increase in dark respiration in the uninfected fourth leaf in cv. Prisma and line B19909, none of the differences were significant. Thus, apart from the increase in the rate of Pg_{max}, Pn_{max} and quantum efficiency in B19909, none of the parameters of photosynthesis measured were affected in any line.

These results are in line with those from previous studies, which have shown that powdery mildew infections of barley and also of pea stimulated photosynthesis in the upper, uninfected leaves of infected plants (Ayres, 1981b; Williams & Ayres, 1981; Walters & Ayres, 1983).

However, although line I-17-40 was shown in growth analysis experiments to compensate for the loss in vegetative growth caused in the early stages by mildew infection, no significant compensatory photosynthesis was observed in its uninfected fourth leaf on infected plants of this line. This could be explained by the fact that the mechanism or mechanisms responsible for compensatory photosynthesis occur in its higher order and flag leaf.

The stimulation of net photosynthesis in uninfected leaves of mildewed barley plants was attributed, in part, to an increase in the amount and activity of RuBPcase (Walters & Ayres, 1983). An increase in the activities of phospho-enol-pyruvate carboxylase (PePcase) and NADP malic enzyme was also observed. Walters (1985) suggested that the changes in the nitrate/ammonium balance in infected shoots may have affected the activities of RuBPcase. He suggested also that the increased uptake of ^{32}P-labelled phosphate in mildewed barley could stimulate photosynthesis, either by

increasing RuBPcase activity or by affecting the ratio of ATP/ADP.

The stimulation of photosynthesis in uninfected leaves of infected plants observed in wild barley B19909 and in other susceptible cultivars in other studies, may allow the plant to compensate for the loss of activity in the infected tissues and for the loss of photoassimilates to the pathogen. The increase in the compensatory photosynthesis in uninfected tissues of infected plants of the wild barley B19909 must play at least some part in compensating for plant growth losses to the pathogen. Thus compensatory activity may play a role in tolerance.

9.4. Conclusions

In conclusion, this study has shown that growth and development as well as photosynthesis, respiration and stomatal function responded differently to given levels of mildew infection in the different lines (see Appendix Tables 13 to 16). The wild line B19909 was the least affected by even heavy levels of fungus development indicating that this line is more tolerant of infection than the other lines. These results support the suggestion that some wild relatives of crop plants may possess higher levels of tolerance of parasites than crop plants do. Since tolerance was reflected in the responses of a range of growth and metabolic processes, it is likely to be determined polygenically and therefore it could be difficult to breed for. However, a genetic analysis of tolerance in the wild barley line B19909 is urgently needed to see how tolerance is inherited and this could indicate the potential value of tolerance as a breeding objective for crop improvement.

The fact that tolerance is not likely to provide a high level of disease protection suggests that it should be used in combination with other measures such as incomplete resistance, variety mixtures and fungicide.

References

Agrios, G. N. (1997) Plant Pathology. Fourth Edition. pp. 607-618. Academic Press. USA.

Ahmad, I.; Farrar, J. F. & Whitbread, R. (1983) Photosynthesis and chloroplast functioning in leaves of barley infected with brown rust. *Physiological Plant Pathology* **23,** 411-419.

Ahmad, I.; Farrar, J. F. and Whitbread, R. (1984) Fluxes of phosphorus in leaves of barley infected with brown rust. *New Phytologist* **98,** 361-375.

Ahmad, I.; Owera, S. A. P.; Farrar, J. F. and Whitbread, R. (1982) The distribution of five major nutrients in barley plants infected with brown rust. *Physiological Plant Pathology* **21,** 335-346.

Allen, P. J. & Goddard, D. R. (1938) A respiratory study of powdery mildew of wheat. *American Journal of Botany* **25,** 613-621. Cited in **Scott, K. J.** (1972).

Allen, P. J. (1942) Changes in the metabolism of wheat leaves induced by infection with powdery mildew. *American Journal of Botany* **29,** 425-435.

Allen, P. J. (1953) Toxins and tissue respiration. *Phytopathology* **43,** 221-229.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidases in Beta vulgaris. *Plant Physiology* **24,** 1-14.

Asher, M. J. C. & Thomas, C. E. (1987) The inheritance of mechanisms of partial resistance to *Erysiphe graminis* in spring barley. *Plant Pathology* **36,** 66-72

Asher, M. J. C. (1972) Effect of *Ophiobolus graminis* infection on the growth of wheat and barley. *Annals of Applied Biology* **70,** 215-23.

Askeer, F. A. (1993) Symptoms expression and virus multiplication in Rhabdovirus-infected plants. Ph.D. Thesis. Department of Botany, University

of Glasgow.

Ayres, P. G. (1981b) Powdery mildew stimulated photosynthesis in uninfected leaves of pea plants. *Phytopathologische Zeitschrift* **100**, 312-318.

Ayres, P. G. & Jones, P. (1975) Increased transpiration and the accumulation of root absorbed ^{86}Rb in barley leaves infected by *Rhyncosporium secalis* (leaf blotch). *Physiological Plant Pathology* **7**, 49-58.

Ayres, P. G. (1972) Abnormal behaviour of stomata in barley leaves infected with *Rhynchosporium secalis* (Oudem) J. J. Davis. *Journal of Experimental Botany* **23**, 683-691.

Ayres, P. G. (1976) Patterns of stomatal behaviour, transpiration, and CO_2 exchange in pea following infection by powdery mildew (*Erysiphe graminis* f.sp. *pisi*). *Journal of Experimental Botany* **27**, 354-363.

Ayres, P. G. (1978) Water Relations of Diseased plants. In: Water Deficits and Plant Growth. Vol. 5. Water and Plant Disease. Ed. by Kozlowski, T. T. pp. 1-60. Academic Press. London.

Ayres, P. G. (1979) CO_2 exchanges in plants infected by obligately biotrophic pathogens. In: Photosynthesis and Plant Development. pp. 343-354. Ed. by Marcelle, R.; Clijsters, H. and Van Poucke, M. Junk, The Hague.

Ayres, P. G. (1980) Responses of stomata to pathogenic microorganisms. In: Stomatal physiology. Ed. by Jarvis, P. G. & Mansfield, T. A. pp. 205-221. Cambridge University Press. Cambridge.

Ayres, P. G. (1981a) Effects of disease on plant relations. In: Effects of disease on the physiology of growing plant. pp. 131-148. Ed. by Ayres, P. G. Cambridge University Press.

Ayres, P. G. (1984) Effects of infection on root growth and function; consequences for plant nutrient and water relations. In: Plant diseases Infection, damage and loss. pp. 105-117. Eds. Wood, R. K. S. and Jellis G. J.

Blackwell Scientific Publications. Oxford.

Ayres, P. G.; Press, M. C. and Spencer-Phillips, P. T. N. (1996) Effects of pathogens and parasitic plants on source-sink relationships. In: Photoassimilate distribution in plants and crops source-sink relationships. pp.479-499. Ed. by Zamski, E. & Schaffer, A. A. Marcel Dekker, USA.

Ayres, P.G. & Zadoks, J. C. (1979) Combined effects of powdery mildew disease and soil water level on the water relations and growth of barley. *Physiological Plant Pathology* **14,** 347-361.

Bengtsson, B. O. (1992) Barley genetics. *Trends in Genetics.* **8,** 3-5.

Ben-Kalio, V. D. & Clarke, D. D. (1979) Studies on tolerance in wild plants: Effects of *Erysiphe fischeri* on the growth and development of *Senecio vulgaris. Physiological Plant Pathology* **14,** 203-211.

Ben-Kalio, V. D. (1976) Effects of powdery mildew (*Erysiphe cichoracearum* D.C Ex Merat) on the growth and development of groundsel (*Senecio vulgaris* L.). Ph.D Thesis. Botany department. University of Glasgow.

Bennet,J. & Scott, K. J. (1971) Inorganic polyphosphates in the wheat stem rust fungus and in rust infected wheat leaves. *Physiological Plant Pathology* **1,** 185-198.

Biscoe, P. V.; Gallagher, J. N.; Littleton, E. J.; Monteith, J. L. and Scott, R. K. (1975) *Journal of Applied Ecology* **12,** 295-318. Cited in **Marshall & Biscoe** (1980).

Blackman, F. F. (1905) Optima and Limiting Factors. *Annals of Botany* **19,** 281-295.

Blackman, V. H. (1919) The compound interest law and plant growth. *Annals of Botany* **33,** 353-360.

Braun, U (1995) The Powdery Mildews (Erysiphales) of Europe. pp. 147. Gustave Fischer Verlag, Germany.

Briggs, G. E.; Kidd, F. & West, C. (1920b) A quantitative analysis of plant growth. *Annals of Applied Biology* 7, 202-223.

Brooks, D. H. (1970) Powdery mildew of barley and its control. *Outlook on Agriculture* **6,** 122-127.

Brooks, D. H. (1972) Observations on the effects of mildew, Erysiphe graminis, on growth and of spring and winter barley. *Annals of Applied Biology* **70,** 149-156.

Buchanan, B. B.; Hutcheson, S. W.; Magyarosy, A. C. and Montalbini, P. (1981) Photosynthesis in healthy and diseased plants. In: Effects of disease on the physiology of growing plant. pp. 12-28. Ed. by Ayres, P. G. Cambridge University Press.

Bushnell, W. R. & Allen, P. J. (1962) Induction of disease symptoms in barley by powdery mildew. *Plant Physiology* **37,** 50-59.

Bushnell, W. R. (1970) Patterns in growth, oxygen uptake, and nitrogen content of single colonies of wheat stem rust on wheat leaves. *Phytopathology* **60,** 92-99.

Caldwell, R. M.; Kraybill, H. R.; Sulivan, J. T. and Compton, L. E. (1934) Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. *Journal of Agricultural Research* **48,** 1049-71. Cited in **Schafer** (1971).

Cameron, P. (1993) The relative tolerance of two barley cultivars to powdery mildew. Unpublished undergraduate studies. University of Glasgow.

Carver, T. L. W. & Griffiths, E. (1981) Relationship between powdery mildew infection, GLA and grain yield of barley. *Annals of Applied Biology* **99,** 255-266.

Carver, T. L. W. & Griffiths, E. (1982) The effects of barley mildew on GLA and grain yield in field and greenhouse experiments. *Annals of Applied Biology* **101,** 561-572.

Chakravorty, A. K. & Scott, K. J. (1982) Biochemistry of host rust interactions: Changes in the gene expression of host plants during the early stages of rust infection. In The rust fungi. Ed. by Scott, K. J. & Chakravorty, A. K. Academic Press London.

Chartier, M. & Catsky, J. (1970) A model of CO_2 assimilation in the leaf. In: Prediction and Measurement of Photosynthetic Productivity. Ed. Setlik, I. pp. 307-315. Pudoc, Wageningen.

Clarke, D. D. (1988) Tolerance of pathogenic infections in plants and its potential for crop protection. *Aspects of Applied Biology* **17,** 125-132.

Clarke, D.D. (1986) Tolerance of parasites and disease in plants and its significance in host-parasite interactions. In: Advances in Plant Pathology. Vol. 5. pp. 161-198. Ed. by Ingram, D. S. & Williams, P. H. Academic Press. London.

Clarke, H. H. (1967) *Agricultural History Review* **15,** 1-18. Cited in Barley. Ed. by Briggs, D. E. (1978). pp. 76-88, Chapman & Hall, London.

Clarkson, D. T.; Drew, M. C.; Ferguson, I. B. and Sanderson, J. (1975) The effect of take-all fungus, *Gaeumnanomyces graminis*, on the transport of ions by wheat plants. *Physiological Plant Pathology* **6,** 75-84.

Coombe, D. E. (1960) An analysis of the growth of *Trema guineensis*. *Journal of Ecology* **48,** 219-231.

Crowdy, S. H. & Manners, J. G. (1971) Microbial disease and plant productivity. In: Symposium 21, Society for General Microbiology. Ed. by Hughes, D. E. & Rose, A. H.

Cruickshank, I. A. M. & Rider, N. E. (1961) *Peronospora tabacina* in

tobacco: Transpiration, growth and related energy considerations. *Australian Journal of Biological Science* **14,** 45-57.

Daly, J. M. (1976) The Carbon Balance of Diseased Plant Changes in Respiration, Photosynthesis and Translocation. In: Encyclopedia of Plant Physiology. Vol. 4, pp. 450-479. Ed. by Heitefuss, R. & Williams, P. H. Springer-Verlag, Berlin.

Davidson, R. L. (1969) Effect of root / leaf temperature differential on shoot / root ratios in some pasture grasses and clover. *Annals of Botany* **33,** 561-569.

Delieu and Walker, (1972) An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. *New Phytologist* **71,** 201-225.

Delieu, T. & Walker, D. A. (1981) Polarographic measurement of photosynthetic O2 evolution by leaf discs. *New Phytologist* **89,** 165-175.

Delieu, T. & Walker, D. A. (1983) Simultaneous measurement of O_2-evolution and chlorophyll fluorescence from leaf pieces. *Plant Physiology* **73,** 534-541.

Doodson, J. K.; Manners, J. G. and Myers, A. (1964) Some effects of yellow rust (*Puccinia stiiformis*) on the growth and yield of spring wheat. *Annals of Botany* **28,** 459-472.

Doodson, J. K.; Manners, J. G. and Myers, A. (1965) Some effects of yellow rust (*Puccinia stiiformis*) on ^{14}C assimilation and translocation in wheat. *Journal of Experimental Botany* **16,** 304-317.

Duniway, J. M. & Durbin, R. D. (1971a) Some effects of *Uromyces phaseoli* on the transpiration rate and stomatal responses of bean leaves. *Phytopathology* **61,** 114-119.

Duniway, J. M. & Durbin, R. D. (1971b) Detrimental effect of rust

infection on the water relations of bean. *Plant Physiology* **48,** 69-72.

Duniway, J. M. & Slatyer, R. O. (1971) Gas exchange studies on the transpiration and photosynthesis of tomato leaves (infected) affected by *Fusarium oxysporum* f.sp. *lycopersici. Phytopathology* **61,** 1377-1381.

Dyer, T. A. & Scott, K. J. (1972) Decrease in chloroplast polysome content of barley leaves infected with powdery mildew. *Nature* **236,** 237-8.

Edwards, H. H. (1970) Biphasic inhibition of photosynthesis in powdery mildewed barley. *Plant Physiology* **45,** 594-597.

Edwards, H. H. (1971) Translocation of carbon in powdery mildewed barley. *Plant Physiology* **47,** 324-328.

Edwards, H. H. and Allen, P. J. (1966) Distribution of the products of photosynthesis between powdery mildew and barley. *Plant Physiology* **41,** 683-688.

Ellis, M. A.; Ferree, C. D. and Spring, D. E. (1981) Photosynthesis, transpiration and carbohydrate content of apple leaves infected by powdery mildew (*Podosphaera leucotricha). Phytopathology* **71,** 392-395.

Evans, G. C. (1972) The quantitative Analysis of Plant Growth. Blackwell Scientific Publications.

Farkas, G. L.; Dezsi, L.; Horvath, M.; Kisban, K. and Udvardy, J. (1964) Common pattern of enzymic changes in detached leaves and tissues attacked by parasites. *Phytopathologische Zeitschrift* **49,** 343-354.

Farrar, I. J. & Lewis, D. H. (1987) Nutrient relations in pathogenic and mutualistic associations. In: Fungal infection of plants. Ed. by Pegg, G. F. & Ayres, P. G. pp. 92-132. Cambridge Cambridge University Press.

Farrar, J. F. & Rayns, F. W. (1987) Respiration of leaves of barley infected with powdery mildew Increased engagement of the alternative oxidase. *New*

Phytologist **107**, 119-125.

Farrar, J. F. (1984) Effects of pathogens on plant transport systems. In: Plant diseases Infection, damage and loss. pp. 87-104. Eds. Wood, R. K. S. and Jellis G. J. Blackwell Scientific Publications. Oxford.

Farrar, J. F. (1985) Carbohydrate metabolism in biotrophic plant pathogens. *Microbiological Sciences* **2**, 314-317.

Farrar, J. F. (1992) Beyond photosynthesis The translocation and respiration of diseased leaves. In: Pests and Pathogens Plant Responses to Foliar Attack. Ed. by Ayres, P. G. pp. 107-124. Bios Scientific Publishers Ltd., Oxford.

Farrell, G. M.; Preece, T. F and Wren, M. J. (1969) Effects of infection by *Phytophtora infestans* (Mont.) De Bary on the stomata of potato leaves. *Annals of Applied Biology* **63**, 265-275.

Finney, M. E. (1973) Growth analysis of barley (cv. Zephyr) infected with *Erysiphe graminis* DC. PhD. Thesis, University of London.

Fisher, R. A. (1921) Some remarks on the methods formulated in a recent article on the ' Quantitative Analysis of Plant Growth'. *Annals of Applied Biology* 7, 367-372.

Fitt, B. D. L. & Hornby, D. (1978) Effects of root-infecting fungi on wheat transport processes and growth. *Physiological Plant Pathology* **13**, 335-346.

Fraser, J. M. (1981) Astudy of the effects of powdery mildew *Sphaerotheca epilobii* on the growth and development of *Epilobium montanum.* Unpublished undergraduate studies. University of Glasgow.

Fric, F. (1975) Translocation of ^{14}C-lablled assimilates in barley plants infected with powdery mildew (*Erysiphe graminis* f.sp. *hordei* Marchal). *Phytopathologische Zeitschrift* **84**, 88-95.

Gerwitz, R. & Dubrin, R. D. (1965) The influence of rust on the distribution of ^{32}P in the bean plant. *Phytopathology* **55**, 57-61.

Giese, H. (1981) Powdery mildew resistance genes in the Ml-a and Ml-k regions on barley chromosomes 5. *Hereditas* **95,** 51-62.

Giese, H., Jørgensen, J. H., Jensen, H. P. and Jensen, J. (1981) Linkage relatioships of ten powdery mildew resistance genes on barley chromosome 5. *Hereditas* **95,** 43-50.

Gordon, T. R. & Duniway, J. M. (1982a) Photosynthesis in powdery mildewed sugar beet leaves. *Phytopathology* **72,** 718-723.

Gordon, T. R. & Duniway, J. M. (1982b) Effects of powdery mildew infection on the efficiency of CO_2 fixation and light utilisation by sugar beet leaves. *Plant Physiology* **69,** 139-142.

Griffiths, E.; Jones, D. G. and Valentine, M. (1975) Effects of powdery mildew at different growth stages on grain yield of barley. *Annals of Applied Biology* **80,** 343-349.

Groth, J. V. (1976) Multilines and "super races" A simple model. *Phytopathology* **66,** 937-939.

Habeshaw, D. (1979) The effect of foliar pathogens on the leaf photosynthetic carbon dioxide uptake of barley. In: Photosynthesis and plant development. pp. 355-373. Ed. by Marcelle, R.; Clijsters, H. and Van Poucke. Junk. The Hague.

Habeshaw, D. (1984) Effects of pathogens on photosynthesis. In: Plant diseases. Infection, damage and loss. pp. 63-72. Ed. by Wood, R. K. S. & Jellis, G. J. Blackwell Scientific Publications.

Haigh, G. R.; Carver, T. L. W.; Gay, A. P. and Farrar, J. F. (1991) Respiration and photosynthesis in oats exhibiting different levels of partial resistance to *Erysiphe graminis* D.c. ex. Merat f.sp. *avenae* Marchal. *New Phytologist* **119,** 129-136.

Hall, A. E. & Loomis, R. S. (1972) An explanation for the difference in

photosynthetic capabilities of healthy and beet yellow virus-infected sugar beets (*Beta vulgaris* L.). *Plant Physiology* **50,** 576-580.

Harlan, J. R. (1995) Barley. In: Evolution of crop plants. Eds, Smartt, J. & Simmonds, N. W. London Longman, 140-147.

Harlan, J. R.. & Zohary, D. (1966) Science, 153, 1074-1080. Cited in Barley. Ed. by Briggs, D. E. (1978). pp. 76-88, Chapman & Hall, London.

Harris, G. A. & Campbelle, G. S. (1989) Automated quantification of roots using a simple image analyser. *Agronomy Journal* **81,** 935-938.

Harry, I. B. & Clarke, D. D. (1992) The effects of powdery mildew (*Erysiphe fischeri*) infection on the development and function of leaf tissue by Senecio vulgaris. *Physiological and Molecular Plant Pathology* **40,** 211-224.

Harry, I. B. (1980) Studies on the *Senecio vulgaris* L.-*Erysiphe fischeri* Blumer, Plant Pathosystem. Ph.D. Thesis. Department of Botany. University of Glasgow.

Hewitt, H. & Ayres, P. G. (1977) Changes in Glycolate Oxidase and Glyoxylate Reductase in *Quercus robur* infected by *Microsphaera alphitoides* (powdery mildew). *Physiologia Plantarum* **41,** 25-28.

Hewitt, H. G. & Ayres, P. G. (1975) Changes in CO_2 and water vapour exchange rates in leaves of *Quercus robur* infected by *Microsphaera alphitoides* (Powdery mildew). *Physiological Plant Pathology* **7,** 127-137.

Hewitt, H. G. & Ayres, P. G. (1976) Effect of infection by *Microsphaera alphitoides* (powdery mildew) on carbohydrate levels and translocation in seedlings of *Quercus robur*. *New Phytologist* 77, 379-390.

Hewitt, H. G. (1976) The effects of infection by *Microshaera alphitoides* upon the physiology and growth of *Quercus robur*. Ph.D. Thesis. University of Lancaster. Cited in **Walters** (1985).

Hibberd, J. M.; Richardson, P.; Whitbread, R. and Farrar, J. F. (1996) Effects of leaf age, basal meristem and infection with powdery mildew on photosynthesis in barley grown in 700 μmol mol^{-1} CO_2. *New Phytologist* **134,** 317-325.

Higgins, C. M.; Manners, J. M. and Scott, K. J. (1985) Decrease in three messenger RNA species coding for chloroplast proteins in leaves of barley infected with *Erysiphe graminis* f.sp. *hordei. Plant Physiology* **78,** 891-894.

Hipkins, M. F. & Baker, N. R. (1986) Photosynthesis Energy transduction, a practical approach. pp. 62-64. IRL Press.

Holden, M. (1965) Chlorophylls. In: Chemistry and Biochemistry of Plant Pigments. Ed. by Goodwin, T. W. pp. 461-485. Academic Press. London and New York.

Holloway, P. J.; Maclean, D. J. and Scott, K. J. (1992) Electron transport in thylakoids isolated from barley leaves infected by the powdery mildew fungus (*Erysiphe graminis* DC. ex Merat f.sp. *hordei* Marchal). *New Phytologist* **120,** 145-151.

Huang, R.; Kranz, J. and Welz, H. G. (1994) Selection of pathotypes of *Erysiphe graminis* f.sp. *hordei* in pure and mixed stands of spring barley. *Plant Pathology* **43,** 458-470.

Hughes, A. P. & Evans, G. C. (1962) Plant Growth and the aerial environment II. Effects of light intensity on *Impatiens parviflora. New Phytologist* **61,** 154-174.

Hunt, R. (1978) Plant Growth Analysis. Studies in Biology No 96. Edward Arnold.

Hunt, R. (1990) Basic Growth Analysis. Plant Growth Analysis for Beginners. London. Unwin Hyman.

Jenkyn, J. F. (1976) Effects of mildew (*Erysiphe graminis*) on green leaf

area of Zephyr spring barley, 1973. *Annals of Applied Biology* **82,** 485-488.

Jenkyn, J. F.; Hirst, J. M. and King, G. (1973) An apparatus for the isolated propagation of foliar pathogens and their hosts. *Annals of Applied Biology* **73,** 9-13.

Johnson, C. O. & Miller, E. C. (1934) Relation of leaf-rust infection to yield, growth and water economy of two varieties of wheat. *Journal of Agricultural Research* **49,** 955-981. Cited in **Walters** (1985).

Johnson, C. O. & Miller, E. C. (1940) Modification of diurnal transpiration in wheat by infections of *Puccinia triticina. Journal of Agricultural Research* **61,** 427-444. Cited in **Walters** (1985).

Jones, G. D. & Clifford, B. C. (1983a) Cereal diseases: Their Pathology and Control. Ed. by Jones, G. D. & Clifford, B. C. pp. 37-56. John Wiley & Sons Ltd.

Jones, G. D. & Clifford, B. C. (1983b) Cereal diseases: Their Pathology and Control. Ed. by Jones, G. D. & Clifford, B. C. pp. 102-141. John Wiley & Sons Ltd.

Jones, G. D. (1987) Disease resistance. In: Plant pathology, principles & practice. Ed. by D. Gareth Jones. pp. 52-72. Open University Press. Milton Keynes. England.

Jørgensen, J. H. (1994) Genetics of powdery mildew resistance in barley. *Critical Review of Plant Science* **13,** 97-119.

Kakkar, R. K. (1966) Decline in glycolic acid oxidase activity in flax leaves infected with *Melampsora lini* (Pers.). *Phytopathologische Zeitschrift* **55,** 172-176.

King, J. E. (1972) Surveys of foliar diseases of spring barley in England and Wales 1967-70. *Plant Pathology* **21,** 23-35.

King, J. E. (1977) Surveys of foliar diseases of spring barley in England and Wales 1972-75. *Plant Pathology* **26,** 21-29.

Kiraly, Z & Farkas, G. L. (1957) Decrease in glycolic acid oxidase activity in wheat leaves infected with *Puccinia graminis* f.sp. *tritici. Phytopathology* **47,** 277-278.

Kosuge, T. & Kimpel, J. A. (1981) Energy use and metabolic regulation in plant-pathogen interactions. In: Effects of disease on the physiology of growing plant. pp. 29-45. Ed. by Ayres, P. G. Cambridge University Press.

Kramer, B. H.; Gildemacher, M.; Van Der ster and Parlevliet, J. E. (1980) Tolerance of spring barley cultivars to leaf rust, *Puccinia hordei. Euphytica* **29,** 209-216.

Kramer, P. J. (1983) Water Deficits and Plant Growth. In: Water Relations of Plants. Ed. by Kramer, P. J. pp. 342-389. Academic Press. London.

Krog, N. E.; Le tourneau, D. and Helen, H. (1961) The sugar content of wheat leaves infected with stem rust. *Phytopathology* **51,** 75-77.

Last, F. T. (1962) Analysis of the effects of *Erysiphe graminis* DC. on the growth of barley. *Annals of Botany* N.S. **26,** 279-289.

Last, F. T. (1963) Metabolism of barley leaves inoculated with *Erysiphe graminis* Mérat. *Annals of Botany* **27,** 685-690.

Lewis, S. J. & Deacon, J. W. (1982) Effects of shading and powdery mildew infection on senescence of the root cortex and coleoptile of wheat and barley seedlings, and implications for root- and foot-rot fungi. *Plant & Soil* **69,** 401-411.

Lim, L. G. & Gaunt, R. E. (1986) The effect of powdery mildew (*Erysiphe graminis* f.sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. I Epidemic development, green leaf area and yield. *Plant Pathology* **35,** 44-53.

Livne, A. & Daly, J. M. (1966) Translocation in healthy and rust affected beans. *Phytopathology* **56,** 170-175.

Livne, A. (1964) Photosynthesis in healthy and rust affected plants. *Plant Physiology* **39,** 614-621.

Lupton, F. G. H. & Sutherland, J. (1973) The influence of powdery mildew (*Erysiphe graminis*) infection on the development of four spring wheats. *Annals of Applied Biology* **74,** 35-39.

Mackinney, G. (1941) Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* **140,** 315-32.

Magyarosy, A. C. & Malkin, R. (1978) Effect of powdery mildew infection of sugar beet on the content of electron carriers in chloroplasts. *Physiological Plant Pathology* **13,** 183-188.

Magyarosy, A. C.; Schűrmann, P. and Buchanan, B. B. (1976) Effects of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. *Plant Physiology* **57,** 486-489.

Majernik, O. (1965) Water balance changes of barley infected by *Erysiphe graminis* D. C. f.sp. *hordei* Marchal. *Phytopathologische Zeitschrift* **53,** 145-153.

Majernik, O. (1971) A physiological study of the effects of SO_2 pollution, phenylmercuric acetate sprays, and parasites on stomatal behaviour and ageing in barley. *Phytopathologische Zeitschrift* **72,** 255-268.

Manners, J. G. & Myers, A. (1973) Means by which Cereal Rusts and Smuts Affect Host Translocation and Growth. Ed. by Byrde, R.J.W. and Cuttings, C.V. pp. 319-330. Academic Press, London.

Manners, J. G. (1993) Principles of Plant Pathology. Cambridge University Press.

Manners, J. M. & Scott, K. J. (1984) The effect of infection by *Erysiphe graminis* f.sp. *hordei* on protein synthesis in vivo in leaves of barley. *Plant and Cell Physiology* **25(7),** 1307-1311.

Marshall, B. & Biscoe, P. V. (1980) A model for C3 leaves describing the dependence of net photosynthesis on irradiance I- Derivation. *Journal of Experimental Botany.* **31**(120), 29-39.

Martin, J. F.; Stuckey, R. E.; Safir, G. R. and Ellingboe, A. H. (1975) Reduction of transpiration from wheat caused by germinating conidia of *Erysiphe graminis* f.sp. *tritici. Physiological Plant Pathology* **7,** 71-77.

Mason, P. A. (1973) Studies on the biology of *Bremia lactucae* (Regel). Ph.D. Thesis. University of Cambridge. Cited in **Walters** (1985).

McAinsh, M. R.; Ayres, P. G. and Hetherington, A. M. (1989) The respiration of protoplasts from leaves of barley infected by powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Plant Science* **64,** 221-230.

McIntosh, R. A. (1978) Breeding for resistance to powdery mildew in the temperate cereals. In: The powdery mildews. Edited by Spencer, D. M. (Acad. Press. London), pp. 238-257.

Meek, A. (1981) An investigation into the effects of *Erysiphe graminis* DC. on aspects of the growth and development of *Agropyron repens* (L.) Beauv. and barley. Unpublished undergraduate studies. University of Glasgow.

Millerd, A. & Scott, K. J. (1956) Host-pathogen relations in powdery mildew of barley II. Changes in respiratory pattern. *Australian Journal of Biological Science* **9,** 37-44.

Minarčic, P & Paulech, C. (1975) Influence of powdery mildew on mitotic cell division of apical root meristems of barley. *Phytopathologische Zeitschrift* **83,** 341-347.

Misaghi, I. J. (1982) Physiology and biochemistry of plant-pathogen

interactions. Plenum Press, New York and London. Cited in **Scholes** (1985).

Mitchell, D. T. (1979) Carbon dioxide exchange by infected first leaf tissues susceptible to wheat stem rust. *Transactions British Mycological Society* **72,** 63-68.

Montalbini, P. & Buchanan, B. B. (1974) Effect of a rust infection on photophosphorylation by isolated chloroplasts. *Physiological Plant Pathology* **4,** 191-196.

Moseman, J. G. (1957) Host-parasite interactions between culture 12A1 of the powdery mildew fungus and the Ml_k and ML_g genes in barley. *Phytopathology* **47,** 453.

Moseman, J. G. (1959) Host-parasite interaction of the genes for resistance in Hordeum vulgare and for pathogenicity in *Erysiphe graminis* f.sp. *hordei*. *Phytopathology* **49,** 469-472.

Murphy, H. C. (1935) Effect of crown rust infection on yield and water requirement of oats. *Journal of Agricultural Research* **50,** 387-401. Cited in **Walters** (1985).

Murphy, H. C. (1936) Effects of crown rust on the composition of oats. Phytopathology **26,** 220-234.

Newman, E. L. (1966) A method for estimating the total length of root in a sample. *Journal of Applied Ecology* **3,** 139-145.

Newton, M.; Peturson, B. and Meredith, W. O. S. (1945) *Canadian Journal of Research* **C, 23,** 212-218. Cited in **Clarke** (1986).

Owera, S. A. P.; Farrar, J. F. and Whitbread, R. (1981) Growth and photosayntheiss in barley infected with brown rust. *Physiological Plant Pathology* **18,** 79-80.

Parlevliet, J. E. (1981) Race non-specific disease resistance. In: Strategies

for the control of cereal disease. Edited by Jenkyn, J. F. & Plumb, R. T. pp. 47-54. Blackwell Scientific Publications. Oxford.

Paul, N. D. & Ayres, P. G. (1984) Effects of rust and post-infection drought on photosynthesis, growth and water relations in groundsel. *Plant Pathology* **33,** 561-569.

Paul, N. D.; Ayres, P. G. and Wyness, L. E. (1989) On the use of fungicides for experimentation in natural vegetation. *Functional Ecology* **3,** 759-769.

Paulech, C. & Haspelová - Harvatoviăová, A. (1970) Photosynthesis, plant pigments and transpiration in healthy barley and barley infected with powdery mildew. *Biologia, Bratislava.* **25,** 477-487.

Paulech, C. (1969) Influence of *Erysiphe graminis* DC. On the amount of dry substance and on the growth of vegetative organs. *Biologia, Bratislava* **24,** 709-720. Cited in **Scott & Griffiths** (1980).

Peat, W. E. (1970) Relationships between photosynthesis and light intensity in the tomato. *Annals of Botany* **34,** 319-328.

Person, C.; Samborski, D. J. and Forsyth, F. R. (1957) Effects of benzimidazole on detached wheat leaves. *Nature* **180,** 1294.

Poszar, B. I. & Király, Z. (1958) Effects of rust infection on oxidative phosphorylation of wheat leaves. *Nature* **182,** 1686-7.

Prakash, N. (1986) Mehtods in Plant Microtechniques. pp. 52. Second edition. The university of New England. Armidale, N.S.W. Department of Botany.

Rabinowitch, E. I. (1951) Photosynthesis and related processes. **Vol. 2(1),** pp. 831-1191. Inter Science, New York.

Radford, P. J. (1967) Growth analysis formulae Their use and abuse. *Crop*

Science **7,** 171-175.

Raggi, V. (1980) Correlation of CO_2 compensation point (Γ) with photosynthesis and respiration and CO_2 sensitive in rust-infected bean leaves. *Physiological Plant Pathology* **16,** 19-24.

Rea, B. L. & Scott, R. K. (1973) The effects of mildew (*Erysiphe graminis*) on leaf growth and yield of spring barley. Proceedings of the 7th British Insecticide and Fungicide Conference, pp. 29-37.

Reyes,A. A. (1975) Phytotoxicity of Benomyl to crucifers. *Phytopathology* **65,** 535-539.

Richards, D. (1977) Root : Shoot interactions. A functional equilibrium for water uptake in peach (*Prunus persica* L. Batsch). *Annals of Botany* **41,** 279-281.

Richards, D. (1978) Root : Shoot interactions. Functional equilibria for nutrient uptake in peach (*Prunus persica* L. Batsch). *Annals of Botany* **42,** 1039-1043.

Roberts, A. M. & Walters, D. R. (1988) Photosyntheisis in discrete regions of leek infected with the rust, *Puccinia allii* Rud. *New Phytologist* **110,** 371-376.

Roberts, J.J & Caldwell, R. M. (1970) General resistance (slow mildewing) to *Erysiphe graminis* f.sp. *tritici* in Knox wheat. *Phytopathology* **60,** 1310 (Abstract).

Russell, G. E.; Andrews, C. R. and Bishop, C. D. (1975) Germination of *Erysiphe graminis* f.sp. *hordei* conidia on barley leaves. *Annals of Applied Biology* **81,** 161-169.

Ryrie, I. J. & Scott, K. J. (1968) Metabolic regulation in diseased leaves II. Changes in nicotinamide coenzymes in barley leaves infected with powdery

mildew. *Plant Physiology* **43**, 687-692.

Sabri, N. & Clarke, D. D. (1995) The relative tolerances of wild and cultivated oats to infection by *Erysiphe graminis* f.sp. *avenae* I. The effects of infection on vegetative growth and yield. *Physiological and Molecular Plant Pathology* **49**, 405-421.

Sabri, N. (1993) A study of the effects of powdery mildew *Erysiphe graminis* f.sp. *avenae*, on the growth and development of wild and cultivated oats. Ph.D. Thesis. Botany Department. University of Glasgow.

Sabri, N.; Dominy, P. J. and Clarke, D. D. (1997) The relative tolerances of wild and cultivated oats to infection by *Erysiphe graminis* f.sp. *avenae* II. The effects of infection on photosynthesis and respiration. *Physiological and Molecular Plant Pathology* **50**, 321-335.

Salmon, S. & Laude, H. H. (1932) Twenty years of testing varieties and strains of winter wheat. *Kansas Agricultural Experimental Station Bulletin* 30 / pp. 73. Cited in **Schafer** (1971).

Schafer, J. F. (1971) Tolerance to plant disease. *Annual Review of Phytopathology* **9**, 235-152.

Schaller, C. W. (1951) The effect of mildew and scald infection on yield and quality of barley. *Agronomy Journal* **43**, 183-188.

Scholes, J. D. & Farrar, J. F. (1985) Photosynthesis and chloroplast functioning within individual pustules of *Uromyces muscari* on bluebell leaves. *Physiological Plant Pathology* **27**, 387-400.

Scholes, J. D. & Farrar, J. F. (1986) Increased rates of photosynthesis in localised regions of a barley leaf infected with brown rust. *New Phytologist* **104**, 601-612.

Scholes, J. D. (1992) Photosynthesis Cellular and tissue aspects in diseased leaves. In: Pests and pathogens Plant responses to foliar attack. pp. 85-106.

Ed. by Ayres, P. G. BIOS Scientific Publishers. Oxford.

Scholes, J. D.(1985) Effects of biotrophic pathogens on photosynthesis. Ph.D. Thesis. University of Wales.

Scott, K. J. (1972) Obligate parasitism by phytopathogenic fungi. *Biological Review* **47**, 537-572.

Scott, K. J. & Smillie, R. M. (1963) Possible relationship between photosynthesis and the rise in respiration in diseased leaves. *Nature* **197**, 1319-1320.

Scott, K. J. & Smillie, R. M. (1966) Metabolic regulation in diseased leaves. I. The respiratory rise in barley leaves infected with powdery mildew. *Plant Physiology* **41**, 289-297.

Scott, K. J. (1965) Respiratory enzymic activities in the host and pathogen of barley leaves infected with *Erysiphe graminis*. *Phytopathology* **55**, 438-441.

Scott, S. W. & Griffiths, E. (1980) Effects of controlled epidemics of powdery mildew on grain yield of spring barley. *Annals of Applied Biology* **94**, 19-31.

Shaw, M & Hawkins, A. R. (1958) The physiology of host-parasite relations. V. A preliminary examination of the level of free endogenous indol-acetic acid in rusted and mildewed cereal leaves and their ability to decarboxylate exogenously supplied radioactive indol-acetic acid. *Canadian Journal of Botany* 36, 1-16.

Shaw, M & Samborski, D. J. (1956) The physiology of host-parasite relationships I. The accumulation of radioactive substances at infections of facultative and obligate parasites, including tobacco mosaic virus. *Canadian Journal of Botany* **34**, 387-405.

Shaw, M & Samborski, D. J. (1957) The physiology of host-parasite relationships III. The pattern of respiration in rusted and mildewed cereal

leaves. *Canadian Journal of Botany* **35**, 389-406.

Siddiqui, M. Q. & Manners, J. G. (1971) Some effects of yellow rust (*Puccinia stiiformis*) infection on ^{14}C-assimilation and growth in spring wheat. *Journal of Experimental Botany* **22**, 792-799.

Simon, M. D. (1966) Relative tolerance of oat varieties to the crown rust fungus. *Phytopathology* **56**, 36-40.

Slootmaker, L. A. J. & van Essen, A. (1969) Neth. J. Agric. Sci. **17**, 279-282. Cited in The powdery mildews. Edited by Spencer, D. M. (Acad. Press. London), pp. 283-321.

Smedegaard-Petersen, V. & Stolen, O. (1981) Effect of energy requiring defence reactions on yield and grain quality in a powdery mildew-resistant barley cultivar. *Phytopathology* **71**, 396-399.

Smedegaard-Petersen, V. (1980) Increased demand for respiratory energy of barley leaves reacting hypersensitively against *Erysiphe graminis*, *Pyrenophora teres* and *Pyrenophora graminea*. *Phytopathologische Zeitschrift* **99**, 54-62.

Smith, P. R. & Neales, T. F. (1977) Analysis of the effect of virus infection on photosynthetic properties of peach leaves. *Australian Journal of Plant Physiology* **4**, 723-732.

So, M. G. & Thrower, L. B. (1976) The host-parasite relationship between *Vigna sesquipedalis* and *Uromyces appendiculatus* II. Movement of photosynthates and growth substances. *Phytopathologische Zeitschrift* **86**, 252-265.

Søgård, B. & Jørgensen, J. H. (1987) Supplementary list No. 1 (to masterlist of barley genes) Genes for reaction to *Erysiphe graminis* f.sp. *hordei* (powdery mildew). *Barley Genetics Newsletter* **17**, 120-134.

Staples, R. C. (1957) Changes in the organic acid composition of wheat

leaves infected with the leaf rust fungus. Contribution of the Boyce Thompson Institute. Cited in **Scholes** (1985).

Tarr, S. A. J. (1972) Principles of Plant Pathology. Macmillan Press. London.

Thanassoupoulos, C. C.; Gianopolitis, C. N. and Kitsos, G. T. (1970) Control of *Fusarium* wilt of tomato and watermelon with Benomyl. *Plant Disease Reporter* **54,** 561.

Thomas, R. G. & Duniway, J. M. (1982) Effects of powdery mildew infection on the efficiency of CO_2 fixation and light utilisation by sugar beet leaves. *Plant Physiology* **69,** 139-142.

Thornley, J. H. M (1972) A balanced quantitative model for shoot : root ratios in vegetative plants. *Annals of Botany* **36,** 431-441.

Thornley, J. H. M. (1976a) Mathematical models in plant physiology. Eds J. F. Sutcliffe and P. Mahlberg. pp. 11-14. Academic Press, London.

Thornley, J. H. M. (1976b) Mathematical models in plant physiology. Eds J. F. Sutcliffe and P. Mahlberg. pp. 92-110. Academic Press, London.

Thrower, L. B. (1965) Host physiology and obligate fungal parasites. *Phytopathologische Zeitschrift* **52,** 319-334.

Vizárová, G. & Minarčic, P. (1974) The influence of powdery mildew upon the cytokinins and the morphology of barley roots. *Phytopathologische Zeitschrift* **81,** 49-55.

Waggoner, P. E. & Berger, R. D. (1987) Defoliation, disease and growth. *Phytopathology* 77, 393-398.

Walker, D. (1987) The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Packard Publishing, Chichester, Sussex.

Walker, D. (1993) Polarographic measurement of oxygen. In Photosynthesis and Production in a Changing Environement A field and Laboratory Manual. Ed. by Hall, D. O.; Scurlock, J. M. O.; Bolhar-Nordenkampf, H.R.; Leegood, R. C. & Long, S. P. pp. 168-180. Chapman & Hall, London.

Walter, D. R. (1981) Root function in mildewed barley. Ph.D. Thesis. University of Lancaster.

Walters, D. R. & Ayres, P. G. (1980) Effects of powdery mildew disease on uptake and metabolism of nitrogen by roots of infected barley. *Physiological Plant Pathology* **17**, 369-379.

Walters, D. R. & Ayres, P. G. (1981a) Growth and branching pattern of roots of powdery mildew infected barley. *Annals of Botany* **47**, 159-163.

Walters, D. R. & Ayres, P. G. (1982) Translocation of 14C-labelled photoassimilates to roots in barley Effects of mildew on partitioning in roots and the mitotic index. *Plant Pathology* 31, 307-313.

Walters, D. R. & Ayres, P. G. (1983) Changes in nitrogen utilisation and enzyme activities associated with CO_2 exchanges in healthy leaves of powdery mildew infected barley. *Physiological Plant Pathology* **23**, 447-459.

Walters, D. R. & Ayres, P. G. (1984) Ribulose bisphosphate carboxylase and enzymes of CO_2 assimilation in a compatible barley / powdery mildew combination. *Phytopathologische Zeitschrift* **109**, 208-218.

Walters, D. R. (1985) Shoot : Root interrelationships The effects of obligately biotrophic fungal pathogens. *Biological Review* **60**, 47-79.

Walters, D.R. & Ayres, P. G. (1981b) Phosphate uptake and transport by roots of powdery mildew infected barley. *Physiological Plant Pathology* **18**, 195-205.

Wanzhoug, Tan (1988) Relationships between foliar disease and loss of grain yield in barley with particular reference to powdery mildew. Ph.D.

Thesis. University of Wales. Aberystwyth.

Waygood, E. R.; Pao, L. Y. and Godavari, H. R. (1974) Stimulation of phosphoenolpyruvate carboxylase activity in rust-infected wheat leaves. *Experientia* **15,** 986-988. Cited in **Walters** (1985).

Whipps, J. M. & Lewis, D. H. (1981) Patterns of translocation, storage and inter-conversion of carbohydrates. In: Effects of disease on the physiology of growing plant. pp. 47-83. Ed. by Ayres, P. G. Cambridge University Press.

Wilkinson, H. P. (1979) The plant surface (mainly leaf). In: Anatomy of the dicotyledons. Ed. by Metcalfe, C. R. & Chalk, L. pp. 97-165. Oxford Clarendon Press.

Williams, G. M. & Ayres, P. G. (1981) Effects of powdery mildew and water stress on CO_2 exchanges in uninfected leaves of barley plants. *Plant Physiology* **68,** 527-530.

Williams, R. F. (1946) The physiology of plant growth with special reference to the concept of net assimilation rate. *Annals of Botany* **10,** 41-72.

Wolfe, M. S. & Schwarzbach, E. (1978) The recent history of the evolution of barley powdery mildew in Europe. In: The powdery mildews. Edited by Spencer, D. M. (Acad. Press. London), pp. 129-157.

Wolfe, M. S. (1972) The genetics of barley mildew. *Review of Plant Pathology* **51,** 507-522.

Wolfe, M. S. (1984) Trying to understand and control powdery mildew. *Plant pathology* **33,** 451-466.

Wolfe, M. S. (1985) The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* **23,** 251-273.

Wright, D. P. (1992) The effect of powdery mildew on the photosynthetic

and carbohydrate metabolism of wheat leaves. Ph.D. Thesis. University of Sheffield. Cited in **Scholes** (1992).

Wrigley, C. W. & Webster, H. L. (1966) The effect of stem rust infection on the soluble proteins of wheat. *Australian Journal of Biological Science* **19**, 895-901.

Wynn, W. K. (1963) Photosynthetic phosphorylation by chloroplasts isolated from rust-infected oats. *Phytopathology* **53**, 1376-1377.

Yahyaoui, A. H., Reinhold, M. and Scharen, A. L. (1997) Virulence spectrum in populations of the barley powdery mildew pathogen, *Erysiphe graminis* f.sp. *hordei* in Tunisia and Morocco in 1992. *Plant pathology* **46**, 139-146.

Yarwood, C. E. (1934) The effect of mildew and rust infection on dry weight and respiration of excised clover leaflets. *Journal of Agricultural Research* **49**, 549-558.

Yarwood, C. E. & Jacobsen, L. (1955) Accumulation of chemicals in diseased area of leaves. *Phytopathology* **45**, 43-48.

Zadoks, J. C.; Chang, T. T. and Konzak, C. F. (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.

Zohary, D. (1969) The progenitors of wheat and barley in relation to domestication and agriculture dispersal in the world. In: The domestication and exploitation of plants and animals. Ed. by Ucko, P. J. & Dimbleby, G. W. pp. 47-66. Duck Worth, London. Cited in Zohary & Hopf (1994) Domestication of plants in the Old World. Clarendon Press, Oxford.

Zulu, J. N.; Farrar, J. F. and Whitbread, R. (1991) Effects of phosphate supply on wheat seedlings infected with powdery mildew Carbohydrate metabolism of first leaves. *New Phytologist* **118**, 553-558.

Appendix Figures

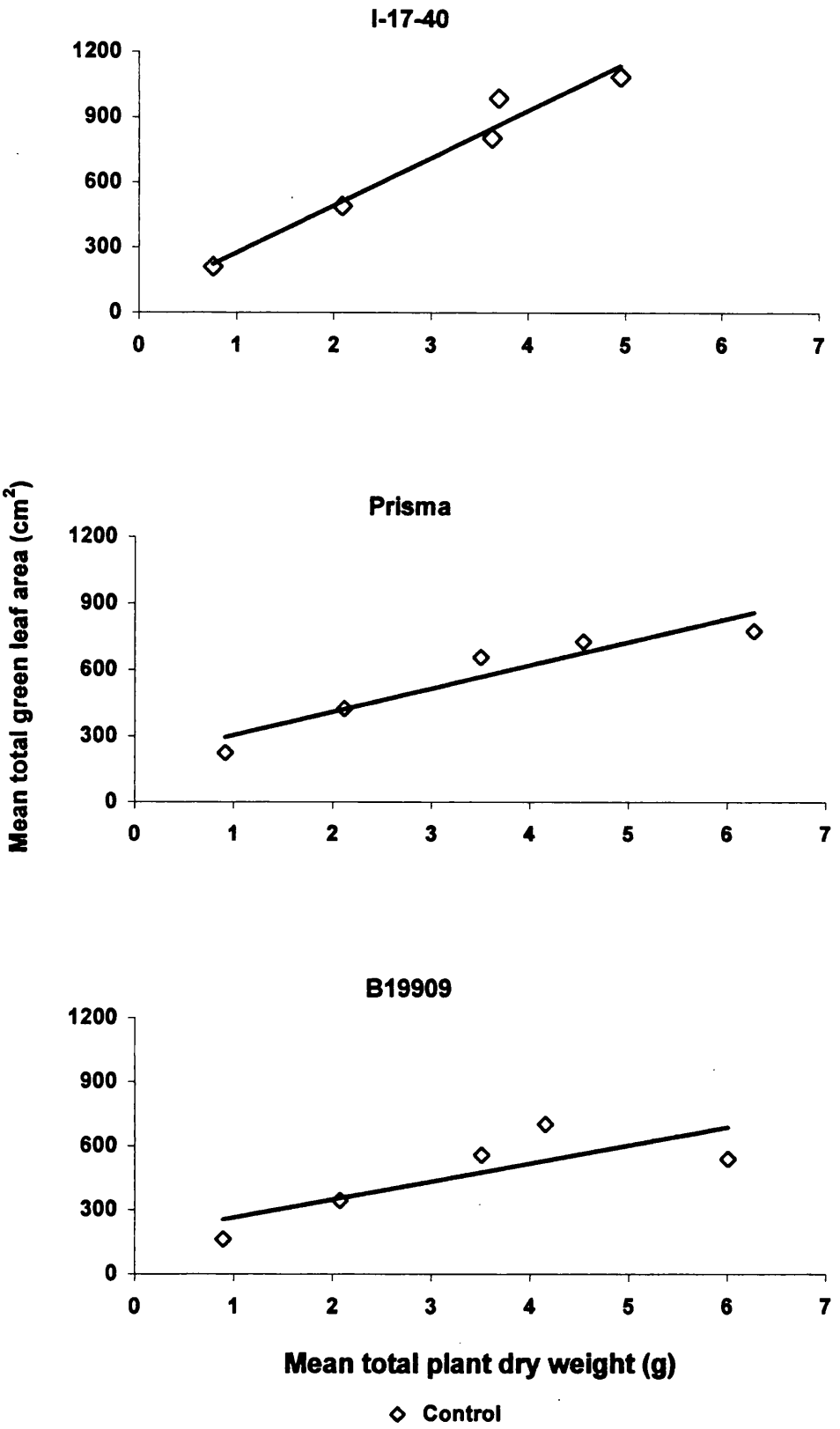


Fig. 1: Relationship between total plant dry weight and total green leaf area in the three barley lines.

Appendix Tables

Appendix Tables 1 to 3

Data on some primary values of growth of *Blumeria graminis* infected and uninfected plants of wild and cultivated barleys taken at weekly intervals during the growth period between the second and the sixth week after inoculation (the fourth and the eighth week after planting).

LS : Leaf sheaths + Primary shoot tissues

SLB : Leaf blades of the primary shoot

LT : Leaf sheaths + Tillers tissues

TLB : Leaf blades of tillers

Appendix: Table 1A. 1st Harvest (Two weeks after inoculation). Line I-17-40.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	7	0	102.94	0.078	0.233	0.138	0.239	0.131	0.819
2	0	7	0	80.25	0.066	0.177	0.125	0.253	0.136	0.757
3	0	7	0	69.26	0.069	0.151	0.126	0.251	0.106	0.703
Mean	0	7	0	84.150	0.071	0.187	0.130	0.248	0.124	0.760
SE	0	0	0	9.916	0.004	0.024	0.004	0.004	0.009	0.034
Infected										
1	4.6	7	1	90.60	0.059	0.152	0.131	0.226	0.119	0.687
2	15.1	7	1	84.55	0.061	0.171	0.118	0.226	0.115	0.691
3	9.7	7	0	101.60	0.084	0.190	0.138	0.242	0.155	0.809
Mean	9.8	7	0.667	92.25	0.068	0.171	0.129	0.231	0.130	0.729
SE	3.0	0	0.333	4.991	0.008	0.011	0.006	0.005	0.013	0.040

Appendix: Table 1B. 2nd Harvest (three weeks after inoculation). Line I-17-40.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	8	1	99.31	0.086	0.189	0.617	0.881	0.285	2.058
2	0	9	1	107.36	0.106	0.229	0.724	0.923	0.303	2.285
3	0	8	1	127.89	0.096	0.242	0.599	0.763	0.216	1.916
Mean	0	8.333	1	111.52	0.096	0.220	0.647	0.856	0.268	2.086
SE	0	0.333	0	8.508	0.006	0.016	0.039	0.048	0.027	0.107
Infected										
1	19.2	9	3	71.95	0.093	0.220	0.578	0.553	0.173	1.617
2	14.5	9	3	55.70	0.087	0.195	0.587	0.552	0.178	1.599
3	17.5	9	4	74.63	0.146	0.227	0.595	0.529	0.186	1.683
Mean	17.1	9	3.333	67.427	0.109	0.214	0.587	0.545	0.179	1.633
SE	1.4	0	0.333	5.914	0.019	0.010	0.005	0.008	0.004	0.026

Appendix: Table 1C. 3rd Harvest (Four weeks after inoculation). Line I-17-40.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	11	3	132.08	0.131	0.344	1.142	1.538	0.606	3.761
2	0	10	3	102.78	0.127	0.270	1.395	1.695	0.506	3.993
3	0	10	3	88.81	0.092	0.205	0.929	1.461	0.448	3.135
Mean	0	10.333	3	107.89	0.117	0.273	1.155	1.565	0.520	3.630
SE	0	0.333	0	12.750	0.012	0.040	0.135	0.069	0.046	0.256
Infected										
1	25.8	9	6	31.73	0.067	0.194	0.406	0.773	0.223	1.663
2	21.0	10	7	30.06	0.061	0.194	0.381	0.740	0.200	1.576
3	36.2	9	6	38.77	0.082	0.243	0.365	0.776	0.210	1.676
Mean	27.7	9.333	6.333	33.52	0.070	0.210	0.384	0.763	0.211	1.638
SE	4.5	0.333	0.333	2.669	0.006	0.016	0.012	0.012	0.007	0.031

Appendix: Table 1D. 4th Harvest (Five weeks after inoculation). Line I-17-40.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	11	7	57.44	0.112	0.233	1.021	1.827	0.621	3.814
2	0	11	6	73.53	0.109	0.290	0.673	1.300	0.389	2.761
3	0	11	5	108.46	0.122	0.296	1.270	2.089	0.744	4.521
Mean	0	11	6	79.81	0.114	0.273	0.988	1.739	0.585	3.699
SE	0	0	0.577	15.059	0.004	0.020	0.173	0.232	0.104	0.511
Infected										
1	31.4	11	8	52.22	0.102	0.304	0.776	1.629	0.383	3.194
2	22.2	11	8	57.09	0.120	0.285	0.479	1.104	0.219	2.207
3	12.2	11	8	60.01	0.123	0.403	0.818	1.603	0.306	3.253
Mean	21.9	11	8	56.44	0.115	0.331	0.691	1.445	0.303	2.885
SE	5.6	0	0	2.272	0.007	0.037	0.107	0.171	0.047	0.339

Appendix: Table 1E. 5th Harvest (Six weeks after inoculation). Line I-17-40.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	12	7	80.22	0.130	0.258	1.788	2.617	0.757	5.55
2	0	11	7	98.49	0.154	0.329	0.998	2.048	0.591	4.12
3	0	11	6	70.04	0.115	0.291	1.449	2.625	0.723	5.203
Mean	0	11.333	6.667	82.917	0.133	0.293	1.412	2.430	0.690	4.958
SE	0	0.333	0.333	8.323	0.011	0.021	0.229	0.191	0.051	0.431
Infected										
1	10.1	12	9	52.24	0.122	0.314	1.587	2.996	0.544	5.563
2	22.7	11	8	44.05	0.127	0.393	1.360	2.849	0.601	5.33
3	11.7	11	8	52.27	0.081	0.263	0.590	1.523	0.226	2.683
Mean	14.8	11.333	8.333	49.52	0.110	0.323	1.179	2.456	0.457	4.525
SE	4.0	0.333	0.333	2.735	0.015	0.038	0.302	0.468	0.117	0.924

Appendix: Table 2A. 1st Harvest (Two weeks after inoculation). CV. Prisma.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	7	0	111.76	0.126	0.267	0.162	0.273	0.160	0.988
2	0	7	0	117.67	0.148	0.273	0.145	0.261	0.132	0.959
3	0	7	0	119.99	0.124	0.300	0.108	0.132	0.119	0.783
Mean	0	7	0	116.47	0.133	0.280	0.138	0.222	0.137	0.910
SE	0	0	0	2.450	0.008	0.010	0.016	0.045	0.012	0.064
Infected										
1	11.2	7	1	132.47	0.153	0.325	0.230	0.321	0.19	1.219
2	12.7	7	0	114.00	0.131	0.245	0.076	0.123	0.105	0.68
3	11.5	7	1	131.45	0.138	0.293	0.194	0.258	0.143	1.026
Mean	11.8	7	0.667	125.97	0.141	0.288	0.167	0.234	0.146	0.975
SE	0.5	0	0.333	5.994	0.006	0.023	0.047	0.058	0.025	0.158

Appendix: Table 2B. 2nd Harvest (Three weeks after inoculation). Cv. Prisma.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	8	0	186.15	0.251	0.417	0.418	0.568	0.273	1.927
2	0	9	1	181.25	0.326	0.497	0.616	0.869	0.396	2.704
3	0	8	0	175.00	0.219	0.376	0.359	0.557	0.203	1.714
Mean	0	8.333	0.333	180.80	0.265	0.430	0.464	0.665	0.291	2.115
SE	0	0.333	0.333	3.227	0.032	0.036	0.078	0.102	0.056	0.301
Infected										
1	27.1	8	4	80.06	0.211	0.405	0.306	0.466	0.159	1.547
2	28.7	8	4	76.48	0.222	0.430	0.412	0.582	0.173	1.819
3	27.2	8	4	71.49	0.175	0.350	0.259	0.392	0.122	1.298
Mean	27.7	8	4	76.01	0.203	0.395	0.326	0.480	0.151	1.555
SE	0.5	0	0	2.485	0.014	0.024	0.045	0.055	0.015	0.150

Appendix: Table 2C. 3rd Harvest (Four weeks after inoculation). Cv. Prisma.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	10	3	179.75	0.435	0.559	0.961	1.202	0.527	3.684
2	0	9	4	165.55	0.348	0.507	0.910	1.198	0.373	3.336
3	0	9	4	151.69	0.295	0.474	1.026	1.296	0.383	3.474
Mean	0	9.333	3.667	165.66	0.359	0.513	0.966	1.232	0.428	3.498
SE	0	0.333	0.333	8.10	0.041	0.025	0.034	0.032	0.050	0.101
Infected										
1	23.5	9	6	63.28	0.215	0.432	0.357	0.593	0.147	1.744
2	34.1	9	6	51.83	0.234	0.413	0.396	0.683	0.176	1.902
3	23.0	9	6	60.4	0.191	0.423	0.351	0.608	0.120	1.693
Mean	26.8	9	6	58.50	0.213	0.423	0.368	0.628	0.148	1.780
SE	3.6	0	0	3.439	0.012	0.005	0.014	0.028	0.016	0.063

Appendix: Table 2D. 4th Harvest (Five weeks after inoculation). Cv. Prisma.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	11	6	134.71	0.577	0.623	1.173	1.035	0.654	6.062
2	0	10	6	129.29	0.309	0.500	1.095	1.415	0.459	3.778
3	0	10	6	122.05	0.362	0.544	1.049	1.349	0.472	3.776
Mean	0	10.333	6	128.68	0.416	0.556	1.106	1.266	0.528	4.539
SE	0	0.333	0	3.667	0.082	0.036	0.036	0.117	0.063	0.762
Infected										
1	32.8	10	7	86.92	0.312	0.608	0.698	1.052	0.184	2.854
2	45.6	10	8	35.87	0.233	0.452	0.589	0.955	0.127	2.356
3	38.8	10	8	52.63	0.351	0.550	1.017	1.422	0.283	2.623
Mean	39.0	10	7.667	58.47	0.299	0.537	0.768	1.143	0.198	2.611
SE	3.7	0	0.333	15.024	0.035	0.046	0.128	0.142	0.046	0.144

Appendix: Table 2E. 5th Harvest (Six weeks after inoculation). Cv. Prisma.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	11	7	91.36	0.507	0:473	1.973	2.117	0.534	5.604
2	0	12	7	112.99	0.616	0.579	2.629	2.530	0.747	7.101
3	0	12	7	90.03	0.409	0.464	2.281	2.260	0.701	6.115
Mean	0	11.667	7	98.13	0.511	0.505	2.294	2.302	0.661	6.273
SE	0	0.333	0	7.442	0.060	0.037	0.189	0.121	0.065	0.439
Infected										
1	57.1	10	8	46.21	0.135	0.204	0.590	1.103	0.103	2.135
2	27.2	10	8	40.74	0.134	0.300	0.552	0.932	0.092	2.01
3	37.2	10	8	29.47	0.299	0.562	0.731	1.168	0.135	2.895
Mean	40.5	10	8	38.81	0.189	0.355	0.624	1.068	0.110	2.347
SE	8.8	0	0	4.928	0.055	0.107	0.054	0.070	0.013	0.277

Appendix: Table 3A. 1st Harvest (Two weeks after inoculation). Line B19909.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	6	0	74.43	0.063	0.162	0.099	0.149	0.082	0.910
2	0	6	0	76.68	0.084	0.171	0.077	0.094	0.120	0.947
3	0	6	0	84.38	0.088	0.193	0.128	0.257	0.143	0.807
Mean	0	6	0	78.50	0.078	0.175	0.101	0.167	0.115	0.888
SE	0	0	0	3.013	0.008	0.009	0.015	0.048	0.018	0.042
Infected										
1	10.7	7	0	91.03	0.089	0.193	0.140	0.240	0.166	1.195
2	11.8	6	1	87.75	0.076	0.188	0.138	0.186	0.141	0.716
3	14.0	6	1	94.55	0.080	0.163	0.128	0.231	0.121	1.004
Mean	12.2	6.333	0.667	91.11	0.082	0.181	0.135	0.219	0.143	0.972
SE	1.0	0.333	0.333	1.963	0.004	0.009	0.004	0.017	0.013	0.139

Appendix: Table 3B. 2nd Harvest (Three weeks after inoculation). Line B19909.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	7	1	99.31	0.103	0.211	0.340	0.599	0.293	1.947
2	0	8	1	89.88	0.906	0.217	0.401	0.635	0.278	2.586
3	0	8	1	107.73	0.100	0.230	0.281	0.492	0.182	1.693
Mean	0	7.667	1	98.97	0.370	0.219	0.341	0.575	0.251	2.075
SE	0	0.333	0	5.156	0.268	0.006	0.035	0.043	0.035	0.266
Infected										
1	25.5	7	3	57.97	0.117	0.233	0.283	0.432	0.157	1.545
2	28.3	7	3	63.70	0.091	0.242	0.209	0.336	0.124	1.770
3	24.4	7	3	60.87	0.086	0.210	0.232	0.382	0.137	1.313
Mean	26.1	7	3	60.85	0.098	0.228	0.241	0.383	0.139	1.543
SE	1.2	0	0	1.654	0.010	0.010	0.022	0.028	0.010	0.132

Appendix: Table 3C. 3rd Harvest (Four weeks after inoculation). Line B19909.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	9	4	94.35	0.153	0.293	0.585	0.906	0.31	3.467
2	0	9	4	91.51	0.155	0.292	0.773	1.155	0.415	3.378
3	0	10	4	104.36	0.306	0.412	0.945	1.546	0.596	3.687
Mean	0	9.333	4	96.74	0.205	0.332	0.768	1.202	0.440	3.511
SE	0	0.333	0	3.897	0.051	0.040	0.104	0.186	0.084	0.092
Infected										
1	43.5	8	6	36.54	0.096	0.232	0.279	0.590	0.14	1.737
2	31.3	9	6	60.71	0.199	0.366	0.472	0.978	0.311	2.037
3	27.2	9	6	49.30	0.121	0.295	0.442	0.910	0.242	1.815
Mean	34.0	8.667	6	48.85	0.139	0.298	0.398	0.826	0.231	1.863
SE	4.9	0.333	0	6.981	0.031	0.039	0.060	0.120	0.050	0.090

Appendix: Table 3D. 4th Harvest (Five weeks after inoculation). Line B19909.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	10	6	79.00	0.128	0.268	0.821	1.561	0.419	4.827
2	0	11	6	92.08	0.214	0.381	1.012	1.905	0.519	3.838
3	0	11	6	87.51	0.257	0.396	0.709	1.246	0.509	3.813
Mean	0	10.667	6	86.20	0.200	0.348	0.847	1.571	0.482	4.159
SE	0	0.333	0	3.833	0.038	0.040	0.088	0.190	0.032	0.334
Infected										
1	33.8	10	7	55.36	0.182	0.363	0.367	0.869	0.162	2.832
2	31.8	10	7	43.80	0.107	0.341	0.550	1.300	0.187	2.416
3	54.5	10	7	49.35	0.160	0.331	0.568	1.124	0.215	2.555
Mean	40.0	10	7	49.50	0.150	0.345	0.495	1.098	0.188	2.601
SE	7.2	0	0	3.338	0.022	0.009	0.064	0.125	0.015	0.122

Appendix: Table 3E. 5th Harvest (Six weeks after inoculation). Line B19909.

Rep.	% Mildew	Total No of Leaves	No of Senesc. Leaves	Leaf Area (cm^2)	Dry Weight (g)					Total Plant (g)
					LS	SLB	LT	TLB	Root	
Control										
1	0	11	7	74.33	0.339	0.364	1.104	2.327	0.394	5.464
2	0	12	7	53.2	0.489	0.368	1.616	1.726	0.447	6.801
3	0	12	7	53.7	0.355	0.359	1.561	1.810	0.342	5.756
Mean	0	11.667	7	60.41	0.394	0.364	1.427	1.954	0.394	6.007
SE	0	0.333	0	6.961	0.048	0.003	0.162	0.188	0.030	0.406
Infected										
1	44.1	11	8	51.63	0.217	0.470	0.635	1.516	0.243	2.275
2	35.9	10	8	49.09	0.143	0.474	0.874	2.078	0.355	2.273
3	51.6	10	8	43.91	0.169	0.349	0.725	1.864	0.228	2.988
Mean	43.9	10.333	8	48.21	0.176	0.431	0.745	1.819	0.275	2.512
SE	4.6	0.333	0	2.272	0.022	0.041	0.070	0.164	0.040	0.238

Appendix Tables 4 to 6

Data on the effects of infection on the development of individual leaves on the primary shoot. Measurements were taken at weekly intervals during the growth period between the second and the sixth week after inoculation (the fourth and the eighth week after planting).

Appendix: table 4 A, 1st Harvest (Two weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	6.44	3.44	4.26	4.713	0.895
2	10.99	8.44	7.85	9.093	0.964
3	13.96	13.63	12.24	13.277	0.527
4	18.14	17.95	13.28	16.457	1.589
5	23.51	19.22	15.31	19.347	2.368
6	20.67	14.56	12.47	15.900	2.460
7	9.23	3.01	3.85	5.363	1.948
8					
9					
10					
11					
12					

Control Plants

Appendix: table 4 A, 1st Harvest (Two weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	2.47	0.5	0.823	0.823	0.167
2	5.34	0.25	6.62	0.5	3.7	0.5	5.220	0.845	0.417
3	10.89	0.15	9.54	0.25	10.37	0.25	10.267	0.393	0.217
4	19.73	0.05	16.54	0.25	18.97	0.15	18.413	0.962	0.150
5	22.7	0.01	18.17	0.15	22.01	0.05	20.960	1.409	0.070
6	22.34	0	22.15	0.01	23.81	0.01	22.767	0.525	0.007
7	9.6	0	11.53	0	20.27	0	13.800	3.283	0.000
8									
9									
10									
11									
12									

Infected Plants

Appendix: table 4 B, 2nd Harvest (Three weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–		
2	3.94	3.28	3.84	3.687	0.205
3	13.90	9.71	11.79	11.800	1.210
4	16.39	11.88	15.87	14.713	1.425
5	19.55	13.75	20.20	17.833	2.050
6	20.66	15.20	27.21	21.023	3.472
7	13.20	17.82	27.32	19.447	4.156
8	2.20	18.12	17.09	12.470	5.144
9	–	8.29	–	2.763	2.763
10					
11					
12					

Control Plants

Appendix: table 4 B, 2nd Harvest (Three weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	2.04	0.75	2.5	0.75	–	–	1.513	0.768	0.500
5	2.66	0.75	2.64	0.75	2.79	0.75	2.697	0.047	0.750
6	8.96	0.5	6.1	0.5	11.04	0.5	8.700	1.432	0.500
7	21.51	0.15	17.88	0.05	27.38	0.15	22.257	2.768	0.117
8	21.15	0.05	19.87	0.01	24.04	0.01	21.687	1.233	0.023
9	7.75	0	6.23	0	3.14	0	5.707	1.356	0.000
10									
11									
12									

Infected Plants

Appendix: table 4 C, 3rd Harvest (Four weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	12.44	11.39	14.32	12.717	0.857
5	14.70	16.59	16.64	15.977	0.638
6	17.67	16.00	14.80	16.157	0.832
7	20.46	16.96	16.04	17.820	1.346
8	23.13	16.28	15.40	18.270	2.443
9	20.37	15.98	13.27	16.540	2.069
10	18.76	8.80	2.64	10.067	4.696
11	4.55	–	–	1.517	1.517
12					

Control Plants

Appendix: table 4 C, 3rd Harvest (Four weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	8.91	0.75	–	–	3.76	0.75	4.223	2.583	0.500
8	18.03	0.1	9.16	0.5	26.07	0.4	17.753	4.883	0.333
9	6.23	0.01	18.21	0.1	7.8	0.05	10.747	3.759	0.053
10	–	–	3.1	0	–	–	1.033	1.033	0.000
11									
12									

Infected Plants

Appendix: table 4 D, 4th Harvest (Five weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	7.26	2.420	2.420
7	–	2.29	20.64	7.643	6.532
8	5.18	3.61	21.55	10.113	5.736
9	15.53	22.54	21.65	19.907	2.203
10	21.35	26.80	23.69	23.947	1.579
11	13.32	14.79	10.25	12.787	1.337
12					

Control Plants

Appendix: table 4 E, 5th Harvest (Six weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	5.92	1.973	1.973
8	13.37	22.81	6.29	14.157	4.785
9	17.25	25.16	14.71	19.040	3.147
10	18.40	29.08	23.64	23.707	3.083
11	19.73	18.76	17.32	18.603	0.700
12	9.67	–	–	3.223	3.223

Control Plants

Appendix: table 4 E, 5th Harvest (Six weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	5.92	1.973	1.973
8	13.37	22.81	6.29	14.157	4.785
9	17.25	25.16	14.71	19.040	3.147
10	18.40	29.08	23.64	23.707	3.083
11	19.73	18.76	17.32	18.603	0.700
12	9.67	–	–	3.223	3.223

Control Plants

Appendix: table 4 E, 5th Harvest (Six weeks after inoculation). Line I-17-40.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–
9	–	–	11.98	0.75	6.37	0.75	6.117	3.461	0.500
10	18.83	0.25	19.98	0.05	26.58	0.05	21.797	2.415	0.117
11	22.5	0.02	12	0	19.24	0	17.913	3.103	0.007
12	9.73	0	–	–	–	–	3.243	3.243	0.000

Infected Plants

Appendix: table 5 A, 1st Harvest (Two weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	4.01	4.85	4.24	4.367	0.251
2	7.30	6.77	7.01	7.027	0.153
3	14.53	13.09	14.17	13.930	0.433
4	21.19	19.85	23.57	21.537	1.088
5	25.78	27.78	27.76	27.107	0.663
6	29.20	33.25	34.48	32.310	1.595
7	9.75	12.08	8.76	10.197	0.984
8					
9					
10					
11					
12					

Control Plants

Appendix: table 5 A, 1st Harvest (Two weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	4.43	0.5	–	–	1.477	1.477	0.167
2	8.56	0.2	5.21	0.5	3.68	0.5	5.817	1.441	0.400
3	17.78	0.2	6.49	0.4	12.11	0.5	12.127	3.259	0.367
4	23.26	0.25	21.29	0.25	22.1	0.25	22.217	0.572	0.250
5	29.95	0.1	27.37	0.05	34.3	0.05	30.540	2.022	0.067
6	35.57	0.02	35.68	0.01	41.7	0	37.650	2.025	0.010
7	17.35	0	13.53	0	17.56	0	16.147	1.310	0.000
8									
9									
10									
11									
12									

Infected Plants

Appendix: table 5 B, 2nd Harvest (Three weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	4.44	–	3.54	2.660	1.355
2	4.43	6.10	6.23	5.587	0.580
3	15	10.74	12.00	12.580	1.263
4	22.10	22.15	22.59	22.280	0.156
5	27.53	28.83	26.92	27.760	0.563
6	34.51	35.49	32.26	34.087	0.956
7	42.94	39.85	41.26	41.350	0.893
8	29.22	33.75	28.37	30.447	1.670
9	–	6.80	–	2.267	2.267
10					
11					
12					

Control Plants

Appendix: table 5 B, 2nd Harvest (Three weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	8.56	0.75	7.67	0.75	6.06	0.75	7.430	0.732	0.750
6	10.54	0.5	14.98	0.5	12.55	0.5	12.690	1.284	0.500
7	30.27	0.25	34.03	0.25	31.72	0.25	32.007	1.095	0.250
8	22.48	0.01	19.8	0.01	19.35	0.01	20.543	0.977	0.010
9									
10									
11									
12									

Infected Plants

Appendix: table 5 C, 3rd Harvest (Four weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	12.70	–	–	4.233	4.233
5	20.22	23.70	26.92	23.613	1.935
6	27.73	39.17	37.38	34.760	3.553
7	40.54	41.78	39.90	40.740	0.552
8	29.83	42.80	34.86	35.830	3.775
9	34.91	18.10	10.50	21.170	7.212
10	8.52	–	–	2.840	2.840
11					
12					

Control Plants

Appendix: table 5 C, 3rd Harvest (Four weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	9.76	0.75	11.68	0.75	7.1	0.8	9.513	1.328	0.767
8	27	0.25	32.52	0.25	18.69	0.25	26.070	4.019	0.250
9	24.24	0.01	5.51	0.01	21.06	0.02	16.937	5.787	0.013
10									
11									
12									

Infected Plants

Appendix: table 5 D, 4th Harvest (Five weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	20.75	20.26	13.30	18.103	2.406
9	38.62	32.36	41.62	37.533	2.728
10	37.71	38.08	42.29	39.360	1.469
11	28.86	32.14	17.29	26.097	4.504
12	6.37	_	_	2.123	2.123

Control Plants

Appendix: table 5 D, 4th Harvest (Five weeks after inoculation). Cv. Prisma.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–
9	22.9	0.75	13.7	0.75	19.79	0.75	18.797	2.702	0.750
10	36.82	0.25	19.57	0.25	30.12	0.15	28.837	5.021	0.217
11	24.58	0.05	–	–	–	–	8.193	8.193	0.017
12									

Infected Plants

Appendix: table 5 E, 5th Harvest (Six weeks after inoculation). Cv. Prisma

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	17.25	4.17	8.93	10.117	3.822
9	32.70	29.33	22.18	28.070	3.102
10	32.03	34.64	31.99	32.887	0.877
11	6.88	30.21	21.12	19.403	6.789
12	_	13.33	2.51	5.280	4.090

Control Plants

Appendix: table 5 E, 5th Harvest (Six weeks after inoculation). Cv. Prisma

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–
9	11	0.75	12.89	0.75	25.93	0.75	16.607	4.693	0.75
10	35.12	0.05	27.77	0.05	30.42	0.05	31.103	2.149	0.05
11									
12									

Infected Plants

Appendix: table 6 A, 1st Harvest (Two weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	3.69	4.39	6.35	4.810	0.796
2	5.12	4.56	7.21	5.630	0.806
3	12.92	10.74	15.89	13.183	1.492
4	18.79	19.26	23.11	20.387	1.368
5	22.18	21.96	20.71	21.617	0.458
6	11.73	15.77	11.11	12.870	1.461
7					
8					
9					
10					
11					
12					

Control Plants

Appendix: table 6 A, 1st Harvest (Two weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	4.78	0.15	–	–	–	–	1.593	1.593	0.050
2	6.12	0.15	6.09	0.15	6.4	0.25	6.203	0.099	0.183
3	9.79	0.25	8.83	0.25	15.2	0.4	11.273	1.983	0.300
4	18.04	0.25	15.32	0.25	18.31	0.25	17.223	0.955	0.250
5	23.34	0.05	21.01	0.15	20.47	0.05	21.607	0.881	0.083
6	23.94	0	23.53	0.01	21.31	0	22.927	0.817	0.003
7	5.02	0	12.97	0	12.86	0	10.283	2.632	0.000
8									
9									
10									
11									
12									

Infected Plants

Appendix: table 6 B, 2nd Harvest (Three weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant Individual Leaf area (cm^2)	2nd Plant Individual Leaf area (cm^2)	3rd Plant Individual Leaf area (cm^2)	Mean Individual Leaf Area (cm^2)	SE
1	–	–	–	–	–
2	5.34	4.67	3.15	4.387	0.648
3	11.60	9.80	10.55	10.650	0.522
4	17.10	16.74	16.86	16.900	0.106
5	18.00	16.53	17.95	17.493	0.482
6	25.58	18.68	24.15	22.803	2.103
7	20.51	20.68	21.30	20.830	0.240
8	_	2.50	2.56	1.687	0.844
9					
10					
11					
12					

Control Plants

Appendix: table 6 B, 2nd Harvest (Three weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	4.54	0.75	5.34	0.75	5.79	0.75	5.223	0.366	0.750
5	8.06	0.5	19.44	0.5	13.22	0.5	13.573	3.290	0.500
6	20.12	0.25	24.75	0.15	24.83	0.15	23.233	1.557	0.183
7	20.19	0.05	12.42	0.01	17.03	0.01	16.547	2.256	0.023
8									
9									
10									
11									
12									

Infected Plants

Appendix: table 6 C, 3rd Harvest (Four weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	9.49	11.5	4.46	8.483	2.094
6	19.24	18.30	20.48	19.340	0.631
7	26.82	25.90	26.20	26.307	0.271
8	26.44	25.50	23.32	25.087	0.924
9	11.41	10.31	19.46	13.727	2.884
10	–	–	10.44	3.480	3.480
11					
12					

Control Plants

Appendix: table 6 C, 3rd Harvest (Four weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	15.5	0.75	17.65	0.75	6	0.8	13.050	3.579	0.767
8	17.12	0.15	23.71	0.15	19.56	0.25	20.130	1.924	0.183
9	–	–	14.55	0.05	22.6	0.15	12.383	6.613	0.067
10									
11									
12									

Infected Plants

Appendix: table 6 D, 4th Harvest (Five weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant Individual Leaf area (cm^2)	2nd Plant Individual Leaf area (cm^2)	3rd Plant Individual Leaf area (cm^2)	Mean Individual Leaf Area (cm^2)	SE
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	13.19	13.32	7.30	11.270	1.985
8	23.75	21.50	25.14	23.463	1.061
9	28.00	22.15	21.98	24.043	1.979
10	9.84	24.03	23.07	18.980	4.578
11	_	5.82	3.96	3.260	1.716
12					

Control Plants

Appendix: table 6 D, 4th Harvest (Five weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–
8	10.61	0.75	11.54	0.75	15.51	0.75	12.553	1.503	0.750
9	27.11	0.5	26.98	0.15	26.02	0.5	26.703	0.344	0.383
10	13.43	0.01	1.47	0.01	3.79	0.01	6.230	3.662	0.010
11									
12									

Infected Plants

Appendix: table 6 E, 5th Harvest (Six weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant	2nd Plant	3rd Plant	Mean Individual Leaf Area (cm^2)	SE
	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)	Individual Leaf area (cm^2)		
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	19.11	–	–	6.370	6.370
9	22.06	14.00	13.50	16.520	2.774
10	23.58	12.92	15.80	17.433	3.184
11	9.52	16.82	17.40	14.580	2.536
12	–	8.75	7.00	5.250	2.673

Control Plants

Appendix: table 6 E, 5th Harvest (Six weeks after inoculation). Line B19909.

Leaf No. on Primary Shoot	1st Plant		2nd Plant		3rd Plant		Mean Individual Leaf Area (cm^2)	SE	Mean % Mildew
	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.	Indiv. L. a. (cm^2)	% Mild.			
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–
9	25.77	0.75	17	0.75	28.1	0.75	23.623	3.379	0.750
10	22.73	0.15	31.91	0.15	15.77	0.1	23.470	4.674	0.133
11	3.1	0	–	–	–	–	1.033	1.033	0.000
12									

Infected Plants

Appendix Tables 7 to 9

Data on the effects of infection on the primary shoot height, on the number of tillers and on total leaf blade area on tillers. Measurements were taken at weekly intervals during the growth period between the second and the sixth week after inoculation (the fourth and the eighth week after planting).

Appendix: Table 7: Line I-17-40

(A)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	4.62	11.0	10.5	6	6	122.35	126.12
2	15.11	10.5	12.0	7	6	133.55	129.12
3	9.71	9.0	11.0	8	6	123.50	135.56
Mean	9.81	10.17	11.17	7.00	6.00	126.47	130.27
SE	3.03	0.601	0.441	0.577	0.000	3.557	2.785

(B)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	19.18	13.00	12.25	17	10	414.62	245.35
2	14.48	12.25	11.75	18	11	417.46	231.63
3	17.49	13.00	14.25	14	9	391.51	237.21
Mean	17.05	12.75	12.75	16.33	10.00	407.86	238.06
SE	1.37	0.250	0.764	1.202	0.577	8.218	3.984

(C)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	25.77	14.5	12.25	25	14	682.29	241.42
2	21.01	17.0	15.00	31	14	755.40	251.31
3	36.24	14.0	16.50	27	13	722.50	271.74
Mean	27.67	15.17	14.58	27.67	13.67	720.06	254.82
SE	4.50	0.928	1.244	1.764	0.333	21.140	8.927

(D)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	31.43	18.0	18.00	30	18	903.60	456.60
2	22.22	19.0	17.25	23	12	775.04	349.98
3	12.17	18.5	18.00	29	17	1037.3	450.43
Mean	21.94	18.50	17.75	27.33	15.67	905.31	419.00
SE	5.56	0.289	0.250	2.186	1.856	75.713	34.558

(E)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	10.10	18.5	17.50	49	29	1143.7	598.7
2	22.71	24.5	18.25	31	38	782.3	547.2
3	11.70	17.5	19.00	41	12	1080.8	397.3
Mean	14.84	20.17	18.25	40.33	26.33	1002.27	514.40
SE	3.96	2.186	0.433	5.207	7.623	111.472	60.408

Appendix: Table 8: Cv. Prisma

(A)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	11.16	12.5	17.5	5	5	130.30	150.87
2	12.69	17.5	18.0	4	2	129.24	68.92
3	11.51	16.0	15.5	4	4	67.60	136.61
Mean	11.79	15.33	17.00	4.33	3.67	109.05	118.80
SE	0.461	1.481	0.764	0.333	0.882	20.726	25.277

(B)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	27.12	21.00	22.00	6	6	266.60	151.26
2	28.70	22.75	21.00	9	7	379.41	180.97
3	27.19	22.25	20.75	6	5	280.41	135.94
Mean	27.67	22.00	21.25	7.00	6.00	308.81	156.06
SE	0.516	0.520	0.382	1.000	0.577	35.526	13.218

(C)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	23.46	27.00	23.5	9	6	470.16	144.18
2	34.09	30.25	21.0	8	5	557.06	170.03
3	23.00	26.00	21.0	8	5	592.30	155.79
Mean	26.85	27.75	21.83	8.33	5.33	539.84	156.67
SE	3.622	1.283	0.833	0.333	0.333	36.295	7.475

(D)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	32.75	40.5	28.25	10	6	695.05	275.91
2	45.59	30.0	23.00	8	5	590.50	258.04
3	38.79	37.0	26.75	8	7	550.73	335.51
Mean	39.04	35.83	26.00	8.67	6.00	612.09	289.82
SE	3.708	3.087	1.561	0.667	0.577	43.038	23.420

(E)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	57.11	49.25	21.5	13	5	604.4	110.92
2	27.19	48.00	18.5	12	5	735.4	129.67
3	37.20	40.50	31.0	12	7	645.3	234.600
Mean	40.50	45.92	23.67	12.33	5.67	661.70	158.40
SE	8.794	2.732	3.768	0.333	0.667	38.695	38.484

Appendix: Table 9: Line B19909.

(A)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	10.72	11	10.00	3	4	83.09	122.41
2	11.78	12	11.00	4	5	60.73	98.70
3	14.05	10	11.75	5	5	112.64	118.85
Mean	12.18	11.00	10.92	4.00	4.67	85.49	113.32
SE	0.981	0.577	0.507	0.577	0.333	15.033	7.382

(B)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	25.47	10.25	12.5	10	6	277.61	180.70
2	28.35	10.75	12.0	10	6	268.04	140.02
3	24.39	12.00	13.0	10	7	255.57	162.27
Mean	26.07	11.00	12.50	10.00	6.33	267.07	161.00
SE	1.181	0.520	0.289	0.000	0.333	6.381	11.761

(C)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	43.51	15.00	13.25	15	10	455.63	205.04
2	31.34	15.00	13.50	21	9	481.45	270.16
3	27.20	26.25	15.00	20	11	502.34	276.11
Mean	34.02	18.75	13.92	18.67	10.00	479.81	250.44
SE	4.895	3.750	0.546	1.856	0.577	13.509	22.763

(D)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	33.77	20.25	23.50	25	7	676.78	172.02
2	31.80	27.00	16.25	23	13	696.35	260.82
3	54.46	25.00	17.50	16	10	500.77	295.39
Mean	40.01	24.08	19.08	21.33	10.00	624.63	242.74
SE	7.247	2.002	2.238	2.728	1.732	62.189	36.743

(E)

Repl.	% Mildew	Stem Height (cm)		Tillers No. / Plant		L.a. on Tillers (cm^2)	
		Cont.	Infec.	Cont.	Infec.	Cont.	Infec.
1	44.06	46.5	26.5	21	10	578.70	269.03
2	35.85	48.5	14.5	17	14	351.60	362.20
3	51.63	42.0	23.5	19	11	452.47	305.64
Mean	43.85	45.67	21.50	19.00	11.67	460.92	312.29
SE	4.556	1.922	3.606	1.155	1.202	65.694	27.101

Appendix Table 10

Primary values on the effects of *Blumeria graminis* on yield components and final grain yield of infected (Infec.) and control (Cont.) plants of three barley lines cv. Prisma, line I-17-40 and line B19909.

Appendix: Table 10A: Line I-17-40.

Replicates	No. Tillers		No. Fertile Tillers		No. Grain per Primary Shoot		No. Grain per Plant		1000-Grain Dry Weight on Tillers (g)		Total Grain Yield (g)		Total Plant Dry Weight (g)		Harvest Index	
	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec
1	26	18	21	15	17	17	187	178	18.25	28.60	3.509	5.114	15.70	11.87	0.223	0.431
2	28	50	22	28	25	18	222	63	17.38	16.31	4.062	1.285	22.79	23.19	0.178	0.055
3	20	24	19	18	19	18	217	233	17.69	25.47	3.895	5.915	13.51	15.85	0.288	0.373
4	40	25	24	20	21	21	100	228	19.46	26.19	1.964	6.01	20.18	16.78	0.097	0.358
5	50	18	27	13	18	17	47	94	18.86	19.52	0.904	1.971	13.32	5.953	0.068	0.331
6	41	18	26	12	17	11	103	66	18.62	9.26	1.971	0.703	9.218	8.076	0.214	0.087
7	44	62	33	32	20	14	288	22	20.10	21.75	5.853	0.443	19.43	12.86	0.301	0.034
8	33	51	24	30	16	21	222	160	23.46	21.53	5.251	3.571	13.91	20.73	0.377	0.172
9	57	47	32	27	20	23	49	109	20.28	16.61	1.099	1.9	13.43	17.16	0.082	0.111
10	54	61	30	32	20	17	97	39	14.29	16.27	1.526	0.758	17.01	15.76	0.090	0.048
Mean	39.30	37.40	25.80	22.70	19.30	17.70	153.2	119.2	18.84	20.15	3.003	2.767	15.85	14.82	0.192	0.200
SE	3.924	5.831	1.489	2.517	0.817	1.106	26.56	24.16	0.745	1.832	0.555	0.698	1.276	1.676	0.034	0.049

Appendix: Table 10B: Cv. Prisma.

Replicates	No. Tillers		No. Fertile Tillers		No. Grain per Primary Shoot		No. Grain per Plant		1000-Grain Dry Weight on Tillers (g)		Total Grain Yield (g)		Total Plant Dry Weight (g)		Harvest Index	
	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec
1	18	15	3	12	21	18	134	39	32.5	12.67	4.363	0.641	23.42	12.47	0.186	0.051
2	12	7	7	7	24	13	101	23	42.16	42.00	4.402	0.566	13.77	12.06	0.320	0.047
3	12	11	8	6	20	11	67	37	44.51	54.81	3.087	2.073	15.24	14.38	0.203	0.144
4	15	11	7	9	17	4	137	14	28.53	72.10	4.137	0.976	13.63	14.39	0.304	0.068
5	13	15	10	8	15	12	83	37	37.53	61.04	3.186	2.274	18.78	15.02	0.170	0.151
6	12	10	7	7	21	7	109	36	46.31	46.55	5.054	1.758	15.13	12.51	0.334	0.141
7	11	15	7	9	14	5	78	17	44.42	47.17	3.53	0.822	18.58	11.65	0.190	0.071
8	15	14	12	6	13	16	51	111	40.16	24.84	2.084	2.933	13.47	11.78	0.155	0.249
9	15	17	11	7	13	10	60	49	40.02	43.92	2.481	2.201	15.97	15.45	0.155	0.142
10	14	11	10	6	19	18	73	99	45.15	25.80	3.288	2.834	14.73	12.68	0.223	0.224
Mean	13.70	12.60	8.200	7.700	17.70	11.40	89.3	46.2	40.13	43.09	3.561	1.708	16.27	13.24	0.224	0.129
SE	0.667	0.968	0.827	0.597	1.221	1.593	9.460	10.41	1.840	5.666	0.292	0.283	0.992	0.449	0.022	0.022

Appendix: Table 10C: Line B19909.

Replicates	No. Tillers		No. Fertile Tillers		No. Grain per Primary Shoot		No. Grain per Plant		1000-Grain Dry Weight on Tllers (g)		Total Grain Yield (g)		Total Plant Dry Weight (g)		Harvest Index	
	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec	Cont	Infec
1	15	30	11	23	12	16	102	172	34.34	47.06	3.591	8.249	6.874	17.76	0.522	0.464
2	20	24	12	18	14	17	98	187	34.26	47.47	3.387	9.005	11.33	18.01	0.299	0.500
3	20	17	17	13	11	13	148	86	39.18	40.37	5.891	3.65	14.74	10.82	0.400	0.337
4	27	14	18	11	12	10	183	61	39.25	34.33	7.274	2.202	18.85	9.302	0.386	0.237
5	28	21	26	18	14	16	235	178	33.89	45.46	8.075	8.197	23.57	17.46	0.343	0.470
6	18	24	15	17	13	13	135	135	35.80	40.45	4.922	5.532	12.87	13.24	0.383	0.418
7	26	23	19	19	16	17	169	190	33.74	43.96	5.925	8.499	19.97	17.10	0.297	0.497
8	15	13	15	10	12	14	131	79	41.18	33.17	5.377	2.735	11.99	11.06	0.448	0.247
9	22	13	17	9	12	12	167	38	38.55	32.42	6.5	1.343	16.97	8.545	0.383	0.157
10	24	18	23	13	13	13	216	74	23.92	34.38	5.443	2.628	22.60	9.538	0.241	0.276
Mean	21.50	19.70	17.30	15.10	12.90	14.10	158.4	120.0	35.41	39.91	5.639	5.204	15.98	13.28	0.370	0.360
SE	1.493	1.789	1.453	1.441	0.458	0.737	14.23	18.51	1.534	1.883	0.464	0.959	1.694	1.237	0.026	0.040

Appendix Tables 11 to 12

Data on the effects of infection on stomatal resistance in the light (Table 11A) and in the dark (Table 11B). Table 12 shows the predicted effects of changes in r_p and r_x on Pg_{max} and θ.

Appendix: Table 11A.

Measurements in **the light** of stomatal resistances (s cm^{-1}) of infected and uninfected third leaf of cv. Prisma and the wild lines I-17-40 and B19909.

Days after inocul.	Repli.	I-17-40				Cv. Prisma				B19909			
		Control		Infected		Control		Infected		Control		Infected	
		M	T	M	T	M	T	M	T	M	T	M	T
1	1	1.18	2.78	3.08	3.06	2.63	3.58	3.27	2.28	1.80	2.42	5.05	3.76
	2	1.42	2.09	5.72	2.74	2.28	2.83	4.60	4.47	1.95	1.24	4.98	5.68
	3	2.65	3.07	5.91	4.73	2.83	3.09	5.51	4.65	1.78	4.07	4.66	2.71
	4	2.77	3.08	4.56	3.72	2.77	2.89	6.23	3.99	2.40	2.97	4.37	1.60
	Mean	2.01	2.76	4.82	3.56	2.63	3.10	4.90	3.85	1.98	2.67	4.76	3.44
	SE	0.41	0.23	0.65	0.44	0.12	0.17	0.64	0.54	0.14	0.59	0.16	0.87
3	1	2.60	4.98	5.90	3.27	2.39	5.87	4.39	7.43	2.52	4.34	5.11	5.60
	2	2.55	4.10	4.57	5.60	3.98	6.74	5.06	10.8	3.22	4.99	4.54	5.13
	3	2.96	3.30	3.75	5.72	3.93	4.74	5.41	7.97	2.34	4.24	5.07	6.43
	4	2.78	4.56	6.17	3.99	3.17	4.27	5.50	8.09	2.54	4.57	5.16	5.83
	Mean	2.72	4.23	5.10	4.65	3.37	5.40	5.09	8.57	2.66	4.53	4.97	5.75
	SE	0.09	0.36	0.57	0.60	0.37	0.56	0.25	0.75	0.20	0.16	0.15	0.27
6	1	0.87	0.14	4.87	2.49	0.75	2.61	3.35	3.94	0.25	1.65	2.58	1.74
	2	0.45	0.58	2.84	3.39	1.53	2.90	3.49	2.58	0.97	1.75	3.71	3.05
	3	0.24	0.47	4.27	1.09	1.11	3.82	7.39	5.01	1.29	1.14	3.03	3.05
	4	0.87	0.99	3.92	-0.5	1.26	2.59	4.74	1.36	1.02	1.34	1.78	1.54
	Mean	0.61	0.54	3.97	1.63	1.16	2.98	4.74	3.22	0.88	1.47	2.77	2.35
	SE	0.16	0.17	0.43	0.84	0.16	0.29	0.94	0.80	0.22	0.14	0.40	0.41
8	1	2.54	3.89	4.18	3.78	3.53	5.20	5.69	5.16	2.06	2.88	4.43	3.81
	2	2.79	3.18	3.88	3.97	4.40	4.60	2.21	3.17	2.04	3.08	2.45	3.63
	3	2.07	3.49	3.84	3.58	2.84	3.89	2.20	2.53	1.97	3.17	0.96	1.26
	4	2.40	3.75	3.97	5.50	4.11	4.57	3.61	2.40	2.11	3.45	3.63	3.12
	Mean	2.45	3.58	3.97	4.21	3.72	4.56	3.43	3.32	2.04	3.15	2.87	2.96
	SE	0.15	0.15	0.08	0.44	0.35	0.27	0.82	0.64	0.03	0.12	0.76	0.58
10	1	1.17	2.68	3.91	5.98	3.94	4.19	3.87	4.01	0.72	3.91	0.57	1.64
	2	1.09	2.78	2.94	6.11	3.50	4.94	0.65	-0.5	1.90	2.00	0.79	-0.2
	3	2.05	3.36	1.69	3.45	5.26	3.74	2.69	2.42	1.08	2.09	0.52	0.64
	4	0.98	1.28	1.69	3.01	5.37	5.24	1.37	1.00	1.27	1.85	0.56	1.15
	Mean	1.32	2.52	2.56	4.64	4.51	4.53	2.15	1.73	1.24	2.46	0.61	0.82
	SE	0.25	0.44	0.54	0.82	0.47	0.34	0.71	0.96	0.25	0.48	0.06	0.38

M: Middle region of the leaf, **T**: Tip of the leaf

Appendix: Table 11B.

Measurements in **the dark** of stomatal resistances (s cm^{-1}) of infected and uninfected third leaf of cv. Prisma and the wild lines I-17-40 and B19909.

Days after inocul.	Repli.	I-17-40				Cv. Prisma				B19909			
		Control		Infected		Control		Infected		Control		Infected	
		M	T	M	T	M	T	M	T	M	T	M	T
2	1	13.14	8.32	11.4	18.3	12.2	10.9	8.67	13.8	8.71	10.6	8.54	9.26
	2	12.37	9.00	11.5	17.1	12.4	10.7	8.47	6.62	6.43	9.23	8.42	9.01
	3	8.35	7.66	6.11	9.99	6.99	9.61	7.93	8.55	8.15	9.53	8.60	10.8
	4	7.01	12.9	5.45	8.95	8.23	9.94	8.30	8.17	11.9	10.5	9.46	18.9
	Mean	10.22	9.47	8.61	13.6	9.96	10.3	8.34	9.29	8.80	9.98	8.76	12.0
	SE	1.50	1.18	1.64	2.40	1.38	0.30	0.16	1.56	1.15	0.36	0.24	2.33
4	1	18.2	26.9	11.0	24.4	16.3	21.4	8.45	15.5	12.7	13.6	11.3	13.8
	2	15.9	21.1	10.1	26.1	15.4	23.3	11.7	14.2	13.4	30.0	7.70	25.1
	3	15.3	22.0	9.28	25.6	21.8	18.9	12.6	24.4	12.0	17.8	6.65	19.0
	4	17.5	22.6	8.07	22.9	15.8	19.8	7.91	18.3	7.40	24.8	7.60	20.0
	Mean	16.7	23.2	9.61	24.7	17.3	20.8	10.2	18.1	11.4	21.6	8.30	19.5
	SE	0.66	1.29	0.62	0.71	1.52	0.96	1.17	2.26	1.36	3.63	1.01	2.32
7	1	10.3	10.9	9.54	8.52	11.3	11.2	10.6	11.2	12.6	14.1	7.82	6.61
	2	15.3	13.6	8.44	7.49	11.3	15.9	9.18	10.8	9.67	7.59	7.19	9.56
	3	11.3	9.22	7.13	9.68	12.3	15.5	9.37	11.5	10.8	11.4	7.50	9.82
	4	11.0	10.1	7.80	8.50	12.3	10.4	9.77	11.0	8.62	9.48	7.21	9.59
	Mean	12.0	10.9	8.23	8.55	11.8	13.2	9.74	11.1	10.4	10.6	7.43	8.89
	SE	1.13	0.94	0.51	0.45	0.29	1.42	0.32	0.14	0.86	1.39	0.15	0.76
9	1	8.89	9.04	6.75	5.65	12.4	13.2	9.47	7.09	15.7	19.0	6.66	9.00
	2	8.68	8.87	6.29	6.02	14.2	14.6	7.20	8.89	14.2	18.9	7.69	9.07
	3	10.2	8.80	7.16	7.39	12.6	12.2	7.42	8.60	13.7	14.8	7.50	9.35
	4	10.6	8.95	7.64	8.54	10.9	11.1	8.68	9.95	11.9	15.2	7.41	8.78
	Mean	9.59	8.92	6.96	6.90	12.5	12.8	8.19	8.63	13.9	17.0	7.32	9.05
	SE	0.48	0.05	0.29	0.66	0.67	0.74	0.54	0.59	0.78	1.16	0.23	0.12
11	1	8.11	7.62	6.45	9.33	11.0	14.3	7.75	8.72	13.5	14.3	7.00	9.67
	2	7.71	10.5	7.18	9.79	11.1	15.9	8.04	12.0	8.74	14.1	6.47	9.45
	3	9.17	14.7	7.00	8.97	12.7	13.4	6.95	7.43	11.7	19.0	9.71	8.72
	4	8.17	15.1	7.37	9.01	14.6	15.5	8.29	10.5	12.1	16.2	6.42	6.86
	Mean	8.29	12.0	7.00	9.27	12.3	14.8	7.76	9.67	11.5	15.9	7.40	8.67
	SE	0.31	1.78	0.20	0.19	0.84	0.57	0.29	1.01	1.01	1.14	0.78	0.64

M: Middle region of the leaf, **T**: Tip of the leaf

Appendix: Table 12.

The predicted effects of changes in r_p and r_x on Pg_{max} and θ.

Assumption	Changes	Expected Changes	
		Pgmax	θ
$r_p > r_x$	r_p	√	-
	r_x	-	-
$r_p \approx r_x$	r_p	√	√
	r_x	√	√
$r_p < r_x$	r_p	-	√
	r_x	√	√

Where r_p = physical resistance, r_x = biochemical resistance,

√ = significant changes, (-) = insignificant changes.

Appendix Tables 13 to 16

Summary tables of the results of growth analyses, photosynthesis and stomatal resistance.

Table 13A: Growth analyses summary table

Effects of infection on the growth and development			
Lines	**B19909**	**cv. Prisma**	**I-17-40**
Mildew development			
%GLA colonised	+++	+++	+
Conidial production	+++	++	+
Shoot production			
Primary shoot height	--	---	-
Number of tillers	--	-	---
GLA on primary shoot	-	---	--
GLA on all tillers	--	---	---
Senescence of leaves	+	+	++
No of leaves on the primary shoot	1 or 2 less	1 or 2 less	similar
No of epidermal cells	10 % fewer	7 % fewer	6 % fewer
Size of epidermal cells	-	---	--
Root production			
No of seminal roots	0	0	0
No of laterals on seminal roots	-	---	0
Total length of seminal roots	--	---	-
Diameter of seminal roots	0	0	0
Surface area of seminal roots	--	---	-
No of nodal roots	-	---	--
No of laterals on nodal roots	0	--	0
Total length of nodal roots	-	---	--
Diameter of nodal roots	0	0	0
Surface area of nodal roots	-	---	--
Dry matter accumulation			
Shoot dry weight	0	---	-
Root dry weight	--	---	-
Total plant dry weight	--	---	-
Root : shoot ratio	0	---	--

\+ : Increase

\- : Decrease

0 : No changes

Table 13B: Growth analyses summary table

Effects of infection on the growth and development			
Lines	**B19909**	**cv. Prisma**	**I-17-40**
Efficiency of growth			
Relative growth rate (RGR)	-	-	- and +
Unit leaf rate (ULR)	-	-	- and +
Dry matter distribution			
Leaf weight ratio (LWR)	+++	++	+
Leaf area ratio (LAR)	0	- -	- -
Specific leaf area (SLA)	-	- - -	- -
Root weight ratio (RWR)	0	- -	-
Root area ratio (RAR)	0	-	-
Specific root area (SRA)	0	0	0
Specific root length (SRL)	0	+	0
Root tip cell division	-	0	0
Yield components			
No of grains per primary shoot	0	- -	0
Dry weight of grains on the primary shoot	+	- -	0
1000-grain weight on the primary shoot	+	0	0
No of fertile tillers	0	0	0
No of unfertile tillers	0	0	0
No of grains on tillers	0	-	0
Dry weight of grains on tillers	0	- -	0
1000-grain weight on tillers	0	0	0
Total grain dry weight per plant	0	- -	0
Harvest index	0	- -	0

\+ : Increase

\- : Decrease

0 : No changes

Table 14: Photosynthesis summary table

Photosynthesis in infected third or fourth leaf						
Lines	**Golden Promise** (4^{th} leaf)	**B19909** (3^{rd} leaf)	**cv. Prisma** (3^{rd} leaf)	**cv. Triumph** (4^{th} leaf)	**I-17-40** (3^{rd} leaf)	**B8893** (4^{th} leaf)
Fungal biomass (Number of conidia)	42×10^5	42×10^5	24×10^5	23×10^5	10×10^5	1×10^5
Pg_{max} (per leaf area)	- 89 %	- 80 %	- 80 %	- 85 %	- 83 %	0
Pg_{max} (per mg chlorophyll)	0	0	0	0	0	+ 49 %
Quantum efficiency (α)	- 53 %	- 27 %	- 46 %	- 30 %	- 28 %	0
Convexity (θ)	+	0	+	+	0	0
Total chlorophyll	- 84 %	- 85 %	- 85 %	- 62 %	- 77 %	- 41 %
Chlorophyll a	- 85 %	- 87 %	- 87 %	- 67 %	- 84 %	- 44 %
Chlorophyll b	- 79 %	- 84 %	- 84 %	- 51 %	- 60 %	- 33 %
Chlorophyll a : b ratio	-	0	0	-	-	-
Dark respiration	+ 40 %	+106 %	+178 %	+64 %	+70 %	0

\+ : Increase

\- : Decrease

0 : No changes

Table 15: Stomatal resistance summary table

Stomatal resistance in infected middle region and uninfected tip region of the third leaf			
Lines	**B19909**	**cv. Prisma**	**I-17-40**
Light			
Middle region	++	++	++
Tip region	+	+	+
Dark			
Middle region	-	-	-
Tip region	0	-	-

\+ : Increase (Stomatal closure)

\- : Decrease (Stomatal opening)

0 : No changes

Table 16: Compensatory photosynthesis summary table

Photosynthesis in uninfected fourth leaf of infected and uninfected plants			
Lines	**B19909**	**cv. Prisma**	**I-17-40**
Pg_{max} (per leaf area)	0	0	+
Pg_{max} (per mg chlorophyll)	0	0	0
Quantum efficiency (α)	0	0	+
Convexity (θ)	+	+	0
Total chlorophyll	0	0	0
Chlorophyll a	-	0	0
Chlorophyll b	0	0	0
Chlorophyll a : b ratio	0	0	0
Dark respiration	0	0	0

+ : Increase

- : Decrease

0 : No changes

Analysis of Photosynthetic Light Response Curves using the Leaf Oxygen Electrode.

Akhkha, A., Clarke D.D. and Dominy, P.*+

Division of Environmental & Evolutionary Biology and Division of Biochemistry & Molecular Biology*, Institute of Biomedical & Life Sciences, The Bower Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

+ To whom correspondence should be addressed.

Introduction

Photosynthesis rates are often used as measures of the well-being of plants and so their determination is important in many disciplines within plant science research. Frequently, one of two methods is used to determine photosynthesis rates. In laboratories where the main focus of interest is on photosynthesis, infra red gas analysers (IRGAs) are routinely used as they have excellent sensitivity and are versatile. However, IRGAs and the required accompanying gas mixing / delivery systems are expensive, and are, therefore, not the choice for plant biologists with interests that lie outside photosynthesis. Consequently, the leaf O_2 electrode has gained wide acceptance in many laboratories where photosynthesis rates are only occasionally measured as they are relatively inexpensive and simple to operate.

The leaf O_2 electrode is a closed chamber system that was first introduced by Delieu and Walker (1981). A detached leaf, or leaf piece, is sealed in a chamber containing an atmosphere which should saturate the CO_2 requirement for photosynthesis (1 - 5 kPa partial pressure CO_2, Delieu and Walker, 1981). As there is no simple way of regulating CO_2 levels during an experiment, photosynthesis rates are usually measured as a function of incident light intensity (I), *i.e.* light response curves (P *versus* I) are often measured. Several methods can then be used to determine important photosynthetic parameters such as the apparent quantum yield of photosynthesis (α'), the maximum net photosynthesis rate (Pn_{max}) and the dark respiration rate (Rd). Often, α and Pn_{max} are derived by linearization of the data, which assumes a linear (hyperbolic) relationship between Pn and I (e.g. Rabinowitch, 1951).

Thornley (1976), however, has pointed out that Pg (gross photosynthesis rate) *versus* I plots are rarely hyperbolic, but are better described by a non-linear (quadratic) function, which allows for the often-observed sharp discontinuity between the initial near linear slope (α') and the final near-linear asymtote (Pg_{max}). This discontinuity provides an additional photosynthetic

parameter θ, or the convexity parameter, which can vary between a value of 0 and 1. When θ = 0, the Pg *versus* I curve degenerates into a linear (hyperbolic) function; when θ=1, the relationship converts to a Blackman response (Chartier, 1968). Thornley (1976), and later Marshall and Biscoe (1980) who extended the model to describe the Pn *versus* I relationship, viewed θ as a parameter that expresses the relative limitations placed on rubisco turn-over by the supply of NADPH, ATP and ribulose 1,5-bisphosphate (biochemical processes) and the supply of CO_2 (physical processes). When θ = 0, only the biochemical processes are limiting; when θ = 1, only the physical processes are limiting. However, this view has been challenged (Terashima & Saeki, 1983; Vogelman *et al.*, 1989; Leverenze, 1987 & 1988). These groups have provided evidence that both leaf development and architecture can cause changes in the light gradient penetrating into green tissues and have suggested that these give rise to changes in θ. More recent work has suggested that changes in the intensity and direction of illumination during growth can affect θ, but also provide evidence for a strong dependence of θ on atmospheric CO_2 partial pressures (C_a; Ogren, 1994; Ogren & Evans, 1993; Palmquist et al., 1994). The interpretation of θ is thus still unclear and probably arises from several factors.

In our laboratory, the light response curves from a range of monocotyledonous and dicotyledonous species, determined using the leaf O_2 electrode, rarely conform to either a linear (hyperbolic) or a Blackman relationship. Therefore, the non-linear (quadratic) formulation proposed by Thornley (1976) and Marshall and Biscoe (1980) may provide a better description. The observation that hyperbolic responses are rarely measured in leaf O_2 electrodes when the recommended chamber CO_2 partial pressures are used (C_a <5 kPa) may be interpreted in two ways. Firstly, C_a is saturating (Delieu & Walker, 1980) but θ is unrelated to the supply of CO_2 (Terashima & Saeki, 1983; Vogelman *et al.*, 1989; Leverenze, 1987 & 1988). Secondly,

the supply of CO_2 is limiting photosynthesis when C_a is <5 kPa, and θ is a function of CO_2 supply (Thornley, 1976; Marshall & Biscoe, 1980). In either case, methods for the extraction of P_{max} and α from P *versus* I curves that are based on an assumption of a hyperbolic relationship will incur errors that may prove to be serious.

In this paper we report an investigation using barley, bean (both C-3) and maize (C-4) to investigate the relationship between θ, C_a and the model underlying Pn *versus* I plots determined with a leaf O_2 electrode. Firstly, we established what effect changes in chamber C_a has on θ. Secondly, we show that serious errors can arise when Pn_{max} and α are determined from data assuming a linear (hyperbolic) relationship at the recommended levels of C_a, and make suggestions on how to minimize these errors.

Materials and methods.

Plant material. Seeds of barley (*Hordeum vulgare* L.) cv. Golden Promise, (*Phaseolus coccineus*) cv. Scarlet runner bean and maize (*Zea mays*) cv. , were placed on moistened filter paper and germinated in a growth cabinet. Seedlings, selected for uniformity, were transplanted singly in 15 cm (barley and bean) or 18 cm (maize) plastic pots containing Levington potting compost. The pots were placed in a growth cabinet maintained at a temperature of 19 ± 2°C, which provided RH between 60 to 70%, and a photoperiod of 16 hours (130 μmol quanta m^{-2} s^{-1}).

Photosynthetic measurements. Photosynthesis rates were determined from measurements of O_2 evolution obtained using a Hansatech LD2 leaf disc electrode system (Hansatech Ltd., Paxman Road, Hardwick Industrial Estate, King's Lynn, Nofolk, UK) designed by Delieu and Walker (1981, 1983).

All measurements were made at a constant temperature (25 ± 0.05°C). Illumination of the chamber was provided by a 50W 24° dispersion halogen dichroic lamp (Osram) emitting through a water bath to remove infrared heat.

Neutral density filters (Balzar, Lichtenstein) were used at the surface of the chambers to attenuate the light to the desired levels (0–1363 μmol quanta m^{-2} s^{-1}). Fresh leaf samples were cut (6–10 cm^{-2}) just prior to use and placed in the thermostatted chamber. Chamber volumes were determined using the volume injection method (Delieu & Walker, 1981). Gas mixtures containing the desired CO_2 partial pressures (1, 5 and 10 kPa) were mixed using compressed air and 10% CO_2 in compressed air cylinders. The mixtures were humidified at room temperature (*ca.* 20°C) by bubbling through water traps before passing into the leaf chambers. The chambers were purged at 100 ml min^{-1} for 3 minutes to equilibrate before sealing the valves. All measurements were made as follows. Samples were initially irradiated at 150 μmol quanta m^{-2} s^{-1} for approximately 5 minutes, and then left to dark adapt for at least 15 minutes until a steady rate of dark respiration was attained. The chamber was then flushed for 3 minutes with fresh gas, sealed and the sample then exposed to a new light level until a steady state rate of O_2 evolution was reached. Generally, illumination was altered sequentially, from dark to high light.

Data Analyses. Two models were used in this study to calculate photosynthetic parameters.

Linear (hyperbolic) Model. This model was proposed by Rabinowich (1952), and predicts that at high CO_2 partial pressures, photosynthesis rates are governed by light intensity. Three parameters are included: P_{max} (gross or net), the maximum rate of photosynthesis: α, the quantum efficiency: Rd, the dark respiration rate.

$$P = \frac{P_{max}\alpha I}{P_{max} + \alpha I} \qquad (1)$$

Where P is the (net or gross) photosynthesis rate, P_{max} is the maximum rate of photosynthesis, α is the quantum efficiency at low irradiance, and I is the irradiance (PPFD). The gross or net photosynthesis rates (Pg and Pn, respectively) are related as Pg = Pn + Rd where Rd is the rate of dark respiration.

Thornley (1976) pointed out that the observed light response curve is better described by a quadratic function, *viz.*

$$\theta P^2 - (P_{max} + \alpha I)P + (P_{max}\alpha I) = 0$$

(2)

where θ is the convexity term. Marshall and Biscoe (1980) extended this model to describe Pn *versus* I, *viz.*

$$\theta Pn^2 - (Pg_{max} + \alpha I - \theta Rd)Pn + (\alpha I(Pg_{max} - (1-\theta)Rd) - RdPg_{max} = 0$$

(3)

Pn_{max} is calculated as $Pn_{max} = Pg_{max} - (1-\theta)Rd$ (Marshall and Biscoe, 1980). When θ is zero, equation (3) degenerates into equation (1), when it is unity, equation (3) describes a Blackman curve. Equation (3) is a quadratic (second order polynomial) of the form

$$y = ax^2 + bx + c = 0$$

(5)

which can be solved for its roots using

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

(6)

Equation (6) was used to solve for Pg_{max}, α, θ and Rd using the non-linear Solver routine in Microsoft Excel version 5 (Newon Raphson method, forward derivatives, scaled); solutions were found by minimising the data-minus-fit sum of squares by successive itteration. Values for Pn_{max} were then calculated using the relationship given above. In addition, the linear hyperbolic model (1) was fitted to the same data sets using both an itterative method (Solver) and linear regression techniques after transforming to a linear form.

Results.

The relationships between incident light intensity and net photosynthetic oxygen evolution when bean, barley and maize leaves were exposed to 1, 5 and 10kPa partial pressure CO_2 are presented in Fig. 1. Each curve is the average of 5 separate plots (± standard errors) that were determined from 5 separate leaves. Table 1 presents a summary of the differences in Pn, Pg, α, θ and Rd for each species at each CO_2 partial pressure.

The Effect of C_a on Photosynthesis. The effect of changes in C_a on the photosynthetic parameters determined by the non-linear model are presented in Table 1 and Figure 1. Increasing the CO_2 partial pressure from 1 to 10 kPa produced no significant ($p>0.05$) increase in Pn_{max} for bean. In contrast, Pn_{max} for maize and barley increased significantly ($p< 0.01$) over the same CO_2 concentration range; a similar pattern of change was observed when the effect of C_a on the maximum gross photosynthesis rate was examined (Table 1). Increasing C_a also produced significant ($p<0.05$) changes in the apparent quantum efficiency, α', for barley and bean, but not for maize. No significant changes ($p> 0.05$) were observed for the dark respiration rates (Rd) for any of the species with increasing CO_2 partial pressures. However, C_a had a dramatic effect on the convexity term θ (Table 1 & Fig. 2). Increasing C_a

	Comparisons Between CO_2 Levels (kPa)	Pn	Pg	α	θ	Rd
Bean	**1 *vs.* 5**	*n/s*	*n/s*	*n/s*	0.1	*n/s*
	5 *vs.* 10	*n/s*	*n/s*	*n/s*	0.5	*n/s*
	1 *vs.* 10	*n/s*	*n/s*	0.05	0.10	*n/s*
Barley	**1 *vs.* 5**	*n/s*	*n/s*	0.05	0.1	*n/s*
	5 *vs.* 10	*n/s*	*n/s*	*n/s*	0.1	*n/s*
	1 *vs.* 10	0.01	0.01	*n/s*	0.1	*n/s*
Maize	**1 *vs.* 5**	0.01	0.01	*n/s*	0.1	*n/s*
	5 *vs.* 10	0.01	0.01	*n/s*	0.1	*n/s*
	1 *vs.* 10	0.01	0.01	*n/s*	0.1	*n/s*

Table 1. Summary of the Statistical Analyses of the Effects of CO_2 Partial Pressures on Photosynthetic Parameters from Bean, Barley and Maize.

The non-linear model of Marshall and Biscoe (1980) was used to determine values for Pg_{max}, Pg_{max}, α', θ and Rd from each of the 45 light response curves presented in Figure 1. Analysis of variance was performed on each of these parameters using the GLM routine in Minitab 10.0

Figure 1. Photosynthetic Light Response Curves for Bean, Barley and Maize Measured with Leaf Oxygen Electrodes.

Samples were taken at random from plants in a growth room and immediately placed in the thermostatted leaf chamber (25 ± 0.05°C) and exposed to the initial light and dark pre-adaptation regime as described in the text. The chambers were flushed for three minutes (100 ml. min-1) with fresh humidified gas of 1 (a,d & g), 5 (b,e &h) or 10 (c,f & i) kPa partial pressure CO_2 in air. The chambers were then sealed and exposed to one of the light levels indicated until a steady rate of net oxygen exchange (Pn) was attained. The chambers were then flushed with fresh gas and Pn determined at another light level. Panel a-c, bean: panel d-f, barley: panel g-i, maize. Each panel presents the average and standard errors of the pooled data from 5 separate response curves determined from five separate leaves. Superimposed on these data are the fitted responses: solid line, non-linear model fit: broken line, linear model.

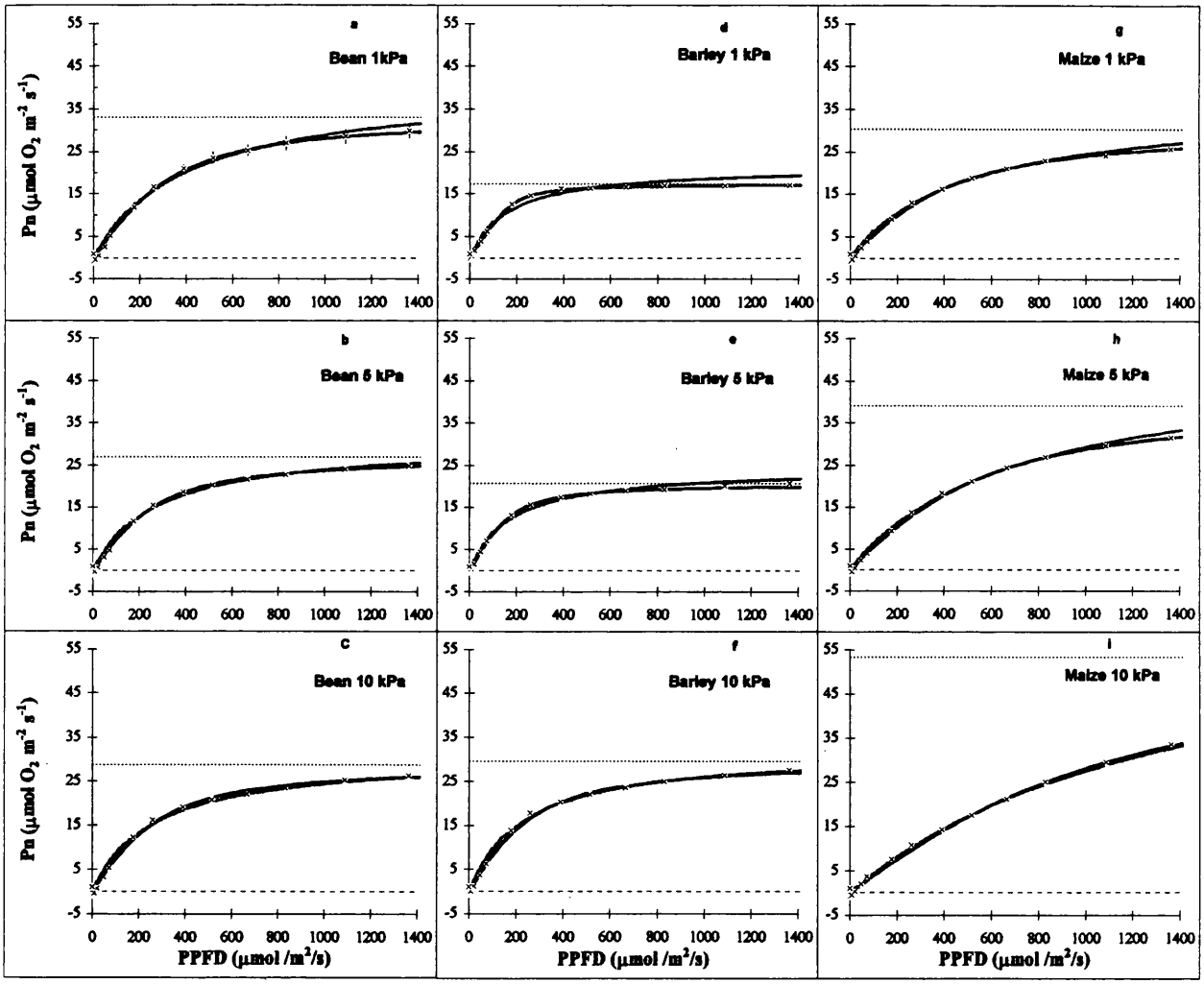
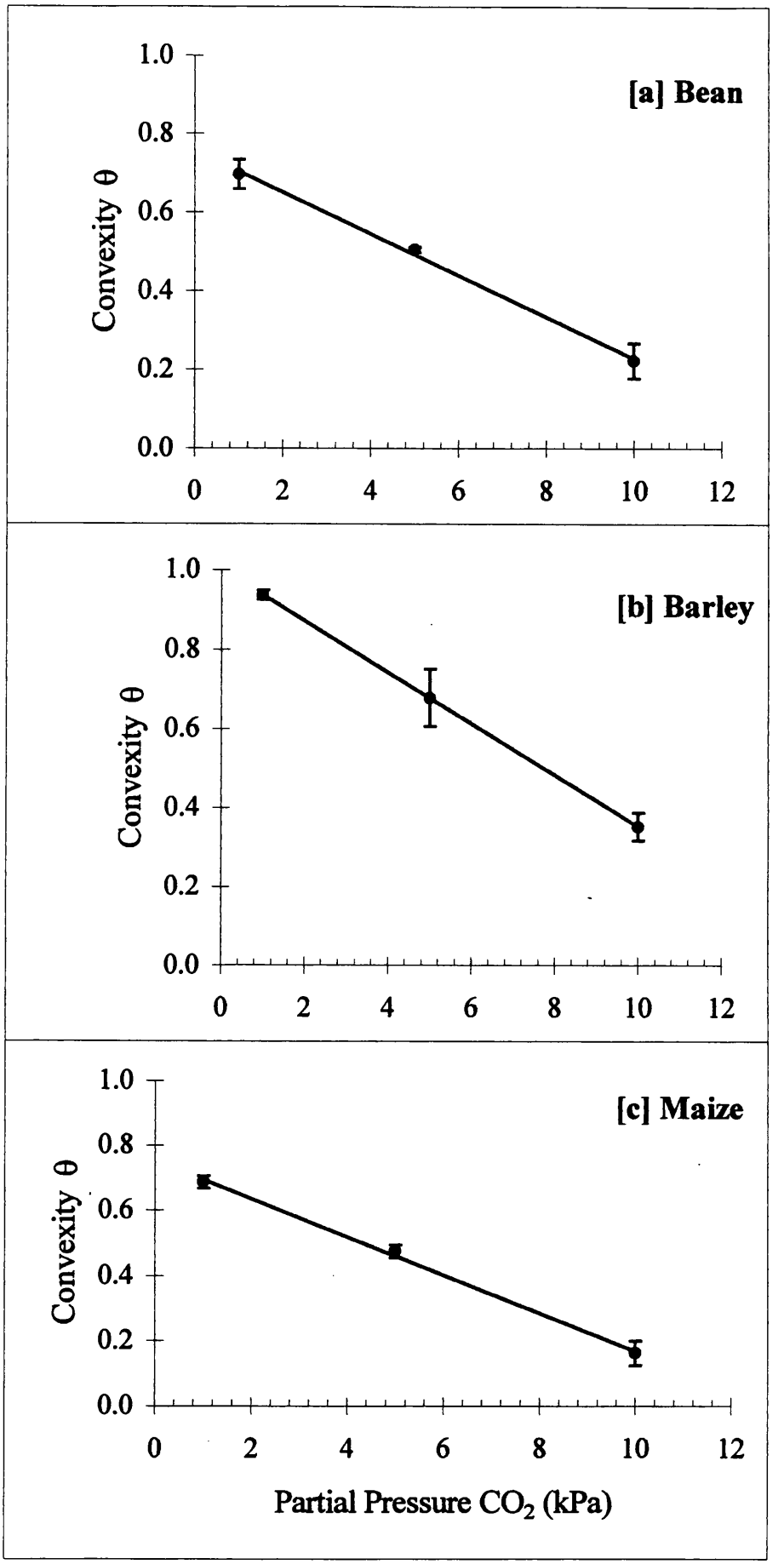

a
Bean 1kPa
d
Barley 1 kPa
g
Maize 1 kPa
b
Bean 5 kPa
e
Barley 5 kPa
h
Maize 5 kPa
c
Bean 10 kPa
f
Barley 10 kPa
i
Maize 10 kPa
Pn (µmol O2 m-2 s-1)
PPFD (µmol /m2/s)
55
45
35
25
15
5
-5
0
200
400
600
800
1000
1200
1400

Figure 2. The Effect of CO_2 Partial Pressures on the Convexity Parameter, θ.

Each data point represents the average (± standard error) value from 5 separate leaf samples at each CO_2 partial pressure.


[a] Bean
Convexity θ
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
[b] Barley
Convexity θ
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
[c] Maize
Convexity θ
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Partial Pressure CO_2 (kPa)

from 1 to 10 kPa produced a significant ($p<0.01$) linear decrease in θ (from 0.697 to 0.222 for bean, from 0.936 to 0.352 for barley and from 0.686 to 0.162 for maize).

Comparison of the Models. At 1 kPa partial pressure CO_2 (Fig. 1a, 1d & 1g), the level recommended for use with the leaf O_2 electrode, the linear model tended to underestimate photosynthesis in the 100- 500 μmol quanta m^{-2} s^{-1} PPFD light range, and over-estimate photosynthesis in the low and high PPFD range. Therefore, one consequence of using the linear model will be the persistent over-estimation of α', the apparent quantum efficiency. For all data sets, the observed data were used to fit both the linear and non-linear models, and in all cases it was found that the linear model gave a poorer fit ($n = 45$). An analysis of variance test was performed on the residual errors for each fit and a significant reduction was found with the non-linear model ($p< 0.004$).

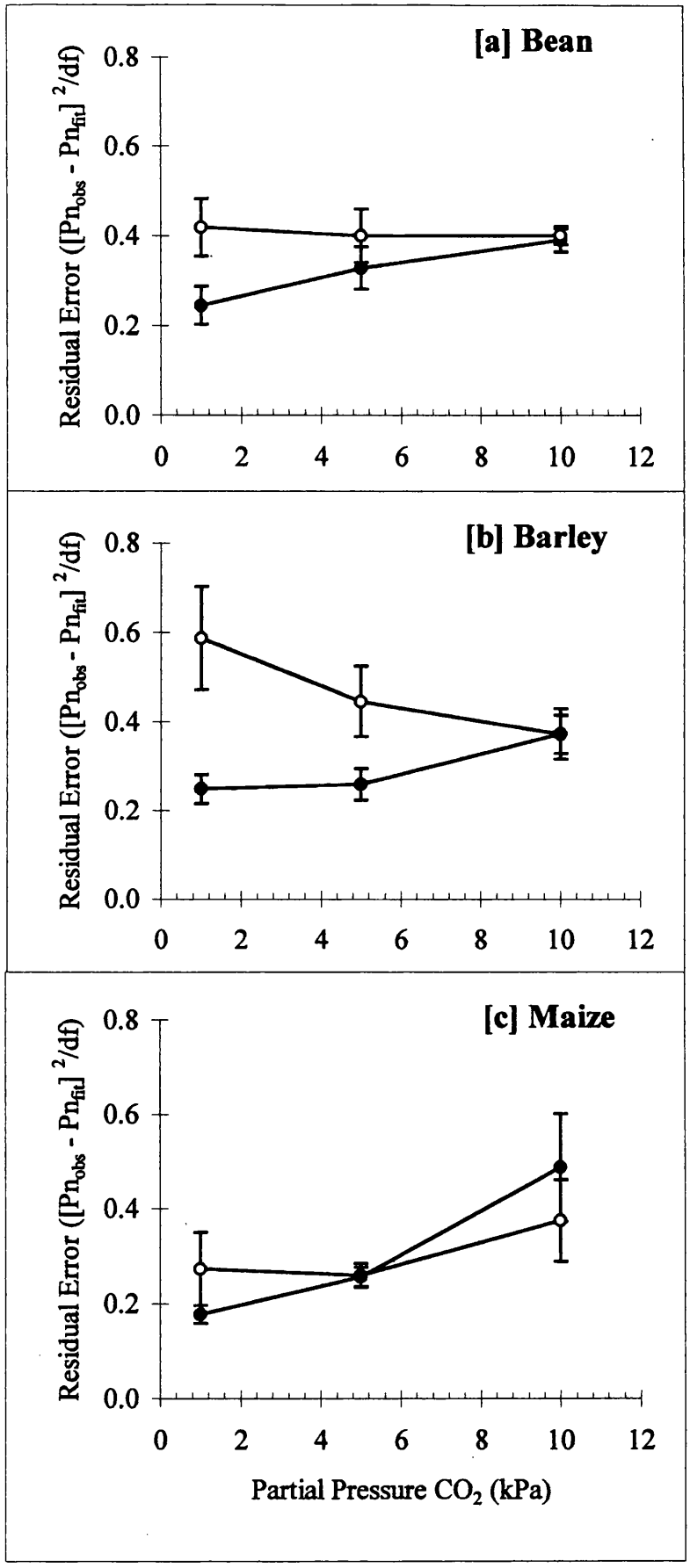
Figure 3 shows that the discrepancies generated by the two models were most noticeable at low CO_2 partial pressures. As CO_2 levels were increased, the average residual errors that arise from the linear model decreased, so that in all cases at 10kPa CO_2, no significant differences ($p>0.05$) were observed between the linear and non-linear model.

Discussion.

In this report we provide evidence that for all three species studied here, θ is strongly inversely related to external CO_2 partial pressures. These observations are consistent with the assertion originally proposed by Thornley (1976) and Marshall & Biscoe (1980) that θ reports the physical diffusive resistance of CO_2 supply to the site of carboxylation. For the experiments we report here, leaf pieces were taken randomly from plants in the growth room

Figure 3. The Effect of CO_2 Partial Pressures on the Linear and Non-Linear Model Residual Errors.

The residual errors were calculated as the mean sum-of-squares. Each data point is the average (± standard error) calculated from the residual errors of 5 separate data sets.


[a] Bean
[b] Barley
[c] Maize
Residual Error ([Pn$_{obs}$ - Pn$_{fit}$] ^{2}/df)
0.0
0.2
0.4
0.6
0.8
0
2
4
6
8
10
12
Partial Pressure CO_2 (kPa)

just prior to measurement, and so differences in θ cannot be attributed to light gradients within the leaves.

A dependence of θ on C_a has been reported before (Ogren & Evans, 1994). In their experiments θ was estimated from measurements on CO_2 exchange using four different partial pressures of CO_2 (~20, ~40, 100 and 5000 Pa) and was shown to decrease as C_a was increased from 20 to 100 Pa. However, on changing C_a from 100 to 5000 Pa, θ increased from ~0.6 to ~0.95 (Fig. 2 in Ogren & Evans, 1994; *cf.* Fig. 2 presented here). There appears to be some discrepancy regarding the behaviour of θ at high values of C_a and these differences may arise from differences associated with the plant material used, the range of C_a employed, or the methodology (IRGA *versus* leaf O_2 electrode).

The residual errors that were generated when curves were fitted to the data using the linear and non-linear models clearly indicate that the latter provides a better description of the Pn *versus* I relationship. The data presented here suggest that only at 10kPa CO_2, as the non-linear model degenerates into the linear form (*i.e.* $\theta \rightarrow 0$), are the two models equivalent in terms of their accuracy. Therefore, if Pn measurements are made using the CO_2 levels recommended with the manufacturer's instructions (Hansatech, 1984; Delieu & Walker, 1981), then the non-linear model should be used. Failure to do this will result in a 50 to 100 % increase in the residual errors and errors will also be propagated in the estimates of P_{max} and α'. Table 2 presents the average values for Pg_{max} and α' determined from the same data sets by the two models (n=45). It is clear that when C_a is 1 kPa, α' can be significantly over-estimated by the linear model in barley by as much as 60% ($p<0.001$) although bean (25%, $p=0.045$) and maize (21%, $p=0.003$) are also significantly affected. Similarly, Pg_{max} is significantly over-estimated at 1 kPa CO_2 when the linear model is used with bean (28%, $p=0.015$); a similar pattern was observed with barley (60%, $p=0.053$) and maize (37%, $p=0.635$) but here, although large, the differences were not significant. With increasing CO_2

		Bean			Barley			Maize		
		Linear	Non-Linear	%	Linear	Non-Linear	%	Linear	Non-Linear	%
Pg_{max}	1	42.00	32.78	28.1	21.68	13.52	60.4	40.42	29.55	36.8
	5	32.69	28.03	16.6	24.50	21.29	15.1	51.42	41.37	24.3
	10	33.44	31.54	6.0	36.88	35.64	3.5	54.12	68.40	-20.9
α	1	0.1111	0.0890	24.8	0.1473	0.0926	59.1	0.0805	0.066	21.1
	5	0.1146	0.1010	13.5	0.1607	0.1164	38.1	0.0745	0.070	5.8
	10	0.1213	0.1154	5.1	0.1368	0.1132	20.8	0.0566	0.049	14.6

Table 2. Comparison of the Estimates of Pg_{max} and α' from the Linear and Non-linear Models.

In this analysis values of Pg_{max} and α' were determined using both the linear and non-linear models for each individual data set (n= 45). The values presented are the average of the 5 independent estimates of Pg_{max} and α' (note: the values presented here are somewhat different to those presented in Fig.1 due to the different ways in which the data were grouped and analysed; however, the trends are similar). Two-factor analysis of variance was then performed on the data for each species using 'CO_2 partial pressures' and 'Model' as the main factors.

levels, the discrepancies between the values of Pg_{max} and α' decreased and were not significant at 10 kPa CO_2.

Figure 1 shows that the Pn *versus* I curves of maize are unusual as Pn does not saturate even at high light intensities, and Pn_{max} increases dramatically as CO_2 partial pressure is increased. The reason for this probably lies with the acidification of the mesophyll cell cytoplasm by the action of PEP carboxylase. The high partial pressures of CO_2 used here are clearly physiological and may have led to an irreversible acidification of the cytoplasm. It is clear that if the photosynthesis rates of maize leaves is to be determined using a leaf O_2 electrode, it should be done using 1 pKa CO_2 partial pressure or perhaps less.

In conclusion, to those investigators using leaf O_2 electrodes we recommend the following:

When chamber CO_2 partial pressures of around 1kPa are used, the non-linear (quadratic) model (equations 2 or 3) should be employed. This general rule should apply for monocot and dicot plants.

If higher chamber CO_2 partial pressures of around 10kPa are used, the linear (hyperbolic) model (equation 1) adequately describes the Pn *versus* I relationship of C-3 monocots and dicots. However, we would urge caution with this approach when C-4 plants are under investigation.

Our data are consistent with the contention that for CO_2 partial pressures of 1kPa and above, θ estimates the relative importance of CO_2 diffusion to the site of carboxylation in the rate of CO_2 fixation. It is therefore likely that θ is a meaningful and significant photosynthetic parameter and its routine measurement may provide important indicators of plant physiology and pathology.

References

1. **Walker D.** 1987. The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Packard Publishing, Chichester, Sussex.

2. **Chartier P.** 1970. A model of CO_2 assimilation in the leaf. In Prediction and Measurement of Photosynthetic Productivity (ed. I Setlik), Pudoc, Wageningen, 307-315

3. **Delieu T, Walker DA.** 1981 Polarographic measurement of photosynthetic O_2 evolution by leaf discs. *New Phytol.* **89**, 165-175.

4. **Delieu T, Walker DA.** 1983: Simultaneous measurement of O_2-evolution and chlorophyll fluorescence from leaf pieces. *Plant Physiol.* **73**, 534-541.

5. **Leverenze JW. 1987.** Chlorophyll content and the light response curve of shade-adapted conifer needles. *Physiol. Plant.* **71**, 20-29.

6. **Leverenze JW.** 1988. The effects of illumination sequence, CO_2 concentration, temperature and acclimation on the convexity of the photosynthetic light response curve. *Physiol. Plant.* **74**, 332-341.

7. **Marshall B, Biscoe PV.** 1980. A model for C3 leaves describing the dependence of net photosynthesis on irradiance: I- Derivation. *J. Exp. Botany.* **31** (120), 29-39.

8. **Ogren E, Evans JR.** 1993. Photosynthetic light-response curves: I. The influence of CO_2 partial pressure and leaf inversion. *Planta* **189**, 182-190.

9. **Ogren E.** 1993. Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. *Plant. Physiol.* **101**, 1013-1019.

10. **Palmquist K, Ogren E, Lernmark U.** 1994. The CO_2-concentration mechanism is absent in the green alga Coccomyxa: a comparative study of

photosynthetic CO_2 and light responses of *Coccomyxa, Chlamydomonas reinhardtii* and barley protoplasts. *Plant Cell & Env.* **17**, 65-72.

11. **Rabinowitch EI** (1951): Photosynthesis and related processes, Inter Science, New York. **Vol. 2 (1),** 831-1191.

12. **Terashima I, Saeki T.** 1985. A new model for leaf photosynthesis incorporating the gradients of light environment and of photosynthetic properties of chloroplasts within a leaf. *Ann. Bot.* **56**, 489-499.

13. **Thornley JHM.** 1976. In Mathematical Models in Plant Physiology (Eds JF Sutcliffe and P Mahlberg). Academic Press, London.

14. **Vogelman TC, Bornman JF, Josserand S.** 1989. Photosynthetic light gradients and spectral regime within leaves of *Medicago sativa. Phil. Trans. Royal. Soc. Lon.* **Ser. B. 323**, 411-421.