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**EXPRESSION OF SYSTEMIC RESISTANCE IN *Hordeum vulgare*
AGAINST *Erysiphe graminis* BY TREATMENT WITH ABIOTIC
ELICITORS**

ANNE FARQUHAR MITCHELL B. Sc.(Hons.)

**A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY AT THE UNIVERSITY OF GLASGOW**

AUGUST 1998

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MITCHELL, A.F. and WALTERS, D.R. (1995) Systemic protection in barley against powdery mildew infection using Methyljasmonate. *Aspects of Applied Biology Conference. Physiological Responses of Plants to Pathogens 1995*, 323-326.

SUMMARY

The effects of 7 chemicals, each at 4 concentrations, were examined to determine their ability to elicit resistance in *Hordeum vulgare* to *Erysiphe graminis*. The 3 chemicals and concentrations selected for further investigation were 15mM Sodium Salicylate, 2.5mM Potassium Phosphate and 20mM Methyljasmonate. Each of these compounds gave significant levels of disease control. A lag period of 48 hours between treatment of the lower leaf with the elicitor and challenge-inoculation of the second leaf was found to give greatest control of powdery mildew. A constant temperature of 22°C and a 16 hour photoperiod were found to be the regimes which gave greatest control of mildew. Each of the 3 chemicals was found to possess antifungal properties *in vitro* to the pathogens *Pyrenophora avenae* and *Crinipellis perniciosus*. *In vivo* only 20mM MJ exhibited antifungal properties against *E. graminis*. Significant levels of resistance were observed up to 42 days post challenge-inoculation. Significant reductions in infection were still observed when challenge-inoculation of the second leaf was delayed by as much as 28 days. Two alternative methods of eliciting systemic resistance, seed treatment and root drench, were evaluated. They did not provide the level of disease control observed by elicitor treatment of the aerial portion of the plant.

Methyljasmonate vapour was evaluated as a potential elicitor of resistance. The vapour from undiluted MJ and the vapour from 20mM MJ were examined. Both were found to give significant reductions in the level of mildew development observed in the second leaf when the lower leaf was vapour treated and the second leaf was challenge-inoculated with mildew conidia. The optimal lag period between exposure of the first leaf to MJ vapour and challenge-inoculation was found to be 24 hours. The vapour from both undiluted MJ and 20mM MJ displayed antifungal properties *in vitro* and *in vivo* against powdery mildew. The MJ precursors Linoleic acid and Linolenic acid were

screened at four concentrations for their ability to elicit resistance. Linoleic acid displayed no activity at any of the concentrations screened, but all concentrations of Linolenic acid were effective elicitors of resistance, with 5mM being the most significant. Again, *in vitro*, both compounds exhibited antifungal properties.

The activity of three defence related enzymes, phenylalanine ammonia lyase, peroxidase and lipoxygenase were monitored in the lower 3 leaves of elicitor treated plants and also in elicitor treated plants which had been subsequently challenge-inoculated. This was carried out for the 3 elicitor chemicals selected from the initial screening, 15mM NaSA, 25mM PP and 20mM MJ. In general terms both sets of plants exhibited elevated levels of enzyme activity, in all 3 leaves.

Field scale trials of the elicitors 15mM NaSA and 25mM PP showed significant reductions in powdery mildew development and significant increases in plant height, plant dry weight and grain weight. A second field scale trial with 15mM NaSA and 20mM MJ showed superior control with both chemicals relative to commercial standards. 15mM NaSA gave significant increases in plant height, plant dry weight and grain weight. 20mM MJ exhibited a significant decrease in plant height, but significant increases in plant dry weight and grain yield.

I. INTRODUCTION

1 INTRODUCTION

Since the beginning of organised food production, man's food supply has always been threatened by drought, pests and diseases. Theophrastus, the Greek philosopher has been credited with certain observational comments regarding cereal diseases, some three centuries before Christ. Although he wrote in ignorance of the causal organism he recognised host variation in susceptibility and the dramatic effect of the environment. In Roman times diseases of cereals and the damage caused was attributed to the wrath of the Gods. In these circumstances nothing was done to contain the spread of the disease, since the preachers of the time advised that this would only provoke the wrath of the Gods further.

The desire for elevated levels of food production to feed the ever-increasing populations, the development of crop improvement schemes, the extensive use of mechanisation in crop husbandry and above all, the increased understanding of the causal organism, have all been important contributors to the increased awareness of the importance of plant pathology. Enormous losses are suffered globally as a result of plant disease. It is estimated that globally, on an annual basis, there is a yield loss of around 35%, as the result of weed, diseases and insect pests. The devastating economic impact of plant pathogens is best left to the professional economist. The financial implications of reduced crop quality and consequently yield has left crop producers with little alternative but to seek some means of reducing crop losses to an acceptable level.

With the twentieth century came a new era of crop protection as greater numbers of chemicals were developed and marketed for the control of plant disease. The 1960's were of significant importance, with the development of highly specific fungicides. This altered the emphasis of cereal production. However, increased pesticide usage has led to the

development of resistant strains of pathogens able to breach chemical control, resulting in a breakdown of the existing control measures. Many modern agricultural practices, designed to maximise short term profitability, have only served to exacerbate the problem. Such practices include the extensive use of irrigation and fertilisers, as well as the use of genetically uniform crop varieties over a large geographical area. In addition, many of these new high yielding cultivars are proving to be susceptible to diseases which were previously of little importance.

Agrochemical companies are faced therefore with a continual struggle to keep one step ahead of resistant pathogen development. This is an extremely expensive operation, an expense which must be borne by someone, and this will ultimately be the grower. In the current climate of increased consumer awareness regarding pesticide residues on food, alternative control measures which do not rely on the heavy use of chemicals are being explored. This type of resistance is being achieved largely through the incorporation of resistance genes into plants by breeding programmes. However, this again has led to resistance which is highly ephemeral in nature, with new virulent strains appearing, which have the ability to overcome the introduced resistance genes in a short period of time.

In recent years the term 'disease management' has been favoured in place of the more emphatic description of 'disease control', although the primary objective of all disease management systems is to effect complete control. This, in reality, is rarely achieved, and the success or failure of a particular scheme will be judged on whether or not it is capable of producing significant economic returns. Most disease management strategies are aimed, principally, at prevention of infection, or if this is unsuccessful, at controlling the spread of the pathogen, and to maintain disease levels below an economic threshold.

The phenomenon of systemic acquired resistance has been observed in numerous host/pathogen interactions. Infection of the lower leaf by a pathogen renders the upper plant more resistant to attack from a wide range of pathogens. The plant's defence mechanisms are primed in readiness for future pathogen attack. In its primed state, pathogen recognition is immediate and defences can be activated in a shorter period of time than in non-induced plants. Certain chemicals have been identified which are capable of eliciting the same plant responses as pathogen infection. This phenomenon may be of significant importance in the future, in developing techniques to aid the management of plant disease.

1.1 Barley

Hordeum vulgare, barley, is the world's fourth most important cereal, after wheat, rice and maize. Growth of barley is possible over a wider environmental range than any other cereal. Its distribution extends into the Arctic, reaches the upper limits of cultivation in the mountainous regions of the Himalayas and far below sea level in Palestine. It is more salt tolerant than any other cereal, thus enabling it to be grown in oases, such as the Saharan and Asiatic oases.

1.1.1 Early History

Barley was one of the first crops to be domesticated in the Near East (Harlan and Zohary, 1966; Harlan, 1968). Archaeological evidence in the shape of what appears to be wild forms with fragile ears, have been found in considerable quantities at Mureybat on the Euphrates in Syria, and are thought to date from around 8,000 BC.

Cultivated barley has been found at sites in Ali Kosh (Iran), Ramad (Palestine), and at Çatal Hüyük and Hacilar (both in Turkey) dating from the period 6,000 BC to 7,000 BC (Renfrew, 1969). All of the earliest barley finds belong to the two-rowed covered sorts, that is non-naked, but both six-rowed and naked types had appeared by 6,000 BC (Helbaek, 1966; Renfrew, 1969). Barley remains have generally been found with other primary crops of the region and period, such as emmer wheat, einkorn wheat, flax, pea, vetch and lentil.

Barley was the most prolific grain of the ancient Near East and the cheapest. Consequently, it was the food of the poor, the ration of the soldier, serf and slave. Ancient gladiators were known as 'hordearii' or 'barley men', since their staple diet when training was barley. Further archaeological evidence indicates that barley reached Spain in the fifth millennium BC and the lower Rhine a little later. By the second millennium BC barley had reached China.

1.1.2 *Recent History*

During the mediaeval period barley was introduced into Northern Europe. By whom or when exactly it was introduced have been lost in the mists of time, but it would appear likely that it was brought back from some Middle-Eastern site discovered by the crusaders.

Columbus was responsible for the introduction of barley to the New world during his second voyage. It was not successful as a crop in Hispaniola, but in the early Sixteenth Century it was introduced into the Highland region of Mexico, where it is still grown as a minor crop often being used as a 'spacer' between rows of maize (Weaver, 1950).

In the early Seventeenth Century a series of introductions are recorded for the release of barley into New England and Virginia. It was a success, and as the settlers moved Westwards so too did barley. Until the middle or late Nineteenth Century, all barleys existed as highly heterogeneous 'land races'. Many barleys still exist in this form, but with the advances in agriculture in the last 100-150 years these 'land races' have almost wholly been displaced by pure-line cultivars. This has resulted in the marked narrowing of the genetic base in many advanced agricultures, though this will have been partially concealed by the diversity of cultivar names (Harlan, 1976).

1.1.3 Taxonomy/Classification

Kingdom-	<i>Plantae</i>
Division-	<i>Anthophyta</i>
Class-	<i>Monocotyledonae</i>
Family-	<i>Poaceae (Graminae)</i>
Sub-Family-	<i>Hordeae</i>
Genus-	<i>Hordeum</i>
Species-	<i>Vulgare</i>

1.1.4 Cytotaxonomic Background

Hordeum vulgare is a self-pollinating diploid with $2n=2x=14$ (Nilan, 1971; Takahashi, 1955). Tetraploids have appeared spontaneously, but these are a negligible component of the crop. Hybrids between wild and cultivated forms occur freely in nature, in situations where the two are found growing together. The resulting hybrids are fully fertile, the chromosomes pair well and segregation is normal.

Barley has been crossed with a number of other *Hordeum* species, but the resulting hybrids are highly sterile or anomalous, leading to the conclusion that no other species was involved in barley evolution (Haralan, 1976).

1.1.5 *Production Statistics*

Barley is the United Kingdom's second most economically important cereal after wheat. Wheat overtook barley production in 1983, before which barley was the United Kingdom's most important cereal. The statistics shown overleaf on tables 1 and 2 illustrate the relative importance of the barley produced in the United Kingdom on a global scale, and that the yield per hectare is almost consistently double the world average.

1.1.6 *Major Uses Of Barley*

There are four principal uses for cultivated barley:

- A- Animal feed
- B- Malting
- C- Human consumption
- D- Seed

The largest single use of barley is as animal feed, primarily supplying carbohydrates and protein to their diet. A high protein content is desirable for barley which is to be used as animal feed. Like other cereal proteins, barley protein is nutritionally unbalanced, due to a deficiency of the essential amino-acid, lysine.

Table 1- *World Barley Production*

Year	Area Harvested (1000 Ha)	Yield (Kg/Ha)	Production (1000 MT)
1996	66551	2333	155261
1995	69404	2054	142535
1994	72705	2221	161501
1993	74549	2285	170364
1992	72335	2276	164670
1991	75593	2225	170364
1990	71493	2524	180437
1989	72741	2308	167897
1988	75527	2215	167319
1987	78741	2267	178518
1986	79391	2294	182109

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Table 2- United Kingdom Barley Production

Year	Area harvested (1000 Ha)	Yield (Kg/Ha)	Production (1000MT)
1996	3417	1672	5714
1995	4413	2183	9633
1994	5092	2849	14509
1993	3215	1164	6013
1992	2905	1297	7365
1991	3084	1393	7627
1990	5215	1515	7900
1989	4885	1652	8070
1988	4638	1875	8710
1987	4970	1831	9100
1986	5224	1917	10014

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Germinating barley seed produces two enzymes, alpha-amylase and beta-amylase, which hydrolyse starch to fermentable sugars. The production of barley for malting is favoured by a long cool growing season with uniform nutrient supply. Barley for human consumption is generally in the form of pearled barley, flour or barley malt for confectionery or breakfast cereals.

The minor uses of barley by products are too numerous to mention, but they include: the production of paper, laminates, cardboard and millboard. Furfuryl alcohol, the reaction

an important industrial chemical used in the production of resins, adhesives, protective lacquers and paint strippers. It is also widely used in the chemical, cosmetic, oil-refining, car, textile and synthetic rubber industries.

1.1.7 *Climate and Soil Requirements*

The broad ecological adaptation of barley sets it apart from other cereals. Best growth is achieved in temperate climates where the seasons are long and cool and rainfall moderate. Barley will withstand high temperatures in a cool climate or high humidity in a cool climate, but does not perform well in hot, humid conditions (Klages, 1942). Barley is grown over a wide range of photoperiods. In regions such as California or North Africa spring barley is grown during the short winter days, but in Europe and Northern Canada it is grown during the long summer days (Guitard, 1960). Water requirements for barley production, per unit weight of grain, is lower than for other cereals. (Carleton, 1916), and its rate of transpiration is amongst the lowest for the small grain cereals (Nuttson, 1957).

The early ripening of the grain means that it reaches maturity before the soil moisture becomes exhausted. Consequently, its production is extended to droughty soils and areas of low rainfall. Best growth is achieved on a well-drained fertile deep loam or light clay (Morgan et al., 1938). Nutritionally, barley responds well to a balanced application of nitrogen and phosphate fertiliser. It is more salt tolerant than other cereals, but less tolerant of acid soils. A pH range of 6.0-8.5 is acceptable for plant growth (Bower and Fireman, 1957).

1.1.8 *Diseases Of Barley*

Barley is subject to attack by a wide range of pathogens. The resultant damage is reflected in a reduction in grain yield and quality. The extent of the damage depends principally on the nature of the pathogen and the severity of the attack, but on a world scale it is estimated that there is a 12% loss annually due to attack by pathogens. Modern agricultural systems only serve to emphasise the problem. The extensive use of monocropping, coupled with the desire for a uniform crop are just two of the factors which accentuate disease problems.

1.1.8.1 Viral Disease

The most significant viral disease of barley is barley yellow mosaic virus (B.Y.M.V.). It is widespread and is capable of causing dramatic yield reductions, in the region of 15-20% (Doodson and Saunders, 1970; Moore and Moore, 1961). The virus is aphid borne, with different aphids transmitting different isolates of the virus (Slykuis, 1967).

1.1.8.2 Bacterial Disease

The number of significant bacterial diseases which affect barley is small. The most conspicuous are bacterial blight, bacterial leaf blight and black leaf chaff (Dickson, 1956; Moore and Moore, 1961). Bacterial diseases are favoured by warm wet conditions and are spread by rain, insects, crop debris and possibly the soil.

1.1.8.3 Fungal Diseases

Fungal diseases are widespread in barley. The most common, and therefore potentially the most damaging is, *Erysiphe graminis*, which is responsible for the disease powdery mildew. This pathogen will be discussed in more detail in section 1.2. Due to the restrictions of space it is impossible to catalogue all fungal pathogens of barley. A representative selection of the most damaging are listed below in Table 3.

Table 3- *Some Fungal Diseases of Barley*

Pathogen	Resultant Disease
<i>Puccinia hordei</i>	Brown rust
<i>Ustilago hordei</i>	Covered smut
<i>Pseudocercospora herpotrichoides</i>	Eyespot
<i>Rhynchosporium secalis</i>	Leaf scald
<i>Helminthosporium gramineum</i>	Leaf spot
<i>Pyrenophora teres</i>	Net blotch

Foliar pathogens are particularly damaging if they become established in the flag leaf and awns. The photosynthetic products of the awns and flag leaf contribute approximately 50-80% of the grain weight. Consequently, if the photosynthetic capacity of these two major contributors is reduced, or if the products of their photosynthetic activity are utilised by the pathogen, which acts as a rival sink to the phloem, then a reduction in grain yield will follow (Carver and Carr, 1987).

1.1.9 *Breeding*

The improvement of barley depends on the selection of superior strains from amongst a range of types. Many of the improvements of the last 200 years were obtained by selection of the outstanding lines from heterogeneous, indigenous 'land-races'.

Modern advances have arisen by the crossing of varieties from all parts of the world, and consequently, 'land-races' have virtually disappeared from 'developed' countries and the range in existence is decreasing rapidly. Thus, the 'genetic base' of cultivated varieties is being reduced, along with the potential for further improvement through hybridisation. 'Genetic erosion' has been slowed marginally by the establishment of world collections of varieties backed by seed stores or 'gene-banks'.

1.2 *Barley Powdery Mildew*

1.2.1 *Erysiphe graminis*

Kingdom-	<i>Mycotae</i>
Division II-	<i>Eumycota</i>
Subdivision-	<i>Ascomycotina</i>
Class-	<i>Plectomycetes</i>
Order-	<i>Erysiphales</i>
Genus-	<i>Erysiphe</i>
Species-	<i>Graminis</i>

Powdery mildew is one of the most conspicuous and widespread of all plant diseases. Mildews are obligate parasites. Hyphal growth is superficial and their feeding structures, haustoria, are normally restricted to the epidermal cells. Individual colonies are elliptical in shape, but if infection is heavy individual colonies may coalesce and form a dense mycelial mat. Conidial chains develop from individual basal cells (Plumb and Turner, 1972). These conidial chains, known as conidiophores, contain around eight immature, but readily dispersible spores which are firmly attached to each other. Mature spores at the tip of the chain are easily detached in the wind (Hammett and Manners, 1973).

1.2.2 *Infection Process*

Primary infection of powdery mildew is a multi-component process distinguishable on the basis of morphology or sensitivity to environmental factors. There are seven distinct stages of primary infection: germination, formation of appressorial initials, maturation of appressoria, penetration, development of haustorial body, appearance of secondary hyphal initials and formation of functional secondary hyphae.

Within one hour of deposition the conidium germinates producing a short aseptate primary germ tube (PGT). The PGT attaches itself firmly to the host epidermal cell surface, producing a small penetration peg 1-4 hours post-inoculation (Carver et al., 1995), but never forming a haustorium. The process of PGT attachment results in a sequence of host epidermal cell responses, one of which is autofluorescence beneath the PGT, which is followed within minutes by local aggregation of the cell cytoplasm. Following this a circular area of the epidermal cell wall surrounding the contact site of the PGT exhibits altered staining and is known as the 'halo' effect. A small refractive papilla appears, during the next 1-2 hours, in the epidermal cell directly beneath the PGT

tip. The halo and papilla areas of host epidermal cells generally contain bound autofluorogens thought to be phenolic compounds.

Soon after the emergence of the PGT from the conidium the appressorial germ tube (AGT) appears. By approximately 9 hours it has reached its mature length of c. $40\mu\text{m}$ and differentiates an apical lobe, the appressorium. From beneath the lobe a penetration peg, with no apparent cell wall at its tip, emerges and attempts primary infection (Carver et al., 1995). At present the relative importance of mechanical and enzymatic forces in appressorial penetration is unknown, although it is probable that some digestion of the host epidermal cell wall occurs (McKeen et al., 1969; Stanbridge et al., 1971; Carver et al., 1995). In response to this attempted penetration by the appressorial germ tube of the epidermal cell, there is a sequential appearance of autofluorescence, cytoplasmic aggregation, halo formation and papilla deposition. This sequence of events is similar to those associated with PGT's but the response is much more pronounced when viewed by microscopy (Carver et al., 1995). During compatible interactions a proportion of the appressorial penetration pegs pass through epidermal cell response sites to form primary haustoria, first visible by 16 hours. In such cases the penetrated papillae remain as collars surrounding the haustorial neck, though the merits or demerits of the collar to the pathogen in its biotrophic relationship is unknown.

Once full sized haustorial bodies are produced, secondary hyphae develop, these are capable of eventually producing secondary infections. When a haustorium is not produced under the primary appressorium, secondary hyphae may begin to form on the appressorium but will not continue to elongate and initiate the formation of secondary appressoria and secondary infections (Masri and Ellingboe, 1966). The formation of elongating secondary hyphae is dependant on the existence of functional haustoria.

Penetration begins twenty four hours post infection and optimal conditions are 20°C and 100% relative humidity. After penetration, a haustorium, which is surrounded by a sheath, is formed in the epidermal cell of the host (Braker, 1964). The formation of a functional haustorium is crucial for the development of a compatible host-parasite interaction.

1.2.3 *Production and Dispersal of Conidia*

Production of conidia is a continuous process (Hammett and Manners, 1973), but is maximal at 20°C and 100% relative humidity. Light intensity and photoperiod would appear to have no effect on the growth of powdery mildew, except when very little light is supplied (Ward and Manners, 1974). Wind speeds of 1ms^{-1} were found to create sufficient drag to release some conidia from infected leaves in a wind tunnel, but main spore dispersal required higher wind speeds (Hammett and Manners, 1973, 1974). Wind speed within cereal crop canopies are usually much less than 1ms^{-1} , but Bainbridge and Legg (1976), concluded that even light winds generate sufficient force to dislodge conidia due to acceleration as leaves move in the wind.

1.2.4 *Ascospore Production*

Globose cleistothecia are formed in the ageing mycelial mat after conidial production has ended. The globose cleistothecia are black with simple appendages. They are interwoven with the mycelial mat and are approximately 200µm in diameter. The cleistothecia house asci, each of which contain eight ascospores. Ascospores are forcibly discharged and wind dispersed. Ascospore discharge is favoured by temperatures in the region of 20°C, provided the cleistothecia are kept moist (Moseman and Powers, 1957).

1.2.5 *Effect of Environmental Conditions on Erysiphe graminis*

The temperature range over which *E. graminis* can grow is wide (Cherwick, 1944), though optimal growth occurs in the region of 15-20°C, which is comparatively low. Infection of barley by *E. graminis* can take place at temperatures as low as 5°C (Ward and Manners, 1974) and spores are capable of germinating at freezing point (Cherwick, 1944). The upper temperature limit for infection is approximately 30°C (Akai, 1952) and germination may be inhibited at temperatures as high as this (Leimon and Kurkela, 1969), or greatly reduced (Manners and Hossain, 1963). However, if germination is successful and appressoria form, haustoria may still fail to develop.

Conidia of *Erysiphe graminis* are tolerant of extremely dry conditions (Boughey, 1949), and though the optimal relative humidity for germination of conidia is 100% (Manners and Hossain, 1963), free water can inhibit germination. Light of wavelengths between 550nm and 750nm was found by Sempio and Castori (1952) to reduce germination, but shorter wavelengths, between 459nm and 549nm, stimulated germination. Ultra-violet (UV) radiation is likely to damage conidia during the primary stages of infection, that is, germination and formation of the first haustorium (Mount and Ellingboe, 1968). Therefore, less UV radiation is needed to inhibit spore germination than kill established infections, or kill spores (Moseman and Greeley, 1966).

1.2.6 *Host Cell Response to Infection by E. graminis*

Evidence suggests that the development of halo and papillae responses of the epidural cell are involved in general resistance to penetration. The halo response may indicate a general strengthening, enabling epidermal cells to slow or stop penetration. The

deposition of papillae is the result of vesicle-mediated deposition activity of the epidermal cell cytoplasmic response, and directly underlies the attempted penetration site. Papillae is a callose matrix infused with autofluorescent substances, proteins, hydrolytic enzymes and various chemical elements (Zeyen et al., 1995).

In Graminae, all known 'resistance' phenomena at cellular level have a penetration resistance element and some are almost exclusively of this type. When penetration resistance is not the exclusive component, host cell death constitutes a second and final defence response in resistance to infection.

1.2.7 General Physiological and Biological Responses of E. graminis

Research has shown increased activity of enzymes key to plant phenolic metabolism (phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase and peroxidase) at the time of host response to PGT contact (Aist and Bushnell, 1991). Later studies of PAL show biphasic activity with peaks occurring at 6 hours and 15 hours post inoculation. These peaks coincide with the host response to PGTs and appressoria, and an intervening period of relative quiescence. Studies have shown a biphasic accumulation of mRNA transcripts of a variety of barley response genes, including β -1,3-glucanase. For all host response genes studied so far, patterns of response gene transcript accumulation were similar in all cultivars up to 20 hours post-inoculation (i.e. after haustoria formed), irrespective of their major mendelian gene controlled compatibility with the fungal isolate used (Boyd et al., 1994; Clark et al., 1993, 1994, 1995).

- | | |
|------------------------------|-------------------------|
| 1- Liberated ascospore | 6- Conidia |
| 2- Germination | 7- Ascocarp development |
| 3- Penetration peg formation | 8- Cleistothicium |
| 4- Haustorium | 9- Ascus |
| 5- Conidiophore | |

1.2.7.1 *Life Cycle of Erysiphe graminis f.sp. hordei*

Powdery mildew overwinters in volunteer crops. In addition to this inoculum source cleistothecia are released from sexually produced ascospores, during the humid weather in spring and autumn. As the temperature rises in the spring dormant mycelium begins to grow and produce conidia.

1.2.8 *Control of Powdery Mildew*

Effective and efficient control of the pathogen *Erysiphe graminis* f.sp. *hordei* is dependent on accurate information regarding life cycle and disease epidemiology. At the present moment in time control lies mainly with the use of systemic fungicides and breeding programmes to develop resistant cultivars. There are, however, a number of factors which can be used individually or combined to relieve the disease pressure on plants. These include:

- A- Cultural control
- B- Biological control
- C- Cultivar diversification
- D- Integrated management
- E- Direct control measures
- F- Disease forecasting

1.2.8.1 *A- Cultural control*

Any practice which limits or reduces the sources of inoculum is desirable, although the success of any such scheme is dependent on the strength of the source and also the area over which the measure is enforced. For pathogens such as *E. graminis*, which would

have a rapid infection rate, the reduction in the source of primary inoculum would have to be very large to significantly affect the development of an epidemic (Van der Plank, 1963). Crop debris acts as a host for the cleistothecial stage of *E. graminis*, therefore destruction of crop remains will only be of value in preventing spread to autumn sown crops if carried out prior to ascospore discharge.

Crops which are grown in close proximity to a source of inoculum e.g. spring barley close to winter barley, are likely to develop severe infections at the seedling stage, which ultimately will be important in determining yield (Yarham et al., 1971). For significant reductions in disease it is necessary to remove major sources from a wide area (Lester, 1971), so that levels of primary inoculum are very small. In the Faeroe Islands, for example, powdery mildew on barley plants only becomes noticeable on adult plants, since overwintering sources are all but absent and the primary source of inoculum is from some distant source elsewhere in Europe (Hermansen and Wibeig, 1972). Other obvious factors such as sowing date, manuring and crop rotation should be considered when using cultural control as a method of reducing disease severity within a crop.

1.2.8.2 Biological Control

Many members of the *Erysiphaceae* are parasitised by *Ampelomyces* species (Rogers, 1957), although to date there are no reports to indicate that it is capable of parasitising *E. graminis*. In 1957, Yarwood concluded that the control of powdery mildews using this hyperparasite was unlikely to be fully successful, since the host and parasite were favoured by different sets of climatic conditions.

Grebenchuck (1965) reported decreases in the levels of powdery mildew infection when barley plants were sprayed with dung infusions containing mycolytic bacteria, or with pure cultures of *Trichoderma viride*. Culture filtrates of *Trichoderma roseum* (Darpoux and Faivre-Amiot, 1952) are also effective against cereal powdery mildews, as indeed are many other antibiotics (Davis et al., 1960; Rhodes et al., 1961), but to date none of the above have been developed for use commercially. More recently, *Tilletiopsis pallescens* was shown to reduce mycelial expansion and spore production by *E. graminis* on barley leaves (Klecan et al., 1990).

1.2.8.3 Cultivar Diversification

The most economic method for the control of powdery mildew would appear initially to lie with the use of cultivars with a high level of disease resistance. Such cultivars have been developed and are commercially available, although their success is usually short lived. In such cultivars resistance is generally specific, based on one or two major resistance genes. Consequently, within a few years their resistance has been overcome by a new race of the pathogen. The concept of diversification has been developed in an attempt to utilise these specific sources of resistance more fully. The underlying principle is to reduce the rate of pathogen development by choosing cultivars with different resistance factors to be grown in adjacent fields. In experimental trials (Gair et al., 1987), this concept has been shown to reduce disease levels by 50-80%, which is reflected in a 6-10% yield increase. An extension of this basic idea is to grow three or more cultivars, each of which contains a different source of resistance.

1.2.8.4 Integrated Disease Management

The hope that breeding programmes selecting the most resistant strains of barley would lead to a stable solution to the problem of powdery mildew control, is now looking extremely unlikely. Fear is growing, that like the new generation of pathogen specific fungicides, the new resistant varieties will be rendered ineffective by strains of virulent pathogen able to overcome host resistance. It would seem that the most likely solution to the problem of stability of control measures for the control of powdery mildew lies in a balanced application of a range of measures. The three basic components for such a system are:

A- Host resistance

B- Fungicides

C- Cultural procedures

This scheme has the great advantage that it reduces the exposure time of each control measure to the pathogen population and consequently, it minimises the selection for virulence or fungicide insensitivity.

1.2.8.5 Direct Control Measures

This takes the form of the application of compounds to restrict the spread or effect of the pathogen, by either killing the pathogen directly or stimulating the host to produce compounds which do so.

Chemical control of plant pathogens was restricted to the application of simple non-systemic compounds such as copper, sulphur and mercury until around the 1940's. These chemicals are still used today, though they have largely been replaced by the more complex non-systemic or systemic fungicides.

Chemical control of plant disease can be a very expensive option. Not only are chemicals often very expensive to buy, due to high development costs, but they require additional equipment for application. In addition many chemicals can have hidden environmental costs. They can be highly toxic to mammals, including man, and may disturb the ecological balance of non-target organisms in the treated environment.

At present the three most frequently used fungicides for the control of powdery mildew are propiconazole, fenpropimorph and tebuconazole. Both propiconazole and tebuconazole are systemic conazole fungicides, with protective and curative action, while fenpropimorph belongs to the family of morpholine fungicides whose action is both contact and systemic.

Economically speaking the stage has been reached where it is no longer viable to produce highly specific pesticides, and in the future only chemicals with a broad biological spectrum of activity will be manufactured.

The largest potential disadvantage of chemical control is the possibility that pathogens can adapt and become resistant or tolerant to certain chemicals, as was the case with *Erysiphe graminis* to ethirimol.

Despite the possible disadvantages associated with the use of chemicals for the control of powdery mildew on barley, chemical control has, to date, been the most successful means of control. Disease would be far more damaging if pesticides were not used. Pesticides should be used to supplement existing control measures or in the situation where no suitable alternative exists.

1.2.8.6 Disease Forecasting

Fungicide use against foliar diseases of cereals is a relatively recent event, since approximately 1970. In order to optimise the new generation of systemic fungicides for the control of powdery mildew, it has been necessary to formulate a protocol for determining the necessity for, and the timing of, fungicide application for maximal protection. Disease forecasting is based partly on the effects of temperature and humidity on conidial growth and germ tube growth, and partly on the rainfall and wind speed. Meteorological data have been described which is thought are related to periods where there is likely to be an increase in the number of conidia above barley crops. These data are described below:

1- Day maximum temperature $>15^{\circ}\text{C}$

2- Day sunshine >5 hours

3- Day rainfall < 1 mm

4- Day run of wind > 15 knots

(Polley and King, 1973)

From these data it is then possible to compute and predict high risk days i.e. days when all four criteria have been satisfied; this denotes the start of a high risk period. This high risk

period ends on a day when none or only one of the criteria have been satisfied, or the third consecutive day when only three factors have been satisfied.

1.3 *Acquired Resistance*

Following Edward Jenner's first demonstration of human immunisation, whereby protection against the smallpox virus could be stimulated by a vaccination of the closely related, but less virulent cowpox virus, the possibility that a parallel system may operate in plants has fascinated plant pathologists. It is not unreasonable to suggest the existence of such a parallel since plants and animals have evolved simultaneously facing similar disease problems.

1.3.1 *Historical Development of Systemic Acquired Resistance*

The first recorded observation of plant immunisation, by methods similar to those used in humans, was in 1911 in the United States of America by Smith et al. They inoculated Paris daisies with *Agrobacterium tumefaciens*, a tumour producing pathogen. Subsequent inoculations did not lead to infection. These observations could not be validated. In 1923 Brown successfully inoculated plants using attenuated spores of the same pathogen. These plants failed to develop tumours when inoculated one day later with virulent spores of *A. tumefaciens*. Similar observations followed, although it became apparent that these responses were non-specific and vastly different from the antigen-antibody responses of animals, and so interest declined.

K.S. Chester in 1933 reviewed over 200 publications describing a phenomenon which he called physiological acquired immunity. This, in retrospect, was actually three different

processes: viral cross-protection, antagonism (or biocontrol) and what is now known as systemic acquired resistance (SAR).

Detailed studies began in earnest in the 1960s with development of reproducible biological models. In 1961 the first systematic study of SAR was published by Ross (1961). It was he who first coined the phrases systemic resistance and localised resistance, the latter describing the resistance induced in inoculated leaves. In the thirty years that have followed, the phenomenon has been widely studied and its validity established.

1.3.2 *Terminology*

The terminology associated with acquired resistance is somewhat confusing. Different authors apply different meanings to the same term, therefore it is necessary from the outset to specify the meaning for each term used.

1.3.2.1 Acquired Resistance- Resistance which is dependent on factors which are only present following host challenge by the pathogen. It is, by definition, the converse of constitutive resistance, which depends solely on preformed factors. These definitions, though seemingly unambiguous, become confused if it is considered that:

A- Resistance can only be induced following an interaction of preformed components of both the host and pathogen.

B- With a particular host-pathogen interaction, resistance may not immediately be apparent, and will have to be measured quantitatively based on a comparison of a susceptible host challenged by the pathogen under identical conditions.

It is true to say that all plants, susceptible included, respond to challenge by a potential pathogen. The difference between resistant and susceptible hosts lies in the speed and magnitude with which the host defences are activated. The point which separates a susceptible reaction from a resistant one is often highly subjective.

Acquired resistance exists in two forms:

1.3.2.2 Localised Resistance- This can only be detected, as the name suggests, in the area immediately surrounding the point of attempted penetration. It is often accompanied by the collapse and necrosis of host tissue known as the hypersensitive response (H.R.). The H.R. may also be accompanied by a sharp increase in phytoalexin production. These are antibiotic compounds which have wide ranging effects on fungi and to some extent bacteria. A distinctive characteristic of phytoalexins is they only accumulate in the area immediately in contact with or adjacent to the pathogen (Küc and Rush, 1985).

1.3.2.3 Systemic Resistance- This resistance occurs at sites distant from the point of attempted penetration. It is detected by challenge-inoculation of the host at a different time and location from the inducing inoculation. Plant tissues which have become resistant as the result of an inducing inoculation are said to be protected. This term, however, should not be confused with the phrase 'cross-protection', which refers to resistance which arises as the result of interactions of closely related pathogens, commonly viruses. The term acquired resistance for the purposes of this thesis is used synonymously with the term induced resistance.

SAR was defined recently by Joseph Küc (1995) as the systemic induction of resistance to disease in a plant by prior inoculation with an infectious agent, exposure to an

environmental influence or treatment with a chemical which may or may not have any anti-microbial activity *in vitro*.

1.3.3 *Signal Production in Systemic Acquired Resistance*

There are numerous reports (Jenms and Kúic, 1979; Richmond, Kúic, and Elliston, 1979; Guedes, Richmond and Kúic, 1980; Kúic, 1984; Tuzan and Kúic, 1985; Dean and Kúic, 1986; Dalisay and Kúic, 1995) which provide strong evidence to indicate that a signal for SAR is produced at an induction site and translocated throughout the plant where it conditions resistance to disease.

Work carried out on cucurbits indicates that the signal for SAR is graft transmissible from rootstock to scion. Protection of the scion is effective if grafting precedes or follows treatment of the rootstock with an inducing agent, and protection would not appear to be cultivar-, genus-, or species-specific (Jenms and Kúic, 1979). Guedes et al. (1980) showed that the effects of immunisation are stronger and more rapid above the inducer leaf than below, suggesting the movement of the signal is stronger upwards than downwards, although induction of resistance in the roots by treatment of the foliage has been reported (Gessler and Kúic, 1982).

If the petiole of the inducer leaf was girdled, resistance was prevented both above and below the ring. If the petiole of the leaves to be challenged were girdled, but the inducer leaf was left intact, resistance was prevented in the challenged leaves with girdled petioles (Guedes et al., 1980). These data indicate strongly that the signal is phloem mobile and that resistance is acquired as a result of a signal transported from the inducer leaf. The

continuous production of the signal is not required once the leaf has been conditioned, and the presence of the inducer leaf is no longer required for the continuation of resistance.

1.3.4 *Characteristics of Induced Resistance*

1.3.4.1 Time-Dependence

The most fundamental characteristic of acquired resistance is the dependence on a time interval between treatment and challenge-inoculations. Protection is not immediate in any system, but can only be recognised after an adequate response period. This time delay is known as the lag period. This time delay corresponds to a delay in the production or release of a signal from the inducer leaf, and not its transport to the distal leaves or the time taken for the initiation of resistance mechanisms, which from time course studies are known to be very rapid (Dean and Kúc, 1986). Work by Guedes et al. (1980) has indicated that the signal is not volatile and does not move outside the plant (Tuzuan and Kúc, 1985). The intensity of the host response is reflected in the total area of the plant to be protected. If the challenge-inoculation is carried out at a point distant from the initial inducing treatment, then protection may not be observed for some time. This can be demonstrated by the work of Cruickshank and Mandryk (1960) who stem inoculated tobacco plants with *Pernospora tabacina*. A full protective response in the plant foliage was not evident until twenty-eight days after the initial inducer treatment.

1.3.4.2 Light Dependence

The protection of plants involves the movement and/or synthesis of plant metabolites, both of which are energy demanding processes. Thus, it is logical that light should

influence the ability of the plant to undertake such energy demanding processes. Lozano and Sequira (1970) demonstrated on tobacco infiltrated with heat killed bacterial cells, that no protection was observed in leaves maintained in the dark. An increasing degree of protection was observed in direct relation to the length of photoperiod.

1.3.4.3 Temperature-Dependence

Resistance elicited by prior treatment appears to be highly temperature sensitive. This property has not been explored in any great detail. Work by Klement and Goodman (1968) indicates that resistance developing in tobacco as a result of local lesion formation is maximal in plants grown at temperatures between 20°C and 24°C, but is not observed in plants grown at 30°C.

1.3.4.4 Systemic Involvement

It is important to note that in many host-parasite interactions the resistance is spread from the site of initial inoculation. Stem lesions caused by *Peronospora tabacina* increased resistance in the upper leaves to TMV and other pathogens (Hecht and Bateman, 1964; Mandryk, 1963). The effect was most pronounced on the first few leaves closest to the point of initial inoculation. Systemic resistance is by no means the rule in certain other host-parasite interactions. There are many instances of phytoalexin induction, the result of prior inoculation with saprophytic or pathogenic fungi, giving evidence for localised effects (Cruickshank and Perrin, 1965).

1.3.4.5 Persistence

One of the most unique characteristics of acquired resistance is the long lasting effect of initial treatment. A study carried out by Kuc et al. (1975) showed that resistance in cucumber repeatedly inoculated with *Colletotrichum lagenarium* persisted for ten weeks against the same organism.

1.3.4.6 Nonspecificity

A major distinguishing feature that separates acquired resistance in plants from that in animals, is the lack of specificity of the former as opposed to the latter. The lack of specificity of the protective responses in plants is reflected not only in terms of the agents that induced the response, but also in the wide range of potential pathogens against which the plant is protected. It is apparent that resistance acquired by prior inoculation is usually non-specific, because it may be related to recognition by the host of compounds of general occurrence in the cell walls of the pathogens. Pathogens appear able to prevent this general resistance response by means that are not entirely understood at present, or alternatively they may be insensitive to or unaffected by metabolites produced as a result of the response.

1.3.5 Criteria For SAR Activators

There are three criteria which must be satisfied before an agent can be classified as an SAR inducer (Kessmann et al., 1994):

A- Neither the agent nor their metabolites should have any significant anti-microbial activity.

B- The same set of genes should be induced in treated tissues to levels comparable to those observed in biologically-induced SAR.

C- Application of an agent should induce local resistance to the same spectrum of pathogens as seen in biologically induced SAR tissues.

The first criterion is in direct conflict with that described by Kuc (1995), who states that whether an agent can be classified as an elicitor of resistance is unrelated to its direct antifungal properties. This is an issue which is still open to debate.

1.3.6 *Methods Used to Induce Resistance in Plants*

Resistance in localised areas is the result of an incompatible interaction between plant and pathogen. Many of the interactions lead to expression of the hypersensitive response and are characteristic of:

A- A normally susceptible cultivar challenged by an avirulent mutant of a pathogen.

B- A cultivar challenged by a pathogen of an unrelated plant species.

C- A resistant cultivar challenged by a strain of pathogen that is virulent to other cultivars.

Unlike localised resistance, induced systemic resistance is separated from the inducing treatment by both time and space. Consequently, the methods used to induce systemic resistance are more complex than those used in expression of localised resistance and for the same reason are more difficult to quantify.

1.3.6.1 Prior Inoculation With Viable Organisms

The principal tenet of systemic acquired resistance is the protection of a plant against further attack from harmful pathogens via prior inoculation with an avirulent pathogen of the plant. The inducing inoculation may use the same pathogen as challenge-inoculation, or may use avirulent or incompatible forms of the same pathogen, or other pathogens or saprophytes (Sequeira, 1979). Induction of this form of resistance can be illustrated using cucumbers. The upper leaves of certain cultivars of cucumber seedlings develop resistance to TMV, *Colletotrichum lagenarium* or *Pseudomonas lachrymans* (Jenns et al., 1979). The resistance is manifested as a reduction in the number of lesions on the upper leaves. The system has a dual action; it is possible to induce resistance by- as well as against- the same pathogen (Caruso and Kùc, 1979; Staub and Kùc, 1980).

1.3.6.2 Prior Inoculation With Attenuated or Heat-Killed Organisms

Reports that heat-killed, sonicated, formalin-treated or otherwise modified fungal spores or bacterial cells are effective, to varying degrees, as elicitors of disease resistance, support the theory that pathogens induce systemic resistance by different methods. Heat-killed cells of *Pseudomonas solanacearum* are less effective inducers of resistance in tobacco than living *Pseudomonas pisi* cells. Attenuated (hypovirulent) strains of TMV have been applied on a practical basis more extensively than any other method for inducing systemic resistance in tomatoes. Plants were pre-treated with appropriately selected strains resulting in almost complete cross-protection against more virulent strains of TMV, yet suffered minimal loss of yield (Rast, 1975).

1.3.6.3 Prior Treatment With Microbial Extract

Relative to localised resistance, induction of systemic resistance with microbial extract has had limited success. Lipopolysaccharide (LPS) containing extract from *Pseudomonas solanacearum* (Graham et al., 1977) as well as various other *Pseudomonas* spp. and *Erwinia chrysanthemi* (Mazzuchi et al., 1976; Mazzuchi et al., 1979) effectively inhibit both hypersensitive response and susceptible response of tobacco to other pathogens. Extracts and culture filtrates from various fungi and bacteria protect bean plants against *Uromyces phaseoli* (Schönbeck et al., 1980). In this case resistance is only induced in certain locations in the plant and is not predicted by the vascular interconnections (Schönbeck., 1980).

1.3.6.4 Prior Treatment With Abiotic Agents

An abiotic agent would be considered an 'activator' of systemic resistance if it induced the same set of biological markers as the biological model and resistance to the same pathogens. It is also important that neither the agent nor its metabolites should have any direct anti-microbial activity.

The abiotic agent must fulfil the three criteria for an SAR activator. At first examination it would appear that there are numerous agents capable of inducing systemic resistance e.g. silicon, (Doubrava et al., 1988), nucleic acids (Cherf et al., 1992), unsaturated fatty acids (Cohen et al., 1991) and many more. However more detailed studies indicate that the vast majority fail to satisfy the necessary criteria. In many cases application of the agent results in local necrosis, which subsequently triggers a salicylic acid dependent

pathway like that induced by pathogen infection and in some cases a direct anti-microbial effect is possible.

Two abiotic agents which satisfy the criteria outlined in section 1.3.5 are salicylic acid and 2,6-dichloroisonicotinic acid. Salicylic acid and its uses as an SAR activator will be discussed in more detail in section 1.8. 2,6-dichloroisonicotinic acid (INA) provides good protection against bacterial and fungal pathogens on cucumber, rice and other crops both in glasshouse and field trials. Extensive studies carried out by Ward and co-workers (1991) shows that in tobacco plants treated with INA, the same set of nine gene families are activated as in plants infected with TMV. A similar correlation is observed in other plant species between genes systemically induced by pathogen treatment and those induced by INA application. INA is also responsible for increases in the level of β -1,3-glucanase, chitinase and 6-phosphogluconate-dehydrogenase activity in tobacco. In addition to the induction of defence enzymes and SAR genes, INA is capable of priming plant tissues to respond more rapidly with additional defence mechanisms than untreated tissues (Kauss et al., 1992). Seguchi et al. (1992) demonstrated that levels of lipoxygenase, peroxidase, and general lipid metabolism, were accelerated in chemically treated and blast-inoculated rice leaves compared to uninoculated controls. The mechanism for such sensitisation remains unknown.

1.3.6.5 Wounding

There is limited evidence that certain types of wounding can induce systemic resistance in plants. The systemic changes which occur following wounding have only recently been studied in any detail (Ryan, 1982). In 1978, Ryan found that crushing or tearing of the lower leaf of young tomato plants caused a significant increase in proteinase inhibitors in

the upper leaves. As little as 48 hours after wounding of a single leaf, proteinase inhibitor accounted for at least 10% of the soluble proteins. It must be remembered however, that the inhibitor proteins are not the only proteins which will increase as a result of wounding. It is possible that the induced mechanism responsible for the synthesis of proteinase inhibitors regulates the synthesis of other defence mechanisms such as lignification and suberization.

1.3.7 *Duration of Induced Resistance*

Kúic and Richmond (1977) have shown that treatment of the first true leaf of cucumber, followed by a booster treatment two to three weeks later, will protect the plant from a variety of diseases through the fruiting period. The same team also demonstrated that a single treatment protects the plant for a four to six week period. Without further booster treatments systemic resistance is lost by the end of this period. However, once cucumbers have started flowering and set fruit it is not possible to induce resistance (Kúic, 1981). One possible explanation of this is that the onset of flowering and fruiting alters plant hormonal balance, thus rendering the induction of resistance impossible. These factors considered, it would appear that the biological programming of a plant for reproduction switches off the ability to induce resistance. It does not, however, prevent the expression of resistance in protected tissues.

1.3.8 *Nature of The Signal*

As yet the identity of the primary signal in response to initial treatment is completely unknown. There have to date been numerous attempts to isolate the signal, but all have been unsuccessful. Extraction of the chemical may be difficult due to its instability *in*

in vitro or it may bind to a moiety during extraction. Its detection may also be hampered by a low concentration *in vitro* or its existence as a previously compartmentalised substance released during extraction in both healthy and infected tissue.

It has been thought by many that salicylic acid may be the systemic signal for the induction of SAR in plants (Ward et al., 1991; Malamy et al., 1990; Metraux et al., 1990). Dramatic increases in salicylic acid levels in TMV-resistant (Xanthi-nc) tobacco, but not in susceptible (Xanthi) tobacco have been observed, which is paralleled by the induction of genes encoding pathogenesis related proteins- (PR) proteins, e.g. PR-1 genes. Furthermore, Ward et al. (1991) confirmed that the exogenous application of salicylic acid induced transcription of an entire set of SAR gene families which are activated by biological induction. These findings at the time were taken as evidence indicating that SA was the systemic signal for SAR.

However, Gaffney and co-workers (1993) used tobacco which was transformed with the *nahG*-gene from *Pseudomonas putida*. This gene encodes a salicylate hydroxylase which catalyses the degradation of salicylate to the non-inducing catechol. The transgenic plants were shown to express the *nahG*-gene and salicylic acid levels did not rise, nor did it accumulate after the onset of SAR. Using tobacco plants *nahG*-gene Vernooij et al. (1994) expressed beyond doubt that salicylic acid was not the signal responsible for the induction of SAR. When *nahG*-gene scions were grafted onto Xanthi-nc tobacco, no SAR was induced despite the presence of salicylic acid in the Xanthi-nc rootstock. Conversely, grafts of Xanthi-nc onto *nahG*-gene plants induced a typical SAR response in the Xanthi-nc scion, though no increase in salicylic acid levels in the rootstock was observed.

The essentiality of SA *per se* for induced resistance is further questioned by the findings of Delaney et al. (1994) who found that INA, a compound capable of inducing resistance in a number of plant species, was capable of restoring resistance to an incompatible race of *Phytophthora parasitica* and induced resistance to a compatible race in *Arabidopsis thaliana* containing the salicylate hydroxylase gene. The authors report that because hydroxylase-containing plants cannot accumulate SA, they become more susceptible to some pathogens and less resistant to incompatible races of *P. parasitica*. Therefore SA is not necessarily essential *per se*, but it may function as part of the signalling process in SAR. It was reported recently that the ability of INA to induce SAR in tobacco does not depend on SA and INA induces systemic increases in PR-1 mRNA in plants expressing the hydroxylase gene (Vernooij et al., 1995). One possible interpretation of this is that INA is a functional analogue of SA (Delaney et al., 1994).

1.3.9 Signal Transduction

It is difficult to examine the mode of action of signal transduction, since the initial signal for SAR is still unreported. Studies have been initiated which may provide an insight into the pathways or early events in signal transduction and may even elucidate the initial signal(s) for SAR (Dixon et al., 1994).

Chen and co-workers (1994) isolated a salicylic acid binding protein with catalase activity; this binding has been suggested to result in the accumulation of transitory levels of hydrogen peroxide and other active oxygen species. It has been suggested that the oxidative burst following catalase inhibition by SA is responsible for SAR. It is uncertain whether SA levels in tissue distant from the site of SAR induction before challenge is sufficient to inhibit catalase and generate sufficient active species (AOS) for SAR.

However, the levels of SA accumulating around infection sites in leaves with SAR induced may be sufficient. The ability of INA to induce SAR without increasing SA levels make it unlikely that the SA-catalase binding is uniquely responsible for SAR (Vernooij et al., 1995), although oxidative stress may be partially responsible for the induction of SAR.

1.4 *Effect of Induced Resistance on Host Physiology*

Resistance activates a multiplicity of defence mechanisms, many already known, others may not yet have been identified. It includes the formation of both physical and chemical barriers and the synthesis of anti-microbial compounds, which limit or restrict the growth of pathogens. Active defence mechanisms have proved an invaluable tool in breeding and hence, modern plant production. However, it has to be considered that while active defence mechanisms are highly efficient at restricting pathogen growth, these processes occur at the expense of the energy of the plant, and therefore may be a limiting factor in plant growth and yield.

1.4.1 *Energy Costs Associated With Disease Resistance*

Incompatibility and active defence are associated with the enhancement of certain biological activities such as altered translocation and an increase in the synthesis of defence related compounds. These increases in biological activity are energy demanding, energy which to a large extent is derived from an increase in respiratory activity. It is well documented that increased respiratory activity is an associated feature of diseased plants (Daly, 1976), although research by Smedegaard-Petersen and Stølen (1981) on the respiratory activity of tissue reacting incompatibly is less conclusive. If the respiration rate of compatible and incompatible interactions between *Erysiphe graminis* and barley

are compared, then typically the resistant plants do exhibit an earlier and more dramatic increase in respiration than susceptible plants (Millard and Scott, 1956; Smedegaard-Petersen, 1980; Smedegaard-Petersen and Stølen, 1981), although there are exceptions to this generalisation (Scott and Smillic, 1966).

Increased photosynthesis in the infected leaves of plants infected with an obligate biotroph has frequently been reported (Ayres, 1979; Walters, 1985) and would appear to be due to the ability of the plant to compensate for a reduction in vitality caused by the pathogen (Edwards, 1970). Falkhof (1990) also reported an increase in the rate of photosynthesis in the upper leaves of induced resistant barley, which may be based on an increase in ribulose-1,5-bisphosphate activity or content (Walters and Ayres, 1983). Interestingly, Murray and Walters (1992) reported increased rates of photosynthesis in upper leaves of broad beans expressing SAR. They found that if this increased photosynthesis was reduced by shading, the magnitude of SAR was substantially reduced, although it was not abolished, illustrating the importance of increased photosynthesis in providing assimilates for SAR. Plants expressing induced resistance have the ability, it would appear, to compensate for the adverse effect of pathogen on their growth and metabolism, which is manifested in a prolonged maintenance of the assimilation rate.

In a recent study, Kehlenbeck et al. (1993) examined the mechanisms underlying this yield increase obtained in mildew-infected barley expressing induced resistance following treatment with *Bacillus subtilis*. They found that flag leaves exhibited elevated rates of photosynthesis, enhanced sucrose levels in the apoplast, and translocation into the grain was unimpaired.

1.5 *Induced Resistance as a Method of Disease Control*

The use of induced resistance, commercially, for the control of plant disease is minimal. In Japan (Komochi et al., 1966) and the Netherlands (Rast, 1975) low virulence strains of TMV have been used to cross-protect tomatoes against virulent strains of the same pathogen. Mild strains of potato virus X have been used similarly to offer considerable protection against more virulent strains of the virus. Other examples of accidental cross-protection can be found in vegetatively propagated crop plants. Cross-protection does not feature largely as a method of disease control in any commercially important crop.

Field scale inoculations with *Colletotrichum lagenarium* against *Colletotrichum lagenarium* were carried out by Kúc (1977) with some degree of success. After challenge-inoculation, sixty-eight percent of unprotected plants died but less than two percent of the protected plants succumbed to the disease. Under normal field trial parameters the results would undoubtedly have been even more impressive, since the natural inoculum levels are likely to have been far lower than those used experimentally.

Glasshouse grown tomatoes in Italy have been protected successfully with a strain of *Verticillium albo-atrum* (Matta and Garibaldi, 1977) which was avirulent on tomatoes. Control was effected by dipping seedling roots into a spore suspension prior to transplanting. However, although the results obtained from these artificially inoculated soils were encouraging, the control achieved on naturally infected soils was less pronounced.

Research at SCRI in Dundee has shown that extracts from the yeast *Saccharomyces cerevisiae* can induce resistance in crop plants (Lyon et al., 1995). These elicitors

enhanced resistance of barley to powdery mildew, lettuce to *Botrytis cinerea* and bean to *Botrytis fabae*. They found that the yeast extract increased PAL activity in barley leaves, and in the field provided substantial control of barley powdery mildew. These extracts are currently being developed for possible commercialisation.

In the summer of 1995 Ciba launched a new crop protection compound, a benzothiadiazole (CGA 2457040), which works by activating SAR. It is the first commercially produced plant protection compound which acts by activating plant defences. Although launched primarily for use on wheat as a 'plant tonic', it induces resistance against a wide range of fungal and bacterial pathogens.

1.6 *The Hypersensitive Response*

This is a common response in plants which are challenged with avirulent pathogens. The challenged cells and on occasion the cells in the immediate vicinity die rapidly, and the associated necrosis is characteristic of resistance of the plant as a whole.

The hypersensitive response is associated with the induction of a number of defence responses including lignification (Beardmore et al., 1983), phytoalexin synthesis (Dixon, 1986), the synthesis of hydroxyproline rich glycoproteins (O'Connell et al., 1990) and the production of hydrolytic enzymes such as chitinases and glucanases (Boller and Métraux, 1988; Castresana et al., 1990).

It is now well established that the hypersensitive reaction induces protection of the plant to challenge by the same or other organisms, i.e. it induces SAR.

1.7 *Defence Responses Induced*

1.7.1 *Phytoalexin Accumulation*

This is one of the most rapid defence response to be activated by pathogen attack. Phytoalexins are low molecular weight anti-microbial substances, which accumulate in the area immediately surrounding infection. They are generally lipophilic substances which are products of secondary metabolism and are not translocated. A signal that conditions plants to accumulate phytoalexins following infection is, however, translocated. Therefore it has been shown that phytoalexins accumulate in tissue which has been primed by prior infection or by treatment with an abiotic elicitor of resistance (Kúic, 1982; Kúic and Caruso, 1977; Kúic and Tuzan, 1983; Kúic, 1984; Kúic and Presig, 1984; Kúic and Rush, 1985). Phytoalexins will therefore accumulate in the required areas following priming of the plant's defences. Some are phytotoxic at concentrations which inhibit pathogen growth. The accumulation of these anti-microbial defence substances has been demonstrated in at least seventeen plant families (Coxon, 1982; Ingham, 1982; Kúic, 1982).

In all reported cases phytoalexin accumulation is not determined by the presence or absence of certain genetic information required for synthesis (Kúic and Rush, 1985). This is therefore compatible with the hypothesis that all plants contain the genetic information required for disease resistance. Phytoalexins are degraded by both plant and microbial enzymes and therefore their accumulation is a function of the rate of synthesis and the rate of degradation. There are two distinct aspects of phytoalexin accumulation. The primary stage is the development of necrosis as a result of either a biotic or an abiotic agent, which releases a nonphytotoxic substance. The second phase is the movement of the

nonphytotoxic signal to healthy non-infected plant cells which then primes them for phytoalexin synthesis, should it be required (Caruso and Kúc, 1977; Bailey, 1982).

1.7.2 Lignification

The process of lignification shares similarities with phytoalexin accumulation. Like phytoalexin accumulation it is brought about by stimulation of the phenylpropanoid pathway, resulting in the formation of sinapyl and coniferyl alcohols. A free radical reaction involving water and peroxidase results in their polymerisation. This polymerisation process gives rise to very complex but highly resistant structures.

Lignification can restrict pathogen growth in a number of ways:

1. Increase the mechanical strength of the host cell wall.
2. Reduce the susceptibility of host cell walls to degradation by extracellular enzymes.
3. Restrict the diffusion of pathotoxins and nutrients.
4. Inhibit the growth of the pathogen by the action of toxic precursors and free radicals.
5. Lignification of the pathogen.

(Kúc, 1983).

In cucumber resistant to *Colletotrichum lagenarium*, lignification occurs rapidly after penetration by the pathogen in numerous cells around the site of pathogen attack. In susceptible cucumber lignification is also observed, but it is delayed until the pathogen has ramified through the host cells and it is diffuse and weak (Hammerschmidt and Kúc, 1980). However, in susceptible plants which have had an immunising treatment with *Colletotrichum lagenarium*, lignification is much more rapid, intense and less diffuse than in non-immunised plants (Hammerschmidt and Kúc, 1980), indicating that

lignification is involved in SAR. It is reported that chitin, a component of the cell wall of many fungi, acts as an elicitor of lignification (Pearce and Ride, 1978) and as a matrix for lignification (Seigel, 1957).

Lignification can therefore create a very hostile environment for plant pathogens. In summary, it generates highly toxic phenolic lignin precursors, toxic free radicals, lignification of the pathogen, binds fungi to host cell walls, renders host polysaccharides and proteins resistant to degradation by fungi. However, to date, although the evidence for lignification as a defence mechanism induced by SAR is strong, much, as yet, is circumstantial.

1.7.3 *Papillae Formation and Callose Deposition*

Papillae are cell wall appositions laid down in response to attempted penetration by both fungal and bacterial infections. At the site of attempted penetration the germ-tube tip swells and adheres to the host. In a bid to prevent penetration, the host deposits a papilla, a host-produced matrix which contains primarily callose. The papilla will slow down the rate of penetration by the pathogen, possibly by physical impedance. This has been extensively studied and papilla formation coincides with failed fungal penetration, and resistance is associated with the chemical composition of the papilla. Callose is a β -1,3-linked glucan which again is laid down in response to pathogen attack or wounding.

Callose formation and papilla deposition were studied in isogenic lines of barley, one containing the *ml-O* mutation for powdery mildew resistance, together with an inhibitor of callose formation (Bayles et al., 1990). It was found that treatment of the resistant barley coleoptile with the inhibitor decreased the formation of callose-containing papillae and

thus increased the efficiency of penetration by the powdery mildew fungus. Time course studies of papilla deposition revealed that the deposition was actually delayed and those which were laid down were breached by the pathogen, but those laid down early were not.

1.7.4 *Hydroxyproline-rich Glycoproteins*

Hydroxyproline-rich glycoproteins (HRGP) are an integral part of plant cell walls which form approximately five to ten percent of the dry weight of the plant cell wall mass (Sauer et al., 1990). Monomeric HRGPs are secreted into the cell wall spaces and rendered insoluble by cell wall bound peroxidases via the formation of isodityrosine linkages (Cooper and Varner, 1983). Esquerré-Tugayé and colleagues (1979) showed that HRGP levels increased following infection of a pathogen known to induce resistance. O'Connell et al. (1990) have shown by immunocytology, using a gold-labelled antibody raised against a major HRGP fraction of melons, that in melon inoculated with *Pseudomonas syringae*, HRGPs accumulate in the walls of living cells which are adjoined to dead hypersensitive cells during resistant reactions. These data support the role of HRGPs in defence. One possible function is that they provide a template for callose deposition in papillae (O'Connell et al., 1990).

1.7.5 *Chitinase Activity*

It has been reported that some plants exhibit an increase in chitinase activity in response to infection by fungi, bacteria and viruses (Métraux and Boller, 1986; Métraux et al., 1988). Chitin is a structural polymer found in the walls of many fungi. Plants however do not contain chitin. Chitinases have been found constitutively in cereals (Schlumbaum

et al., 1986; Ride and Barber, 1990). Two reasons for their possible importance in host parasite relations are:

A- Chitinases are potent inhibitors of fungal growth (Schlumbaum et al., 1986)

B- The chitin oligomer released may act as an elicitor of lignification (Ride and Barber, 1990)

This is analogous to the action of polygalacturonase which releases galacturonate oligomers, which are phytoalexin elicitors. It was shown by Metraux and Bolter (1986) that chitinase was induced in cucumber exhibiting SAR by up to 100 times the base level, following an inducing inoculation of *Colletotrichum lagenarium*. The same authors also reported elevated levels of chitinase, in cucumber plants, treated with known elicitors of SAR. The level of induction was reported to be of a similar magnitude to that induced by the *C. lagenarium*. However, the extent to which chitinase is directly involved in SAR has not been thoroughly investigated. This is an area for future research.

1.7.6 PAL and Peroxidase Activity

PAL and peroxidase are the enzymes which catalyse the primary reaction from phenylalanine (Stafford, 1974) in a pathway which leads to cinnamic acid CoA esters, and is important in many other plant processes including the final step in lignin synthesis from cinnamyl alcohols (Northcote, 1985).

Peroxidases are present in both ionically and covalent forms bound to cell walls, as well as in a soluble state. Associated with the induction of resistance in tobacco and cucumber is a three-fold increase in peroxidase activity (Naldony and Sequeira, 1980; Hammerschmidt and Kuc, 1980). This is systemic and is observed in tissue which is distant from the inducer treatment (Hammerschmidt et al., 1982). Peroxidase activity

increased sooner in induced than in non-induced tissue after challenge. Enhanced activity may also be attributed to an increase in the activity of several peroxidase isozymes (Hammerschmidt and Kúic, 1980). In many cases peroxidase activity in untreated plants will overtake that in immunised tissues after challenge. It is likely that in untreated plants the response is initiated later and less strongly.

It is interesting to note that treatment of cucumber with ethylene also increases the peroxidase level as does the onset of senescence. However, in neither case is disease resistance induced. For induction of resistance enhanced peroxidase activity may require an increase or a non-limiting supply of both hydrogen peroxide and a hydrogen donor. Senescence and treatment with ethylene both fail to provide an adequate supply of these compounds (Kúic, 1983).

Green and co-workers (1975) have noted a doubling in PAL activity within four hours of immunising inoculations, which is surprising due to the low percentage of plant cells in contact with the pathogen at this stage. This rapid and early increase suggests that the changes are induced before appressorium development (McKeen and Rimmer, 1973) and papilla formation. It would appear from the evidence available that the presence of a germinating spore on the leaf surface is all that is required to induce the host response.

1.7.7 *Lipoxygenase Activity*

Lipoxygenase catalyses the incorporation of molecular oxygen into polyunsaturated fatty acids, such as linoleic acid and linolenic acid, the result of which is a conjugated hydroperoxydiene. Lipoxygenase is widespread in the plant kingdom (Shimura et al., 1983), and to date sixty plant families have been reported to contain the enzyme.

Increased lipoxygenase activity was demonstrated in a number of plant-pathogen interactions (Croft et al., 1990; Lupu et al., 1980). The products of lipoxygenase activity may contribute to host defence in a number of ways, including:

A- By direct inhibition of pathogen growth (Namai et al., 1990; Oota et al., 1990)

B- Induction of phytoalexin accumulation (Li et al., 1991)

C- The further synthesis of compounds such as jasmonic acid, with the potential to act as signal messengers (Anderson, 1989; Salt et al., 1986)

Early events in the inoculated leaf which occur prior to the onset of systemic resistance may have a role to play in its establishment. These changes include early alterations in plasma membrane potential (Mayer and Ziegler, 1988), increased membrane permeability (Kepler et al., 1988) and membrane lipid deterioration (Adam et al., 1989).

Aydiushko and co-workers (1993) found a significant increase in lipoxygenase activity in plants expressing SAR. They found all three inducers they tested resulted in elevated levels of lipoxygenase, 2, 3 and 4 days after induction. The increase in lipoxygenase activity precedes or coincides with the onset of induced resistance, therefore it may be of importance in SAR. The systemic enhancement of lipoxygenase activity following inducer treatment with either pathogens or abiotic agents had not previously been reported.

1.7.8 *β -1,3-Glucanase Activity*

It has been recognised for a number of years that many higher plants accumulate β -1,3-glucanase in response to pathogen attack (Ferraris et al., 1987; Joonston and De Wit, 1989; Kauffman et al., 1987), treatment with elicitors of resistance (Kombrink et al.,

1988) or exposure to other stresses (Mauch and Stablin, 1989). In 1987, Kauffman and co-workers identified four induced PR-proteins as β -1,3,-glucanases. It was reported that β -1,3-glucanases hydrolyse β -1,3-glucans, which are cell wall components of many pathogenic fungi (Bartnicki-Garcia, 1969). Consequently, it was suggested that they have an important role to play in plant defence against pathogen attack (Boller, 1987; Vogeli et al., 1988). The enzymes hydrolyse fungal cell walls (Boller, 1987; Young and Pegg, 1982) and also release oligosaccharides capable of eliciting phytoalexin production (Keen and Yoshikawa, 1983; Keen et al., 1983).

Mauch et al. (1988) demonstrated that in some instances β -1,3-glucanases work synergistically with chitinase. This is also true for plants which have been systemically protected (Pan et al., 1989, 1991). A number of reports indicate increases in β -1,3,-glucanases associated with the onset of resistance (Pan et al., 1991; Wyatt et al., 1991; Schneider and Ullrich, 1994).

The principle of SAR presents modern plant pathologists with some interesting possibilities for the control of plant disease, as well as for gaining a deeper insight into the disease resistance mechanisms operational in some plants. Primarily, SAR initiates disease resistance which operates solely on natural defence mechanisms. As demonstrated in much of the work on SAR, it is long lasting and effective against a broad range of pathogens. Finally, the evidence indicates that SAR is responsible for the activation of multiple disease resistance mechanisms. Thus it is less likely the pathogen will overcome this form of resistance, as is the case with resistant varieties and modern single site fungicides.

1.8 *Salicylic Acid*

Many centuries before modern medical scientists identified the multiple therapeutic effects of salicylates, the inhabitants of the old and new world independently observed that the bark and leaves of *Salix* cured fevers and general aches. In 1828 a German, Johann Buchner, successfully isolated a tiny amount of salicin- salicyl alcohol glucoside, the major salicylate in willow bark. The salicylate was named salicylic acid in 1838, the name derived from *Salix*, the Latin name of the willow tree. Commercial production of synthetic salicylic acid first began in Germany in 1874. In 1898, the Bayer company introduced Aspirin, the trade name for acetylsalicylic acid, which went on to become one of the world's best selling drugs.

1.8.1 *General Properties of Salicylic Acid*

Salicylic acid belongs to a diverse group of plant phenolics. Free salicylic acid is a crystalline powder with a melting point in the range of 157-159°C. According to a mathematical model developed in 1988 (Hsu and Kleier, 1990; Kleier, 1988), the physical properties of salicylic acid [pKa=2.98; Minnick and Kilpatrick, 1939 and log Kow (octanol/ water partitioning coefficient)=2.26; Hansch and Anderson, 1967] make it ideal for long distance transport in the phloem. It is possible to assume therefore that unless salicylic acid is actively transported, metabolised or conjugated, it should be translocated from the initial point of application or synthesis to distant tissues. This is further supported by hydrolysis studies (Enyedi et al., 1992; Malamy et al., 1992) which revealed the presence of conjugated salicylic acid, most likely O-β-D glucosylacetylsalicylic acid in TMV treated plants. Such conjugates have not been detected in the phloem exudates or uninoculated tissues of TMV treated plants (Enyedi et

al., 1992), thus supporting the hypothesis that salicylic acid is transported as the free acid. It is possible that conjugation of the salicylic acid by an inducible UDP-glucose-salicylic acid glucosyltransferase provides the mechanism for regulating salicylic acid levels during SAR.

1.8.2 *Salicylic Acid Levels in Plants*

Although it has long been suggested that salicylic acid is present in plants several investigations using modern analytical techniques have verified this (Baardseth and Russwurm, 1978; Metraux and Boller, 1986). Raskin et al. (1990) carried out a comprehensive study of salicylic acid levels in leaves and reproductive structures of over thirty of the most agronomically important plant species. This study confirmed the ubiquitous distribution of this compound in plants, with rice, crabgrass, green foxtail, barley and soybean all recording levels in excess of $1\mu\text{g g}^{-1}$ fresh weight.

1.8.3 *Effects of Exogenously Applied Salicylic Acid*

The first report of salicylic acid being involved in the regulation of flowering in plants followed an experiment in which aphids were allowed to feed on the vegetative and reproductive structures of the short day plant *Xanthium strumarum*. Cleland (1974) hypothesised that the phloem-transmissible factor responsible for inducing flowering could be found in the honeydew excreted by aphids. Flower inducing, as well as flower inhibiting components, were identified in the collected honeydew. It was concluded that the flower inducing factor was of plant origin, since the aphids being fed synthetic diet produced honeydew which was lacking the flower inducing substance. The substance responsible for promoting flowering was subsequently identified as salicylic acid.

Reports soon followed of the florigenic effects of exogenous salicylic acid on *Lemnaceae* and plants of different families. The mechanism by which salicylic acid induces flowering in plants is not yet known. One popular hypothesis is that salicylic acid induces flowering by acting as a chelating agent, since the free O-hydroxyl group confers metal chelating activity on the benzoic acid (Oota, 1975). This hypothesis is further supported by the fact that chelating agents are able to induce flowering in *Lemnaceae* (Oota, 1972; Seth et al., 1970) and the induction resembles that induced by salicylic acid (Pieterse and Müller, 1977).

Certain plants excrete allelopathic chemicals in the rhizosphere to prevent germination of seeds or inhibit growth of neighbouring plants (Einhellig, 1986). It has been suggested that salicylic acid is one such allelopathic chemical. Salicylic acid reduces shoot dry-weight accumulation in several crops and weed species (Shettel and Balke, 1983), possibly by interfering with the membrane ion transport in roots (Glass, 1973; Harper and Balke, 1981).

Concentrations of salicylic acid, between 1 and 10 mM, significantly reduce transpiration in kidney bean (*Phaseolus vulgaris*) leaves (Larque-Saavedra, 1979) and in epidermal strips of *Commelina communis* (Larque-Saavedra, 1978), though because of the relatively high concentrations used it is unlikely that salicylic acid has any significance for the physiological regulation of stomatal behaviour. Salicylic acid also significantly increased pod number and yield in mung beans (Singh and Kaur, 1980) and increased grain number and height of chenopodium millet, *Panicum milaceum* (Datta and Nanda, 1985). At concentrations of between 0.01 and 0.05 mM salicylic acid stimulates the *in vivo* activity of nitrate reductase in maize seedlings, possibly indirectly, by protecting the enzyme from inactivation.

1.8.4 Salicylic Acid Biosynthesis in Plants

Plants are most likely to synthesise salicylic acid from cinnamic acids, important intermediates in the shikimic acid pathway (Gross, 1981; Haslam, 1974). Salicylic acid could therefore be viewed as a derivative of cinnamic acid. The conversion of cinnamic acid to salicylic acid is likely to proceed via one of two pathways as outlined in Figure 1. These two pathways differ in the order of β -oxidation and ortho-hydroxylation and it is feasible that both operate independently in plants.

Work by Chada and Brown (1974) first suggested that both pathways were operational, following the observation that the infection of young tomato plants with *Agrobacterium tumefaciens* increased the ortho-hydroxylation of cinnamic acid. However, in non-infected plants it was the cinnamic acid \rightarrow benzoic acid \rightarrow salicylic acid pathway which was most active.

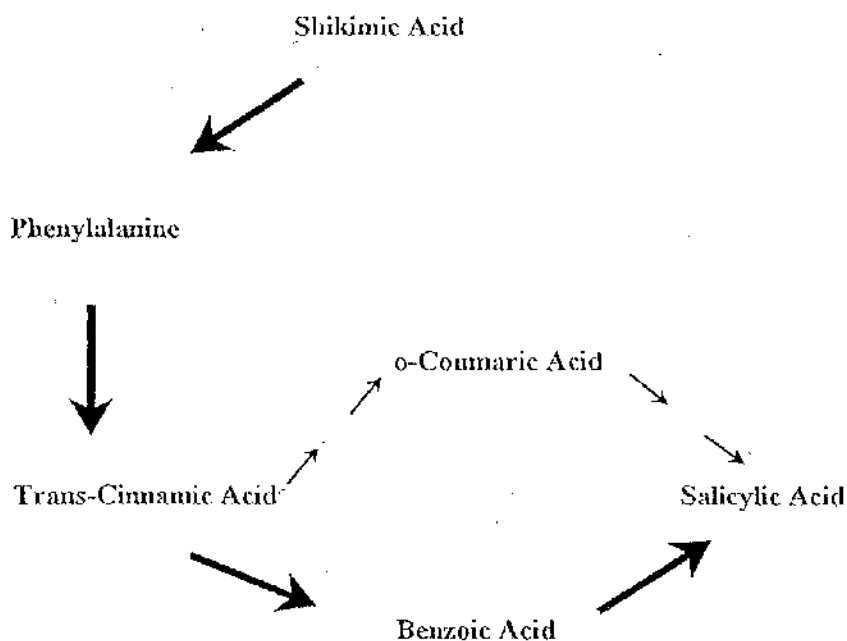


Figure 1. Proposed pathway for salicylic acid biosynthesis

1.9 *Methyljasmonate*

Jasmonic acid [3-oxo-2-(2'-*cis* pentyl)-cyclopentane-1-acetate] and its methyl ester are naturally occurring compounds found in many plant species (Vick and Zimmerman, 1984; Staswick, 1992). Methyljasmonate was first isolated as an odiferous component of the essential oil from *Jasminium grandiflorum* L. (Demole et al., 1962). Early interest in these compounds centred around their fragrant properties, and methyljasmonate has subsequently become a major constituent in the perfume industry. More recently, jasmonic acid and methyljasmonate have been subject to more detailed studies by plant physiologists, who have shown that both these compounds have plant growth-regulating properties (Dathe et al., 1981; Uedo and Kato, 1980; Uedo and Kato, 1982; Yamme et al., 1981), though the general opinion points to their primary action at gene level.

1.9.1 *General Properties of Methyljasmonate*

Methyljasmonate is a volatile, oxygenated fatty acid lipoxygenase product of linolenic acid, which is a liquid at room temperature. It exists as a (-) and a (+) epimer. (-) methyljasmonate has the *trans* configuration of the side chains, with respect to the plane of the ring, and the (+) epimer has the *cis* configuration (Demole et al., 1962). In Vick and Zimmerman's study of plant tissue, results indicated that the *cis* configuration of the side chains was maintained throughout the conversion from 12-oxo-phytodienoic acid to jasmonic acid (Vick and Zimmerman, 1994). Thus, it is believed that the *cis* configuration is the naturally occurring stereoisomer of jasmonic acid. Further support for this hypothesis comes from work by Gundlach et al. (1992) which shows the (+) epimer to have the higher biological activity.

1.9.2 Methyljasmonate Biosynthesis in Plants

The proposed biosynthesis of jasmonic acid and its methyl ester begins with α -linolenic acid and proceeds through the octadecanoid products 13(S)-hydroperoxy-linolenic acid and 12-oxo-phytodienoic acid (Vick and Zimmerman, 1984, 1986; Fig 2). Since a lipoxygenase catalyses the first committed step of the biosynthetic pathway, that is, it converts α -linolenic acid to 13-(S)-hydroperoxy-linolenic acid, potentially jasmonic acid or methyljasmonate could affect their own biosynthesis (Grimes et al., 1992).

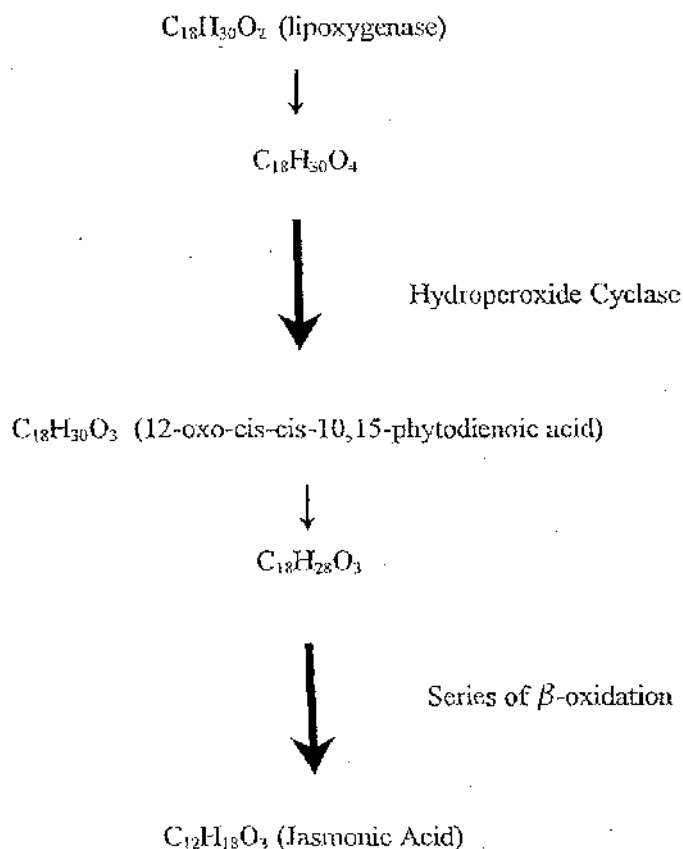


Fig 2. The proposed route for biosynthesis of jasmonic acid from linolenic acid

1.9.3 *Effects of Exogenously Applied Methyljasmonate*

Exogenous application of methyljasmonate elicits a variety of physiological responses in treated plants. It promotes stomata closure (Curtis, 1984), accelerates leaf senescence in barley and oats (Uedo and Kato, 1980; Weidhase et al., 1987), ethylene production in red and green tomato fruits (Saiwski and Czapski, 1985), tendril coiling in wild hops (Falkenstein et al., 1991), and it can also alter gene expression by the rapid induction of protein synthesis (Curtis, 1984; Weidhase, 1987).

An increase in the expression of genes associated with the synthesis of defence related compounds have been noted following methyljasmonate treatment (Gundlach et al., 1992). Soybean cell suspension treated with 250 μ M methyljasmonate exhibited a detectable increase in the level of phenylalanine ammonia lyase (PAL) poly (A) + RNA which was reflected in a significant increase in PAL activity. Tomato leaves exposed to gaseous methyljasmonate responded in a similar manner to those which had resistance induced by insect feeding or wounding (Farmer and Ryan, 1990). The accumulation of proteinase inhibitors in response to this treatment was greater than the detectable level after mechanical injury (Farmer and Ryan, 1990). Application of linolenic acid to tomato leaves induces the same set of proteinase inhibitors, suggesting that the pathway of jasmonic acid biosynthesis is constitutively expressed (Farmer and Ryan, 1992).

1.9.4 *Methyljasmonate Levels in Plants*

Work by Gundlach et al. (1992) indicates that methyljasmonate is partially hydrolysed to free jasmonic acid in the plant by endogenous esterases. This suggests therefore, that exposure of plant tissue to exogenous methyljasmonate results in an endogenous increase

in jasmonic acid levels. At present little is known about the actual alterations in the levels of either methyljasmonate or jasmonic acid following pest or pathogen attack. However, cell suspension cultures of *Rauwolfia canescens* L. and *E. californica* treated with elicitor derived from yeast cell walls exhibited an increase in the endogenous levels of both methyljasmonate and jasmonic acid (Gundlach et al., 1992). The basal level of jasmonic acid increased from 250 ng/g dry weight to 1370 ng/g dry weight in a 45 minute period i.e. a 5-fold increase.

Jasmonic acid is phloem mobile (Anderson, 1985). Its phloem mobility combined with its activation of proteinase inhibitors in distal leaves indicates that it is possibly a signal molecule suitable for transport throughout the plant.

1.9.5 *Methyljasmonate as a Systemic Signal*

In an attempt to verify that methyljasmonate was a systemic signal, possibly in systemic protection against insects, Farmer and Ryan (1992), exposed a single leaf of a two leaf-stage tomato plant to methyljasmonate. Proteinase inhibitors I and II accumulated in treated distal leaves and mRNAs for these proteinase inhibitors accumulated in untreated distal leaves of the methyljasmonate treated plants. Application of jasmonic acid also resulted in the accumulation of proteinase inhibitors I and II (Farmer and Ryan, 1992). Methyljasmonate is also capable of inducing the systemin gene, a peptide shown to possess a hormone-like regulatory function in plants (Farmer and Ryan, 1992). It is possible that methyljasmonate induction of gene expression is a primary step in the signal transduction pathway for the induction of proteinase inhibitors. It is also possible that due to the effect of methyljasmonate and jasmonic acid on the transcription of proteinase I and II mRNAs, the process is systemin mediated. However, before any firm conclusions can be drawn, it

is imperative to determine whether physiologically relevant concentrations of methyljasmonate or jasmonic acid liberated from plant tissues are capable of increasing resistance to pest and disease damage, and also whether systemin and methyljasmonate/jasmonic acid share the same transduction pathway.

1.10 *Potassium Phosphate*

1.10.1 *General Properties*

Potassium phosphate is a white water-soluble crystalline powder at room temperature with a molecular weight of 212g.

1.10.2 *Potassium Phosphate Production*

Production of potassium phosphate was very limited in the early twentieth century AD and though widely studied its commercial production was primarily experimental. Scottish Agricultural Industries has developed a process which involves reacting wet-process phosphoric acid with potassium chloride in a heated rotary drum (Ewart and Raitt, 1969). This process yields approximately 445 kg per hour. Other companies are involved in developing new techniques for the production of potassium phosphate.

1.10.3 *Exogenous Application of Potassium Phosphate*

The exogenous application of potassium phosphate induces systemic protection against a number of plant pathogens (Walters and Murray, 1992; Reuveni et al., 1994; Gottstein and Kúc, 1989). It was suggested that potassium phosphate works on a similar principle

to oxalates (Gottstein and Kúc, 1989), that is by sequestering calcium from host tissues. The sequestration of calcium may affect cell membranes, destroy cell compartmentalisation and cause the synthesis or release of hydrolytic enzymes. These enzymes may then act on cell walls which have been rendered more susceptible to enzymic attack by the removal of calcium. The oligosaccharide or polygalacturonase formed may function as the translocated alarm signal for induced resistance or cause its release.

1.10.4 *Potassium Phosphate as a Fertiliser*

Reuveni et al. (1994a,b) reported that in addition to inducing acquired resistance in cucumbers, potassium phosphate also stimulated a significant increase in plant growth. This phenomenon was also observed in maize treated with potassium phosphate (Reuveni et al., 1992). The effectiveness of foliar fertilisation is limited by several factors, which include poor nutrient uptake due to leaf surface tension and inability to supply the nutrient in large enough quantities.

1.11 *Objectives of The Project*

Although, to date, a substantial amount of research into the phenomenon of systemic acquired resistance has been carried out, little has been focused on the interaction between barley and *Erysiphe graminis*. This is surprising in light of the fact that barley is the worlds fourth most important cereal.

This study was initiated with the aim of fulfilling four primary objectives. These are:

1. To determine the fundamental requirements for optimal induction and maintenance of SAR in barley.
2. To assess the viability of induction of SAR as an alternative, in the field, to proprietary chemicals and to assess the effect on yield.
3. To assess the effects on growth brought about by induction of SAR and challenge-inoculation.
4. To perform a preliminary investigation into enzyme activity in plants exhibiting SAR.

2. MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 *General Materials and Methods*

2.1.1 *Growth and Maintenance of Barley Seedlings*

Seeds of barley, cultivar Golden Promise, were sown individually into FP9, 9 cm pots, filled with Levington M3 compost. The pots were arranged on trays and placed in a ventilated glasshouse. Plants were watered daily with no supplementary nutrition being supplied. The average daytime temperature in the glasshouse during the course of the experiments was 22°C, with a maximum of 25°C. Average night time temperature fell to between 14°C and 17°C. Natural daylight was supplemented by growing plants under 400W mercury vapour lamps, to provide a 16 hour photoperiod. Mean irradiation in the glasshouse at midday was 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation.

2.1.2 *Inoculation and Maintenance of Powdery Mildew*

Barley powdery mildew was maintained on stock barley plants grown in the glasshouse under the conditions outlined in section 2.1.1. Mildew conidia were gathered by gently brushing the upper surface of infected leaves with a fine camel hair brush. The brush was then very gently brushed across the leaf surfaces to be inoculated. This method of inoculation was preferred to the use of a settling tower, since it was more rapid and it consistently provided an inoculum density on leaves of approximately 35 mildew conidia per cm^{-2} .

2.1.3 *Induction of Systemic Protection*

Inducing agents were prepared as millimolar solutions in distilled water, containing 0.01% (v/v) Tween 20 (BDH Chemicals) to act as a surfactant. Systemic protection was elicited by painting the appropriate leaf with the required concentration of the chemical. As a means of applying inducing treatments, painting was ideal, since it allows a single leaf to be treated without accidental treatment of other leaves. Systemic protection was elicited by treatment of the lower leaf in each case unless otherwise stated.

2.1.4 *Challenge-Inoculation of Systemically Protected Plants*

Plants were challenge-inoculated with mildew spores collected as outlined in section 2.1.2. The conidia were gently brushed onto the upper surface of the leaf immediately after they had been gathered as described in section 2.1.2. The stock plants were brushed to remove old spores 24 hours prior to them being used for challenge-inoculations, thus ensuring a supply of young viable spores for use in experimental work. Challenge-inoculation was of the second leaf, unless otherwise stated.

2.1.5 *Assessment of Mildew Infection*

The level of mildew infection was assessed as a percentage of the whole leaf area infected with powdery mildew mycelium. The assessment was visual using a standard area diagram (MAFF, 1988).

2.1.6 *Growth and Maintenance of Fungal Cultures*

The plant pathogen *Crinipellis pernicioso* (cause of Witches broom disease of cocoa) and *Pyrenophora avenae* (cause of rice blast) were maintained by subculturing every 14 days onto potato dextrose agar plates. Cultures were routinely incubated at $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$, in the dark.

2.1.7 *Protein Assay*

The protein content of plant samples were determined using the method developed by Lowry et al., (1951).

2.1.7.1 *Preparation of Protein Assay Reagents*

The following reagents were prepared for use in the assay:

A- 20g anhydrous Na_2CO_3 in 1 litre 0.1M NaOH

B- 0.5% (W/V) copper sulphate solution

C- 1.0% (W/V) sodium potassium tartrate solution

D- A preparation of A:B:C, 48:1:1 was made immediately before use

E- 50% (V/V) solution foline-ciocalteau reagent

2.1.7.2 *Protein Assay Technique*

0.25 ml of reagent D was added to a 0.25 ml sample of crude enzyme extract and incubated at room temperature for 15 minutes. 0.25 ml of reagent E was added to the

reaction mixture, shaken and then incubated for a further 30 minutes, thus allowing the colour to develop. Absorbance at 700 nm was recorded on a Gallenhamp Visi-Spec.

2.2 Experimental Materials and Methods

2.2.1 Screening of Various Concentrations of Potential Elicitors of Systemic Protection

The initial screening programme was carried out in order to determine the concentration of each test chemical which gave the highest level of systemic protection. Each chemical was tested at four concentrations. Plants were grown as described in section 2.1.3. The chemicals used and concentrations tested are listed below:

Salicylic acid- 5, 10, 15, 20 mM

Sodium salicylate- 5, 10, 15, 20 mM

Acetylsalicylic acid- 5,10,15,20 mM

Potassium phosphate- 15, 25, 35, 50 mM

Methyljasmonate- 5,10,15,20 mM

Isonicotinic acid- 0.1, 0.2, 0.3, 0.4 mM

Chitosan- 0.1, 0.2, 0.3, 0.4 %

First leaves were treated with the appropriate compound as described in section 2.1.3. Controls were treated with distilled water containing 0.01% Tween 20. The barley plants were challenge-inoculated with mildew conidia 24 hours after treatment as outlined in section 2.1.4. The plants were then grown for a further 10 days before mildew infection was assessed as described in section 2.1.5.

2.2.2 *Optimal Time Interval Between Inducer Treatment and Challenge-Inoculation*

Based upon the results of the initial screening, three compounds were selected for more detailed study. It was then essential to establish the optimal time interval between treatment of the lower leaf with the chemical and challenge-inoculation of the upper leaf with mildew required for maximum protection.

The plants were grown as described in section 2.1.1. The first leaf was painted with the appropriate concentration of the compound as described in section 2.1.3 and challenge-inoculated with powdery mildew 1, 2, 3, 4, 7 and 12 days later. The plants were grown for a further six days in the glasshouse before visually assessing the percentage leaf area infected with powdery mildew.

Due to the nature of the experiment a separate control was used for each time interval studied.

2.2.3 *Effect of Light on Systemic Protection*

As discussed in section 1.3.4.2 of the introduction, light plays an important role in the synthesis of metabolites which are subsequently utilised in energy demanding defence processes. It was seen as essential therefore to determine the effect of light on the development of systemic protection. Work by Lozano and Sequiera (1970) on tobacco indicated that the degree of protection is directly proportional to the length of photoperiod.

Plants were grown as described in section 2.1.1 and were treated with the inducers of systemic protection as described above in section 2.2.1. Inducing agents were applied 2

days prior to inoculation with mildew. At this point they were transferred to growth chambers (Conviron) where the photoperiod could be regulated and a temperature of 22°C during the light period and 14°C during darkness was maintained. Mean irradiation throughout the period of light was 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$. The photoperiods examined were 7, 12, 16 and 20 hours. The plants were grown under these conditions during the period between elicitor treatment and challenge-inoculation and then for a further 10 days before assessing the percentage of the leaf area covered by mildew infection.

Due to the nature of the experiment each photoperiod required a separate control.

2.2.4 *Effect of Temperature on Systemic Protection*

It has been documented that induced systemic protection is highly temperature sensitive (Klement and Goodman, 1968). Four temperature regimes were examined. The temperatures of 10, 17, 22 and 35°C were examined. The plants were grown under these conditions from elicitor treatment until assessment.

When the seedlings reached growth stage 13 (Zadoks et al., 1974), first leaves were painted with the chemical and the second leaf challenge-inoculated 2 days later with mildew conidia. Powdery mildew infection was assessed 10 days later.

Due to the nature of the experiment, a separate control was used for each of the temperature regimes studied.

2.2.5 *Direction of Signal Movement*

It has been widely documented that the signal for the induction of systemic protection moves from the inducer leaf to the upper portion of the plant via the phloem. Little evidence exists for the movement of the signal downwards, thus protecting the portion of the plant below the inducer leaf. It is important, in terms of potential usage in the field, to establish whether any downward movement exists.

Seedlings were grown as described in section 2.1.1 until they had reached growth stage 31 (Zadoks et al., 1974) i.e. until they had six fully developed leaves. At this stage, the three upper leaves were painted with the appropriate chemical and 48 hours later the three lower leaves were challenge-inoculated with powdery mildew. Mildew infection was assessed on the lower three leaves 6 days later. Leaf one was regarded as the lowest leaf, leaf two the second lowest and leaf three the third lowest. Each leaf was tagged as it was treated in order to avoid possible confusion during the course of the experiment.

2.2.6 *Duration of Systemic Protection*

There are reports that SAR can persist for prolonged periods during the life of a plant, e.g. in cucumber, where SAR to infection by *Colletotrichum lagenarium* was shown to last for 10 weeks (Dean and Kuc, 1985). Such long-lasting protection against pathogen infection would be of considerable advantage in crop protection programmes. It was important therefore to examine the longevity of systemic protection induced by the agents used in this study.

The plants were grown as described in section 2.1.1 until they reached growth stage 13. The leaf which had just emerged (leaf 2) was then tagged to ensure the correct leaf was challenge-inoculated and subsequently assessed. The tagged leaf was challenge-inoculated with mildew 48 hours later. 7, 14, 21, 28, 35 and 42 days post challenge-inoculation, the tagged leaf was visually assessed to determine the level of mildew infection.

2.2.7 Effect of Delaying Challenge-Inoculation on the Magnitude of the Systemic Protection Response

It has been reported by Kúc and Co-workers (1977) that cucumbers may be challenge-inoculated as much as 2 weeks after treatment with the inducing agent and still exhibit systemic protection. The ability to obtain systemic protection several weeks after treatment will be an important factor in determining the efficacy of an inducing agent in the field.

Plants were grown as described in section 2.1.1 until they reached growth stage 13. At this stage the first leaf of each seedling was painted with the chemical, and the second leaf was then challenged with mildew at one of six intervals; 7, 14, 21, 28, 35 and 42 days post-treatment. The second leaf, which had been tagged, was then visually assessed for the percentage mildew infection 10 days after challenge-inoculation.

2.2.8 Seed Priming

There have been a limited number of reports regarding the possibility of pre-soaking seeds as a potential method of inducing systemic protection in plants. If this was possible, it would provide an easy, convenient method of inducing systemic protection, without the

need for aerial application of sprays. Four chemicals were tested using this method of application: sodium salicylate, methyljasmonate, potassium phosphate, and chitosan.

Seeds were pre-soaked for varying lengths of time: 5 minutes, 15 minutes, 1 hour, 2 hours, 3 hours and 6 hours. The treated seeds were then sown as previously described in section 2.1.1 and allowed to germinate. Once the seedlings had reached growth stage 13, the second leaf was challenge-inoculated with mildew and the plants were then grown for a further 10 days before visually assessing the percentage leaf area infected with powdery mildew.

Again due to the nature of the experiment it was necessary to employ a set of controls for each of the time periods examined. In control treatments, seeds were soaked, substituting the chemical with distilled water. From this point onwards the control seeds were treated in the same way as test seeds.

2.2.9 *Root Drenching*

Further investigations were carried out to determine whether it would be possible to induce systemic protection in barley via the root system, by applying the chemicals as a root drench. Plants were grown as described in section 2.1.1 until they had reached growth stage 13. The seedlings were then given a 100 ml root drench of the chemical, made up in distilled water. The drench was applied at 5 separate intervals: 5, 4, 3, 2 and 1 day pre-inoculation of the first leaf. The plants were assessed 10 days after challenge-inoculation.

To examine the possibility that volatile resistance-promoting substances might be released from the jasmonate treated soils, plants in untreated soil were placed in the same chamber during, and for one hour following, the treatment process. For the purposes of this text these plants will be referred to as companion plants. They were then inoculated with mildew and assessed for mildew infection 10 after challenge-inoculation.

Control plants were treated in the same way as test plants, substituting a chemical root drench with distilled water. Controls were carried out for each of the time intervals tested.

2.2.10 Direct Effect of Test Compounds on Powdery Mildew Development

Experimental work to date has indicated that the reduction in the level of mildew infection observed has been due to the priming of the plants natural defence mechanisms, meaning that any subsequent pathogen attack can be dealt with swiftly. It is possible, however, that the observed reduction in the level of pathogen infection may be due simply to the fungitoxic effect of the chemicals being screened. The three chemicals chosen for detailed study were therefore tested for any direct effect on the pathogen. The plants were grown as described in section 2.1.1 under optimal conditions as determined by the results of sections 2.2.3 and 2.2.4. At growth stage 13 (Zadoks et al., 1974), the plants were treated as described below.

2.2.10.1 Fungitoxicity Test One

The second leaf of barley seedlings was inoculated with mildew conidia. One day later the inoculated seedlings were then sprayed with the appropriate chemical. Mildew infection on this leaf was recorded 10 days later.

2.2.10.2 Fungitoxicity Test Two

The second leaves of barley seedlings were sprayed with the chemical agents and were then allowed to dry fully for four hours before they were inoculated with powdery mildew as described in section 2.1.4. Ten days post-inoculation powdery mildew infection was assessed.

2.2.11 *Antifungal Activity of Chemical Elicitors*

In addition to examining the direct effects of the chemicals on mildew development on the leaf surface (see section 2.2.10), it was decided to examine the effects of the compounds on the growth of two plant pathogens *in vitro*. The pathogens tested were *Pyrenophora avenae* and *Crinipellis perniciosa*.

Filter-sterilised aqueous solutions (10ml) containing the compounds were added to 140ml of sterile PDA at 45-47°C to obtain a final concentration of between 0.1-50 mM (see Table 4). Control plates contained PDA only. Sterile media (20 ml) containing the compound was added aseptically to 90 mm single vent sterile plastic Petri dish. To obtain inoculum, a sterile 9 mm diameter core borer was used to remove plugs of mycelium from the plates of stock cultures. The mycelial plugs were inverted and one was placed in the

centre of each petri dish. Inoculated plates were then incubated in the dark at 22°C and colony growth was measured radially, beyond the 9 mm plug of inoculum, 3, 6, 9, 12 and 15 days after inoculation. Results are the mean of 5 replicates.

Because of its oily nature in water, MJ could not be filter-sterilised. It was therefore prepared using sterilised pipette tips and sterile distilled water, and it was assumed that the methyljasmonate was sterile and unable to support the growth of any microbial contaminants.

Final Concentrations of Chemicals in Ammended PDA

5 mM NaSA	0.1 mM MJ	10 mM PP
10 mM NaSA	1 mM MJ	25 mM PP
15 mM NaSA	5 mM MJ	35 mM PP
20 mM NaSA	10 mM MJ	50 mM PP

Table 4

2.2.12 *Effects of Chemical Elicitors on Fungal Growth in Liquid Culture*

The results observed in section 2.2.11 were further investigated using potato dextrose broth as an alternative to potato dextrose agar. It is possible that a pathogen growing on an agar plate is able to avoid contact with any potentially fungitoxic amendments by growing as a very fine film across the surface of the plate, the result being apparently normal growth (Prosser, 1995). It is possible to overcome this problem by growing the fungus in liquid culture on an orbital shaker. The constant motion of the medium within

the flask ensures the pathogen is in constant contact with any chemical amendments. Thus a more accurate picture of the effect of the amendment on the pathogen is achieved.

Filter-sterilised solutions (10 ml) containing the chemical were added to sterile potato dextrose broth (140 ml) in 250 ml flasks to obtain the desired concentration. Each flask was then inoculated with a 9 mm plug of mycelium and placed on a Gallenkamp orbital shaker (100 rpm) at 22°C and a mean irradiance of 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$. After 14 days, the fungus was washed in distilled water through a fine mesh sieve and centrifuged at 16,000g for 15 min. The resulting pellet was then weighed. Methyljasmonate was added to the flasks as described below (section 2.2.13).

2.2.13 *Methyljasmonate Vapour as an Elicitor of Systemic Protection*

There is evidence from earlier research by Farmer and Ryan (1990) that gaseous methyljasmonate induced plant defence responses similar to those promoted by insect feeding or wounding. This hypothesis was tested in barley by isolating the first leaf from the rest of the plant in a plastic chamber and exposing it to vapour from either undiluted methyljasmonate or vapour from 20mM MJ. A cotton tipped applicator soaked in methyljasmonate was sealed in the plastic chamber beside the lower leaf. The exposure times tested in this study were 5 min, 15 min, 30 min, 1 hours and 2 hours. Two days post-exposure, the second leaf was challenge-inoculated with mildew conidia. Ten days later the second leaf was assessed for the percentage leaf area covered with powdery mildew.

A companion plant was placed at the side of the plastic chamber to ensure no substantial leakage of MJ was taking place during the exposure times. Thereafter these companion plants were treated in the same way as test plants.

Control plants were treated in the same way, replacing the methyljasmonate soaked cotton tipped applicator with one soaked in distilled water. There after they were treated in the same way as test plants. A control was used for each of the exposure times.

2.2.14 *Optimal Interval Between Exposure to Methyljasmonate Vapour and Challenge-inoculation*

In order to optimise the exposure time of the plant to methyljasmonate vapour it was necessary to determine the optimal interval between vapour treatment and challenge-inoculation with mildew. A number of intervals were examined ranging from a few hours to several days.

Plants were grown as described in section 2.2.11, until growth stage 13. The lower leaf was exposed to methyljasmonate vapour as described in section 2.2.13, for 30 min. The second leaf was then challenge-inoculated at one of the following eight time intervals: 12 hours, 1 day, 2 days, 3 days, 7 days and 12 days after treatment. Ten days after challenge-inoculation the second leaf was visually assessed to determine the percentage of mildew infection.

Control plants were treated as described in section 2.2.13 and were used for each of the eight time intervals tested.

2.2.15 *Effect of Methyljasmonate Vapour on Germination of Mildew Conidia in vitro*

Following on from the work in described section 2.2.10, the direct effects of methyljasmonate vapour on germination of mildew conidia were investigated *in vitro*.

The following technique was not aseptic and autoclaving was only carried out in order to achieve complete solution of the agar. Tap water agar was made up to 1.2% W/V, autoclaved, poured into Petri dishes and allowed to set. The plates were then inoculated with mildew at approximately 35 spores per cm². Three replicate plates were used per treatment. Open plates were placed inside a plastic chamber for thirty minutes, along with a cotton tipped applicator which had been soaked in either undiluted methyljasmonate or a 15mM solution of methyljasmonate. The plates were then transferred to an incubator and maintained at 22°C in the dark. Twenty-four hours later the plates were examined using light microscopy (x 100 magnification) to assess the percentage germination of the mildew conidia. Each plate was divided into four sections and 100 spores from each quarter counted and the number which had germinated was recorded.

Control plates were prepared and inoculated in the same way. They were placed in a sealed plastic chamber along with a cotton bud which had been soaked in distilled water.

2.2.16 Direct Effect of Methyljasmonate Vapour on Mildew Development in vivo

The direct effect on mildew development *in vivo* from the vapour of both undiluted MJ and a 20mM solution was examined. The second leaf of the barley plant was inoculated with mildew conidia as described in section 2.1.2. The inoculated leaf was then exposed to the vapour from either undiluted MJ or from a 20mM solution by the technique described in section 2.2.13, for a 30 minute period. Ten days later the second leaf was assessed for the percentage leaf area infected with powdery mildew.

Control plants were treated in the same way, replacing the MJ soaked cotton tipped applicator with one soaked in distilled water. Thereafter they were treated in the same way as test plants.

2.2.17 *Effects of Precursors of Methyljasmonate on Induction of Systemic Protection Against Powdery Mildew*

Two precursors of methyljasmonate, linoleic and linolenic acid, were screened for their ability to induce systemic protection against barley powdery mildew. The protocol followed was that described in section 2.2.1 and four concentrations of each were tested: 0.1, 1, 5 and 10 mM. Ten days after challenge-inoculation with powdery mildew the second leaf was visually assessed for the percentage leaf area covered with mildew.

Control plants were grown and treated as described, replacing linoleic acid and linolenic acid with distilled water containing 0.01% Tween 20.

2.2.18 *Effects of Two Methyljasmonate Precursors on Fungal Growth on Agar Plates*

The effects of two methyljasmonate precursors, linoleic acid and linolenic acid, were examined on two plant pathogens, *in vitro*, on amended PDA plates. The two pathogens tested were *P. avenae* and *C. pernicioso*.

The method described in section 2.2.11 was applied throughout.

2.2.19 *Effects of Two Methyljasmonate Precursors on Fungal Growth in Liquid Culture*

The effects of the two methyljasmonate precursors linoleic acid and linolenic acid were further examined on two plant pathogens, *in vitro*, in PDB. The pathogens tested were *P. avenae* and *C. perniciosus*.

The method described in section 2.2.15 was applied throughout.

2.2.20 *Light Microscopy*

To further examine the effect of the chemicals on early mildew development, germling growth was examined by light microscopy. Plants were grown as described in section 2.1.1 until they had reached growth stage 13. The first leaf was treated as outlined in section 2.2.1 and seventy-two hours after challenge-inoculation the infected leaf was harvested and divided into segments of approximately 2-3 cm. The leaf sections were cleared for one hour, longer if required, at 70°C in an ethanol-chloroform (75:25 V/V) mixture containing 15% trichloroacetic acid. The clearing solution was changed at 15 minute intervals. The cleared leaf sections were then stained in a dye solution which was prepared one day prior to use. The dye solution contained one volume 15% trichloroacetic acid and one volume 0.6% Coomassie brilliant blue R-250. Young leaves were stained for 15-25 minutes.

Stained preparations were then preserved in a solution of glacial acetic acid-glycerol-water (2:20:75 V/V) on slides, covered with cover slips and sealed in place with nail polish. If stored in the dark, these slides remain light stable for at least one year. The preparations were then examined under light microscopy (Leitz Dm RB with

photoautomat, Leica, U.K. to observe changes in the germination and fungal development brought about by treatment with the abiotic agents.

Control plants were grown and treated the same way, replacing chemical treatment with distilled water containing 0.01% Tween 20.

2.2.21 *Enzyme Activity*

In order to further understand the physiological changes in the plant brought about as a result of treatment with the chemicals three key enzymes associated with disease resistance were studied, peroxidase, PAL and lipoxygenase. The plants were grown as described in section 2.2.1 and when they reached growth stage 13 the first leaf was treated with the appropriate concentration of the chemical. Tissue from the 1st, 2nd and 3rd leaves of these plants was assayed at 1, 2, 3, 4, 7 and 12 days after treatment for alterations in enzyme activity. In a further set of experiments, when the appropriate period had elapsed following chemical treatment of the first leaf, the second leaf was challenge-inoculated with mildew conidia. The tissue of the first, second and third leaves of these challenged plants were subsequently assayed for alterations in the level of enzyme activity following challenge-inoculation of inducer treated plants.

2.2.21.1 PAL Assay

This assay was based on a method described by Southerton and Deverall (1990). A 250 mg sample of leaf tissue was ground in a cold mortar and pestle containing 2.5 ml sodium borate buffer (0.1 M, pH 8.8). A 1.5 ml aliquot of the homogenate was transferred to a centrifuge tube and 0.75 ml supplemented borate buffer containing 3 mM β -

mercaptoethanol and 3 mM EDTA was added to the homogenate and mixed thoroughly. The tube was then centrifuged at 20,000g for 15 minutes at 4°C. The supernatant was used as the enzyme extract and samples were kept on ice at all times.

A 300 μ l sample of leaf extract was incubated at 40 °C with 0.6 ml borate buffer containing 0.6 μ M L-phenylalanine. A blank with no L-phenylalanine was also prepared. After two hours the reaction was stopped by adding 100 μ l 6M HCl. The product (cinnamic acid) was extracted by adding 1.0 ml of chloroform and mixing thoroughly on a vortex mixer. This was then centrifuged at 1,300g for 5 minutes at 4°C. A 0.5 ml aliquot was then taken from the lower, chloroform phase. The chloroform was then evaporated by blowing nitrogen over the sample and the residue was then redissolved in 0.1 ml borate buffer (0.1 M, pH 8.8). Absorbance was measured at 270 nm on a Gallenkamp visi-Spec spectrophotometer. A standard curve was produced using 0.1-10 μ g/ml Cinnamic Acid. Enzyme activity is expressed as μ g cinnamic acid/mg protein/hour.

2.2.21.2 Peroxidase Assay

This assay is based on the method described by Southerton and Deverall (1990). A 250 mg sample of leaf tissue was ground in a cold mortar and pestle with 2.5 ml sodium borate buffer (0.1M, pH 8.8). A further 0.75 ml sodium borate buffer was added and thoroughly mixed with the leaf preparation. A 1.5 ml sample was then transferred to a centrifuge tube and centrifuged at 20,00g for 15 minutes at 4°C. The supernatant was used as the enzyme extract. Samples were kept on ice until required in the assay.

50 μ l of the enzyme sample was added to 2.95 ml supplemented phosphate buffer (0.1M, pH 7.0) containing 0.9 μ M guaiacol and 0.36 μ M hydrogen peroxide. Absorbance at 470

nm was then recorded for 2 minutes at 25°C on a Gallenkamp Visi-Spec spectrophotometer. Protein was determined using the assay described in section 2.1.7 of the General Materials and Methods (Lowry et al., 1951) and was carried out simultaneously. Enzyme activity was expressed as the Δ Abs/mg protein.

2.2.21.3 Lipoxygenase Assay

This assay is based on the method described by Ocampo et al., (1986). Enzyme extracts were prepared grinding precooled potassium phosphate buffer (0.1M, containing 1 mM EDTA, pH 7.5) with leaf tissue (x ml/ xg) in a precooled mortar and pestle. Samples were then centrifuged at 28,000g for 15 minutes at 2°C. The supernatant was used as the enzyme extract.

The reaction mixture contained 1 ml citrate phosphate buffer (0.1M, pH 6.2) and 100 μ l substrate solution, prepared using 2.5 mM linoleic acid in 0.05 M phosphate buffer, pH 9.0, containing 0.025 % Tween 20. The reaction was initiated by the addition of 20 μ l of enzyme extract and the reaction was followed for 15 minutes at 234 nm using a Gallenkamp Visi- Spec spectrophotometer. Enzyme activity was expressed as Δ Abs/mg protein.

2.2.22 *Growth Analysis of Plants Treated With Chemical Inducers of Systemic Protection*

To further investigate the changes in host physiology following treatment with the abiotic inducing agents, a plant growth analysis was undertaken. Plants were grown as described in section 2.1.1 until growth stage 13 was reached. At this point, plants were treated with

the inducer of systemic protection by painting the three lower leaves with the appropriate chemical. Ten plants per treatment were harvested at weekly intervals for a period of eight weeks. In further studies plants were challenge-inoculated with mildew at the appropriate period after inducer treatment. Plants were assessed at weekly intervals following challenge-inoculation, for a period of eight weeks. Plant material was washed with water to remove extraneous matter and separated into roots and shoots. Wet plant material was blotted dry with absorbent tissue and weighed immediately. It was essential that this element of the growth analysis was carried out immediately after harvest, since any delay would result in moisture loss.

The total leaf area of each plant was measured in cm^2 , using a leaf area meter (Delta Devices, U.K.). Plant material was then put into separate paper bags, labelled and oven dried at 95°C for two days. The plant material was then re-weighed and the dry weight was recorded in milligrams.

From the above measurements it was then possible to calculate the following:

A- FW/W ratio

B- Root/Shoot ratio (Ratio of root dry weight to shoot dry weight)

C- Relative growth rate (R.G.R)- An expression of growth efficiency

$$\text{R.G.R} = \frac{\ln(W)_2 - \ln(W)_1}{T_2 - T_1}$$

This is measured in $\text{mg mg}^{-1}\text{day}^{-1}$.

$(W)_2$ and $(W)_1$ correspond to total dry weights at the times T_1 and T_2 .

D- Net Assimilation Rate (N.A.R.)- An expression of leaf efficiency

$$\text{N.A.R.} = \frac{(W)_2 - (W)_1}{L_2 - L_1} \times \frac{\ln(LA)_2 - \ln(LA)_1}{T_2 - T_1}$$

$$= \text{mg cm}^{-2} \text{day}^{-1}$$

E- Leaf Area Ratio (L.A.R.)- An expression of the leafiness of the plant

L.A.R. = Specific Leaf Area x Leaf Weight Ratio

$$= \frac{1}{2} \frac{(LA)_1 \times (LA)_2}{(W)_1 (W)_2}$$

$$= \text{cm}^{-2} \text{mg}^{-1}$$

F- Specific Leaf Area (S.L.A.)- An expression of leaf density

$$\text{S.L.A.} = \frac{(LA)}{(W) \text{ Leaf}}$$

G- Leaf Weight Ratio (L.W.R.)- An expression of productive investment

$$\text{L.W.R.} = \frac{(W) \text{ Leaf}}{(W) \text{ Total Plant}}$$

2.2.23 Field trials

Spring barley (cv. Golf) was sown at 12 cm row spacing with a seed rate of 190 kg ha⁻¹. Seed was sown in plots (4.0 m x 2.0 m) in a randomised block design with four replicates. Plots were sprayed twice, at the first sign of mildew or at GS 31 (first node detectable) and again at GS 45 (boots swollen). The following treatments were used:

- 1- Control- These plots were left unsprayed.
- 2- Foliar (Bayer)- Containing tebuconazole at 250 g/l. This is a systemic conazole fungicide applied at the rate of 1 l ha⁻¹.
- 3- Corbel (BASF)- Containing fenpropimorph at 750 g/l. This is a contact and systemic morpholine fungicide. It was applied at the normal field rate of 1 l ha⁻¹.
- 4- Radar (Zenica)- Containing propiconazole at 250 g/l. Radar is a systemic, curative and protectant conazole fungicide. This was applied at the normal field rate of 0.5 l ha⁻¹.
- 5- Sodium salicylate- Unformulated, 2.4 g in 1 l distilled water containing 0.5 % (v/v) Agral 90 (Zenica).
- 6- Methyljasmonate -Unformulated 2.06 ml in 1 l distilled water containing 0.5 % (v/v) Agral 90.
- 7- Potassium phosphate- Unformulated 7.42 g in 1 l of distilled water containing 0.5 % (v/v) Agral 90.

Chemicals were applied using an Azo compressed air sprayer with delivery rate of 1 l in 20 seconds and a spray boom width of 1.8 M.

Ten plants per plot were assessed for the percentage mildew infection 7 and 14 days after each spray. Following the first spray the third leaf was assessed. After the second spray the flag leaf was assessed. At the end of the trial, plants were harvested and

measurements made of grain weight. Data was analysed statistically using analysis of variance.

3. RESULTS

3 RESULTS

3.1 *General Screening*

3.1.1 *Screening of Chemical Elicitors*

Screening a number of chemicals at a range of concentrations to determine the most effective elicitors of resistance showed that all concentrations of all the chemicals reduced powdery mildew infection (figs 3-9). The largest reductions in mildew were found on those plants treated with 15mM NaSA, 25mM PP and 20mM MJ. Analysis of variance showed significant differences between treatments and controls.

3.1.2 *Optimal Lag Period*

All time intervals screened as potential lag periods between elicitor treatment and challenge-inoculation gave significant reductions in the level of mildew infection observed (fig 10). Two factor analysis of variance highlighted significant differences between treatments and controls (fig 10). The largest reductions were found when there was a 48 hour lag between elicitor treatment of the lower leaf and challenge-inoculation of the second leaf.

3.1.3 *Optimal Temperature Regime and Effect of Photoperiod on Development of Induced Resistance*

Experiments to investigate the effect of daylength on the development of acquired resistance indicated that a 16 hour photoperiod allowed optimal development of resistance. This is reflected in the largest decrease in mildew development (fig 11). Two factor analysis of variance showed significant differences between treatments and control. Studies examining the influence of temperature on the development of induced resistance showed that 22°C gave the highest levels of systemic protection (fig 12). Two factor analysis of variance highlighted significant differences between treatments and the controls.

3.1.4 *Duration of Induced Resistance*

A tailing off in the level of protection elicited was observed as the period of time between elicitor treatment and challenge-inoculation was increased. The level of protection observed when a period of 42 days had elapsed between treatment and challenge-inoculation was still statistically significant in plants treated with 15mM NaSA or 25mM (fig 13). Plants treated with 20mM MJ no longer gave significant reductions in mildew control after day 35.

3.1.5 *Direction of Signal Movement*

It is possible to afford a degree of protection to the area below the inducer leaf of elicitor treated plants. Thus, all 3 treatments, 15mM NaSA, 25mM PP and 20mM MJ gave

small, but significant reductions in the level of mildew recorded below the inducer leaf (fig 15). The largest reduction was observed in plants treated with 20mM MJ.

3.1.6 *Seed Treatment and Root Drenches as Methods of Inducing Resistance*

Immersion of barley seed in a range of chemicals which elicit resistance in leaves provided variable results. At no treatment time did 15mM NaSA elicit resistance (fig 17). 20mM MJ and 0.1% Ch, at both 5 and 15 minute treatment times, gave significant reductions in mildew development (fig 16). After this time no chemical elicited resistance. Increasing treatment times to 180 and 360 minutes leads to an increase in mildew development on the second leaf. After calculation of the standard errors and examination of the results no further statistical analysis was performed on these data.

3.1.7 *Fungitoxicity Screening*

When the fungitoxicity of the three treatments, 15mM NaSA, 25mM PP and 20mM MJ, was tested using the spray/dry/inoculate technique, there were very large reductions in the level of powdery mildew infection observed (fig 18). When the inoculate/spray technique was used all chemicals provided significant reductions in mildew development although the reductions observed for 20mM NaSA and 25mM PP with this technique were much smaller than observed with spray/dry/inoculate technique (fig 18). MJ gave reductions of a similar magnitude with both techniques.

3.1.8 Effect of Abiotic Elicitors In Vitro

A range of concentrations of NaSA, PP and MJ were all shown to significantly reduce the radial growth of *P. avenae* and *C. pernicioso* grown on amended agar plates (fig 20-25). With each of the three chemicals examined the lowest concentration had the least effect on pathogen growth and the highest concentration the most inhibitory effect on growth (figs 20-25). The largest reduction in growth, for both pathogens, was observed on plates amended with 50mM PP (fig 22-23). On these plates no growth was observed for 9 days after inoculation of the plate. 50mM PP reduced radial growth of *P. avenae* by 85% and *C. pernicioso* by 83%. The same range of chemicals also gave significant reductions in biomass production of *P. avenae* and *C. pernicioso* in amended potato dextrose broth (fig 25-26). Only the lowest concentration of each chemical, 5mM NaSA, 10mM PP and 5mM MJ, supported pathogen growth. The level of growth observed at these three concentrations was significantly lower than the control. All higher concentrations inhibited growth completely (fig 25-26).

FIGURE 3. Effect of treating the lower leaf of barley with various concentrations of the chemical Salicylic Acid on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent the standard errors.

FIGURE 4. Effect of treating the lower leaf of barley with various concentrations of the chemical Sodium Salicylate on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent the standard error.

FIGURE 5. Effect of treating the lower leaf of barley with various concentrations of the chemical Potassium Phosphate on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent standard error.

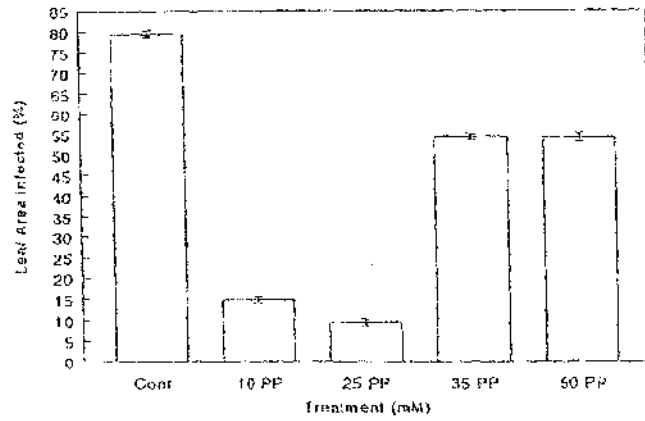
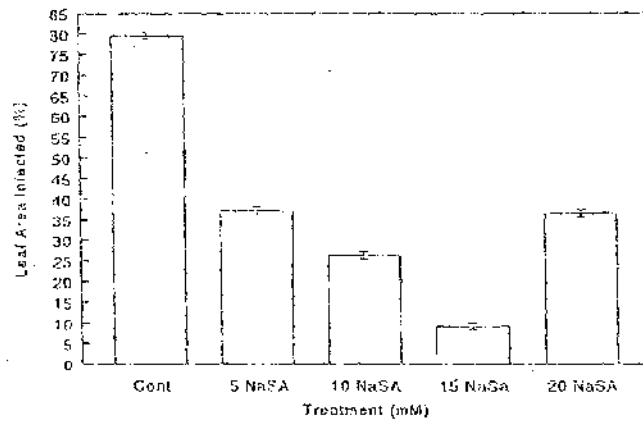
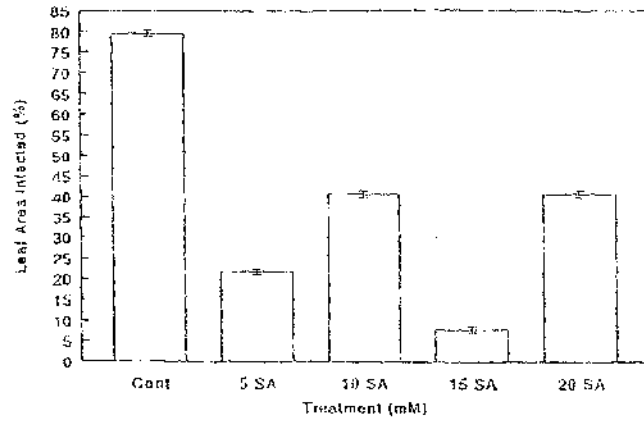


FIGURE 6. Effect of treating the lower leaf of barley with various concentrations of Methyljasmonate on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent the standard error.

FIGURE 7. Effect of treating the lower leaf of barley with various concentrations of Isonicotinic acid on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent standard errors.

FIGURE 8. Effect of treating the lower leaf of barley with various concentrations of Acetylsalicylic acid on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent standard errors.

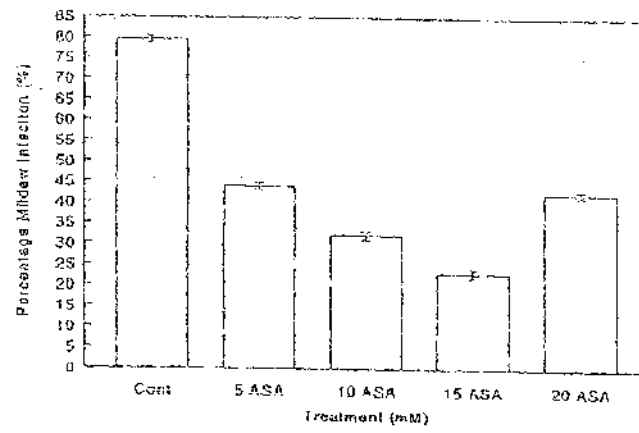
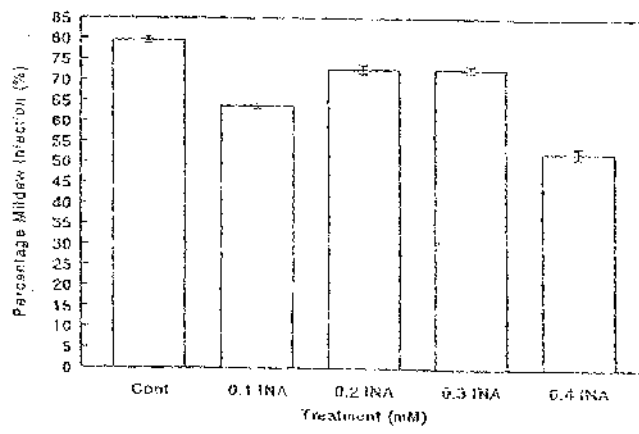
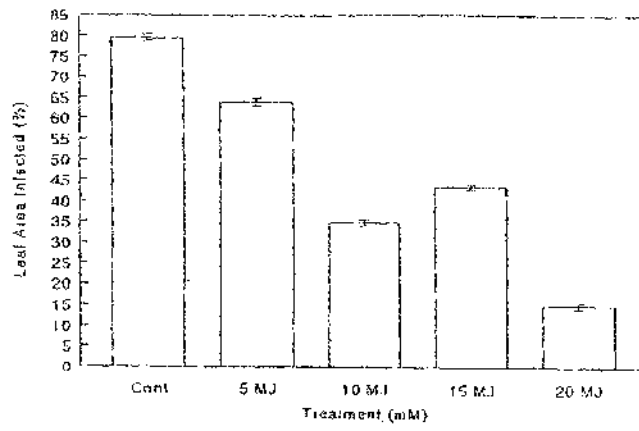


FIGURE 9. Effect of treating the lower leaf of barley with various concentrations of Chitosan on the development of systemic resistance in the second leaf following challenge-inoculation. Results shown are for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bars represent the standard error.

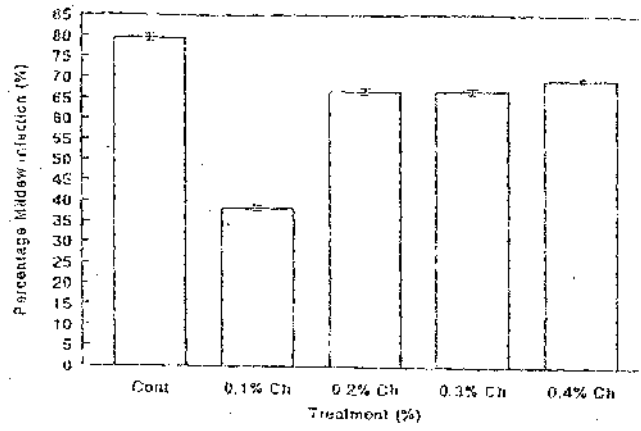


FIGURE 10. Effect of varying the time interval between treatment with abiotic elicitors and challenge-inoculation of the second leaf on the development of SAR on the second leaf. Results shown are for the final assessment of leaves 10 days post challenge-inoculation. Figures are the means of 10 replicates. Standard error of the difference from the control at $P \leq 0.05$, Sed (d.f. = 36) ± 1.023

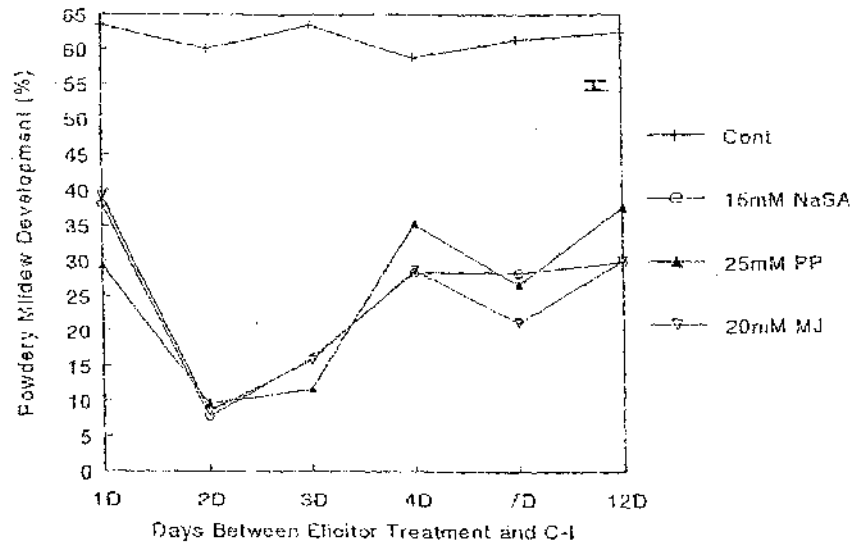


FIGURE 11. Effect of varying the photoperiod on the development of SAR in barley. Results shown are for the final assessment 10 days post challenge- inoculation. Figures are the means of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$, Sed, interaction (d.f. = 9, 144) ± 1.496

FIGURE 12. Effect of varying growth temperature on the development of SAR. Results shown are for the final assessment of leaves 10 days post challenge- inocuation. Figures are the means of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$, Sed, interaction (d.f. = 9, 144) ± 1.2229

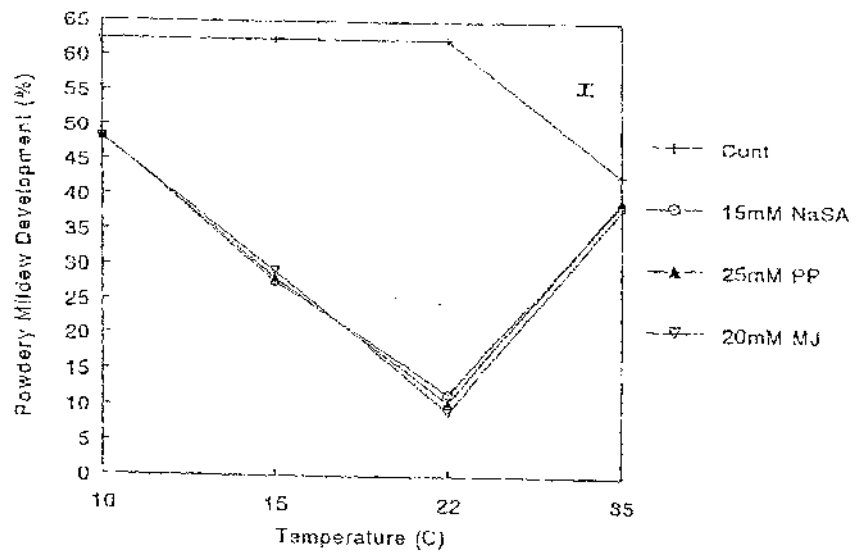
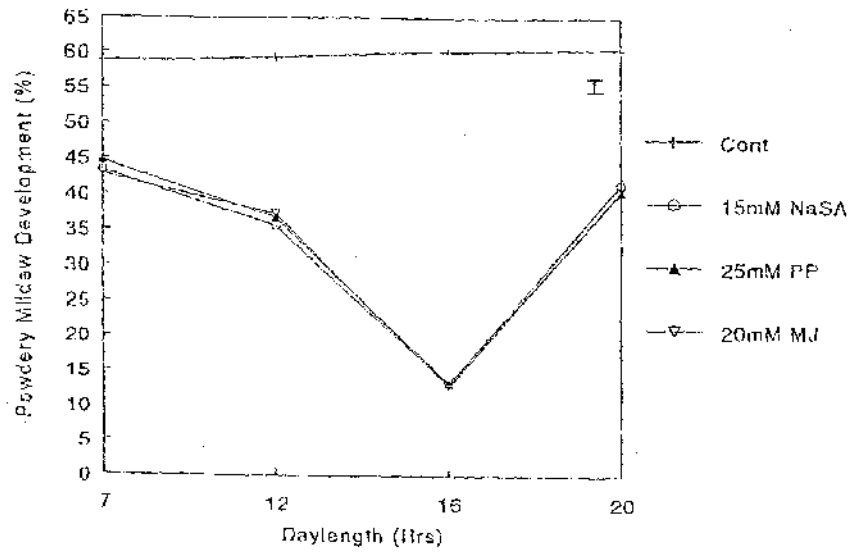


FIGURE 13. Effect of prolonging the time interval between elicitor treatment and challenge- inoculation on the subsequent development of SAR. Results are shown for the final assessment of the second leaf 10 days post challenge- inoculation. Figures are the means of 10 replicates. Standard error of the differences at $P \leq 0.05$, Sed, interaction (d.f. = 4,27) ± 1.48

FIGURE 14. Duration one elicitor treatment of the lower leaf remains effective at eliciting a systemic resistance response in the second leaf. Results shown are for the final assessment of leaves 10 days post challenge-inoculation. Figures are the means of 10 replicates. Standard error of the differences at $P \leq 0.05$, Sed, interaction (d.f. = 5,39) ± 2.26

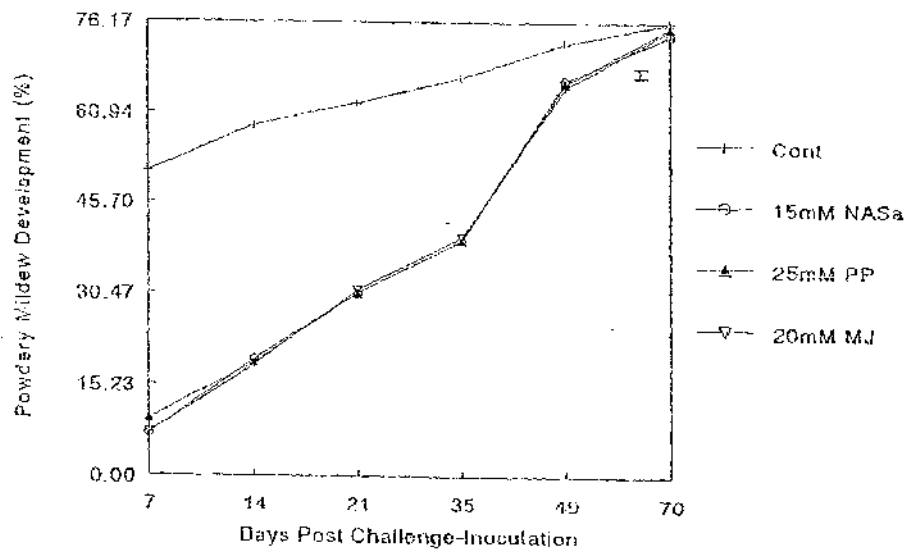
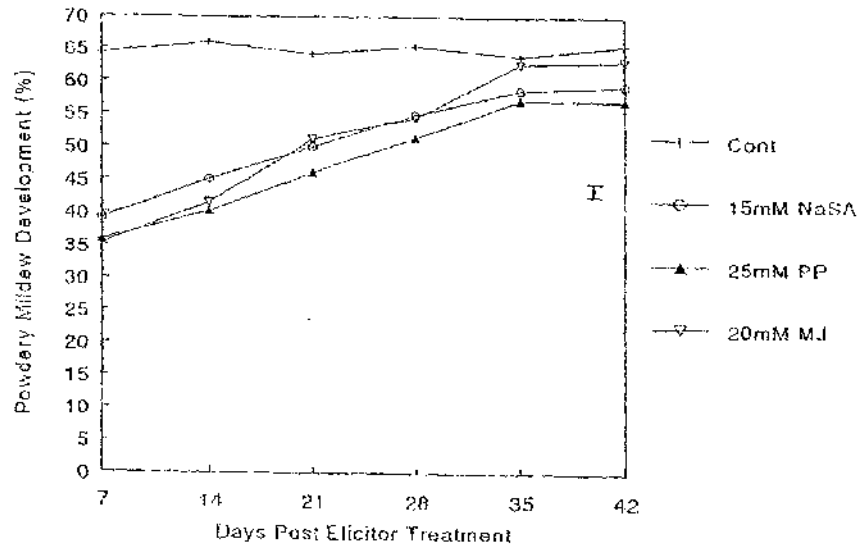


FIGURE 15. Effect of treating the three upper leaves of a plant with leaves on the development of SAR in the lower three leaves. Results are shown for the final assessment of the lower three leaves 10 days post challenge-inoculation. Figures are the means of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$, Sed, interaction (d.f. 6,108) = 1.494

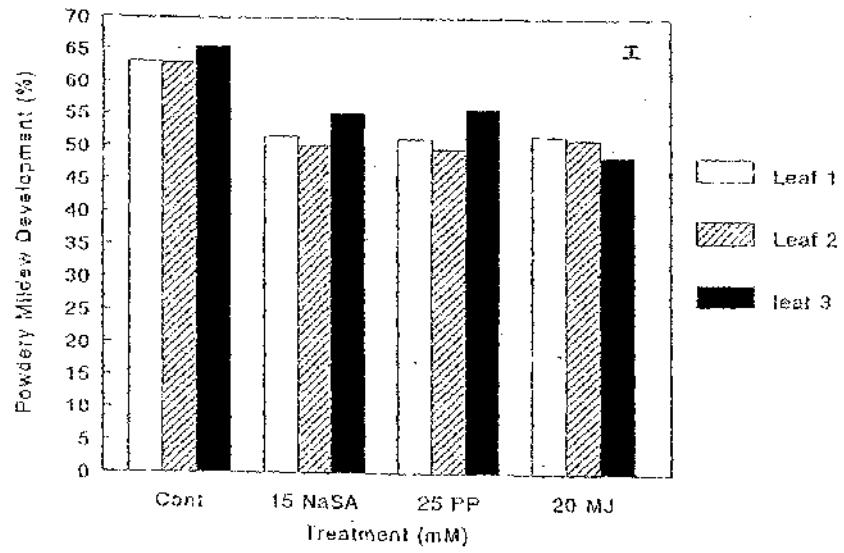


FIGURE 16. Effect of priming seed in elicitors of resistance for varying time periods on the development of systemic resistance in the germinated plant. Results shown are for the final assessment of leaves 10 days post challenge-inoculation. Vertical bars represent standard errors of the mean of 10 replicates.

FIGURE 17. Effect of applying the elicitors as root drenches at various stages pre- and post-inoculation on their ability to induce systemic resistance. Results shown are for the final assessment of leaves 10 days post challenge-inoculation. Vertical bar represents standard errors of the mean of 10 replicates.

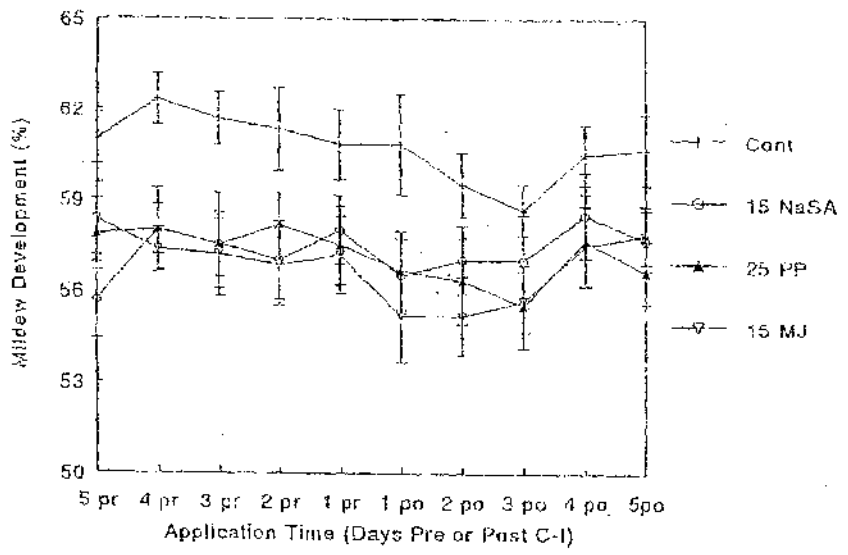
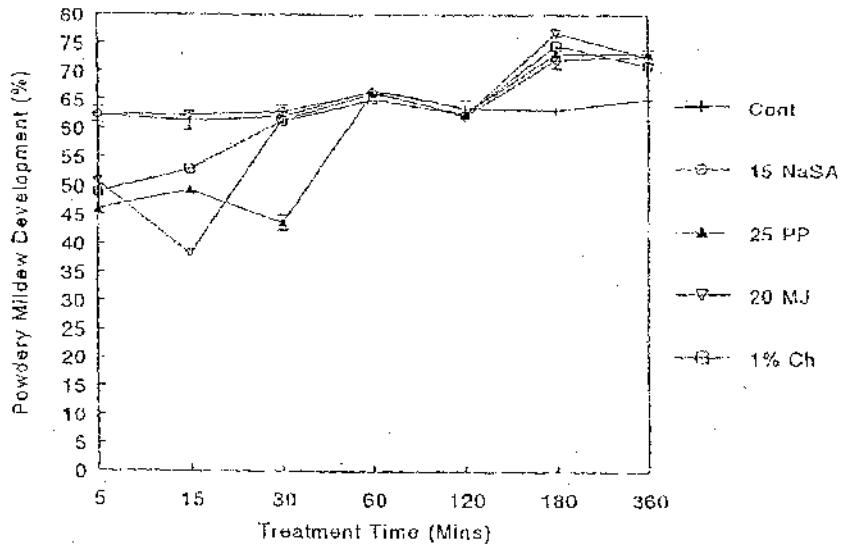


FIGURE 18. Direct effect of the abiotic inducing agents on powdery mildew development treatments applied using two methods; S/D/I- Spray/ Dry/ Inoculate, I/S- Inoculate/ Spray. Results shown are for the final assessment of leaves 10 days after spraying. Figures are the mean of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$, Sed, Interaction (d.f.3,72) ± 0.095 .

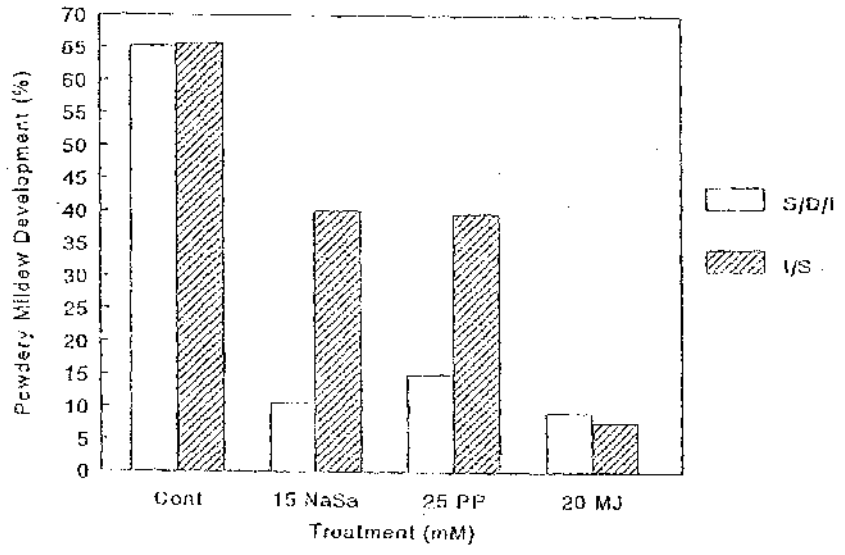


FIGURE 19. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent NaSA on, *in vitro*, radial growth of the pathogen *P. avenae*. Results shown are for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the mean of 9 replicates. Standard error of the differences from the control at $\underline{P} \leq 0.05$, Sed (d.f. = 8) ± 0.058

FIGURE 20. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent NaSA on, *in vitro*, radial growth of the pathogen *C. perniciosus*. Results shown are for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the difference from the control at $\underline{P} \leq 0.05$, Sed (d.f. = 8) ± 0.058

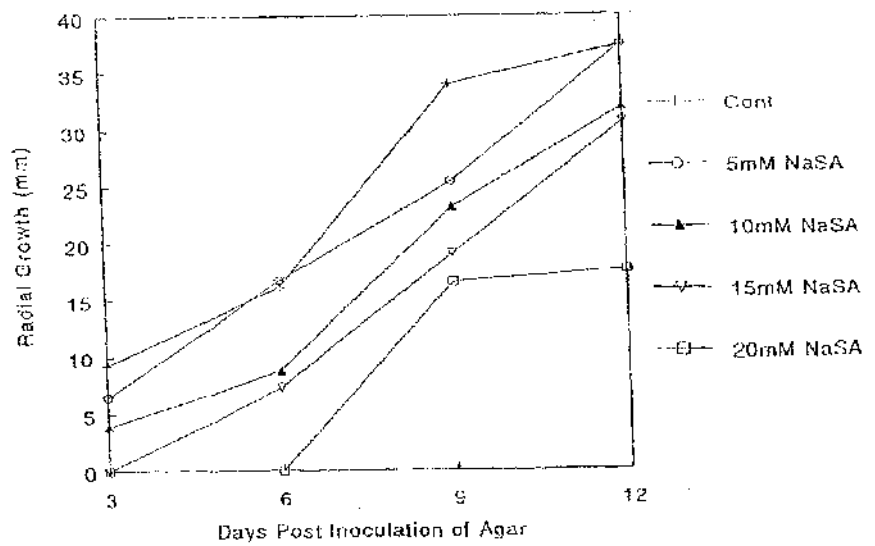
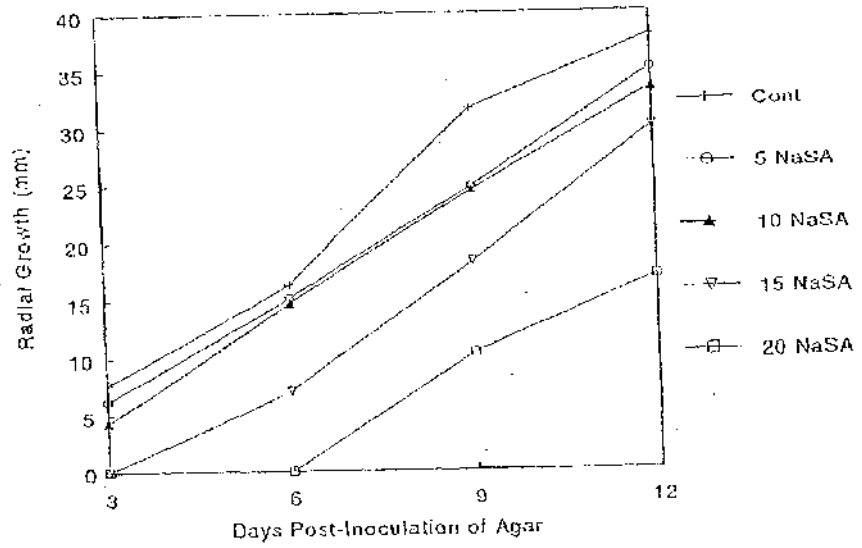


FIGURE 21. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent PP on, *in vitro*, radial growth of the pathogen *P. avenae*. Results are shown for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$, Sed (d.f. = 8) ± 0.058

FIGURE 22. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent PP on, *in vitro*, radial growth of the pathogen *C. perniciosus*. Results shown are for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the difference from the control at $P \leq 0.05$, Sed(d.f. = 8) ± 0.40

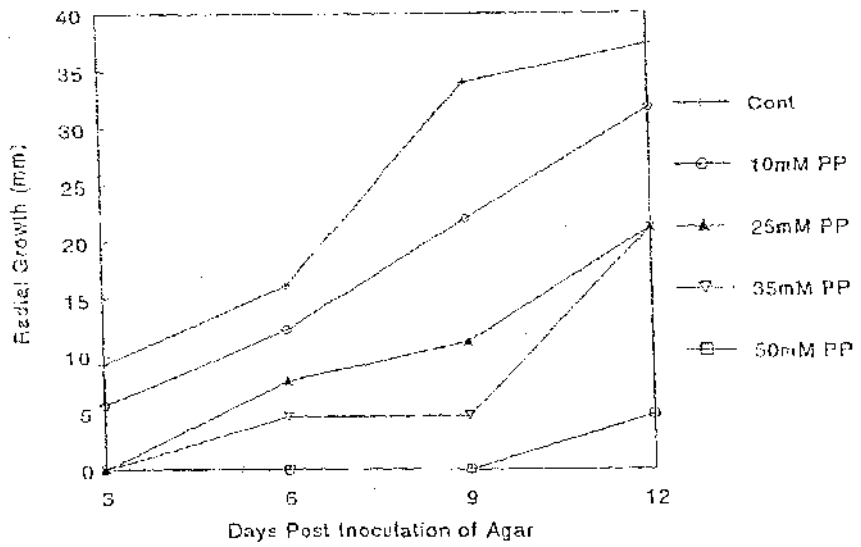
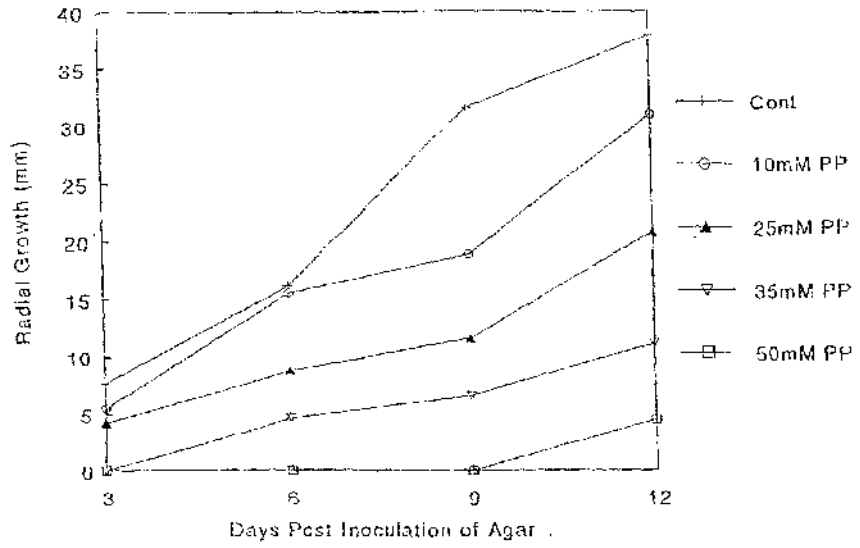
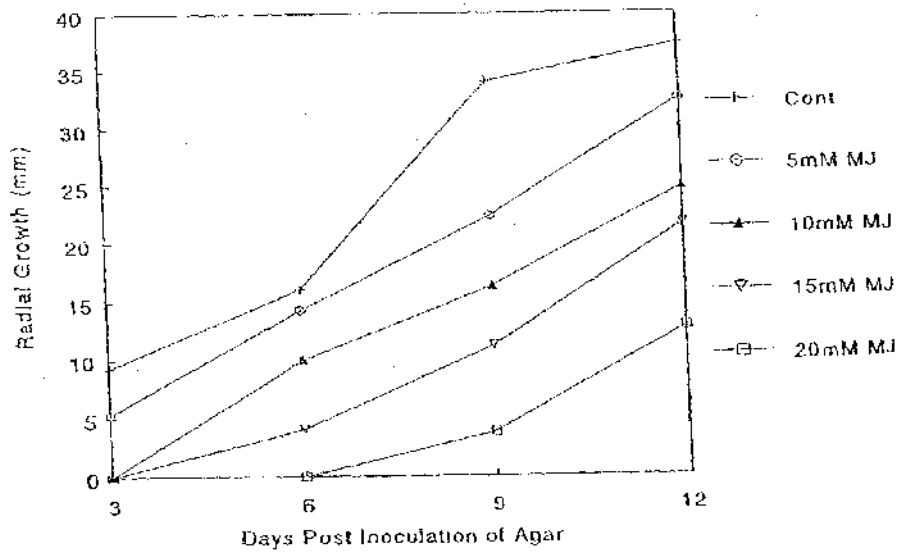
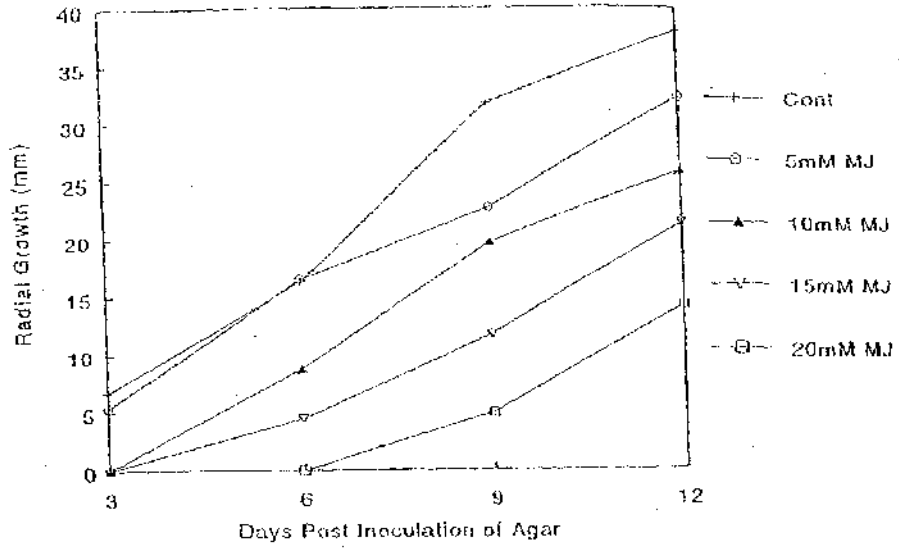


FIGURE 23. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent MJ on, *in vitro*, radial growth of the pathogen *P.avenae*. Results shown are for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the difference from the control at $P \leq 0.05$, Sed (d.f. = 8) ± 0.040

FIGURE 24. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent MJ on, *in vitro*, radial growth of the pathogen *C. perniciosa*. Results are shown for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the difference from the control at $P \leq 0.05$, Sed (d.f = 8) ± 0.040



3.2 *Effect of Methyljasmonate Vapour*

Methyljasmonate vapour was examined as a potential elicitor of systemic resistance. Both the vapour from a 20mM solution of MJ and the vapour from undiluted MJ significantly reduced powdery mildew infection (fig 27). Both treatments gave the largest reductions following a 30 minute exposure time, although the reduction obtained with the undiluted vapour was the highest (93%) (Fig 27). All time intervals examined between exposure to MJ vapour and challenge-inoculation yielded significant reductions in the level of powdery mildew observed (60- 94%) (fig 28). The largest reduction in powdery mildew infection (94%) was found in plants treated with undiluted MJ and was found if 1 day was left between treatment and challenge-inoculation (Fig 28). Exposure of *E. graminis* conidia on the leaf surface, to vapour of either a 20mM solution of MJ or to the vapour from undiluted MJ gave significant reductions in the development of powdery mildew (Fig 29). The largest reductions were obtained with exposure to vapour from undiluted MJ, (62%). Exposure to *E. graminis* conidia, *in vitro*, on tap water agar plates, to the vapour from either 20mM MJ or from undiluted MJ, led to significant reduction in spore germination (fig 30). Reductions of 44% and 70%, respectively, were observed.

3.3 *Effect of Two Methyljasmonate Precursors*

3.3.1 *Screening of Linoleic and Linolenic Acid*

Linoleic acid, a precursor of MJ, gave no significant reductions in mildew development when examined as a potential elicitor of systemic resistance (fig 31). Linolenic acid, also a precursor of MJ, gave significant reductions in mildew development when examined as a potential elicitor of systemic resistance (fig 32). Reductions in mildew infection of up to

90% were observed. A range of concentrations of Linoleic and Linolenic acid were all found to significantly reduce the radial growth of the pathogens *P. avenae* and *C. pernicioso* grown on amended PDA plates (fig 33-36). With both chemicals the highest concentrations exhibited the largest inhibitory effect and the lowest concentration had least effect on growth. The same range of chemicals also gave significant reductions in the growth of *P. avenae* and *C. pernicioso* in amended PDB (fig 37-40). Only the lowest concentrations used, 0.1mM Linoleic Acid and 0.1mM Linolenic acid, supported pathogen growth. No growth was recorded in any of the higher concentrations examined (fig 37-40).

FIGURE 25. Effect of amending potato dextrose broth the abiotic inducing agents 5mM NaSA 10mM PP or 5mM MJ on biomass productivity of the pathogen *P. avenae*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$ Sed (d.f. = 2) ± 0.027

FIGURE 26. Effect of amending potato dextrose broth the abiotic inducing agents 5mM NaSA, 10mM PP or 5mM MJ on biomass productivity of the pathogen *C. perniciosus*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$, Sed (d.f. = 2) ± 0.027

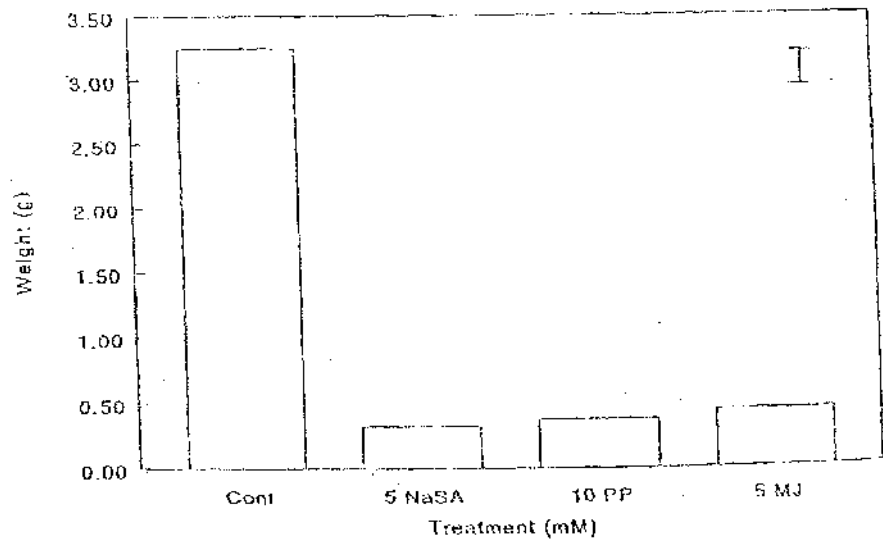
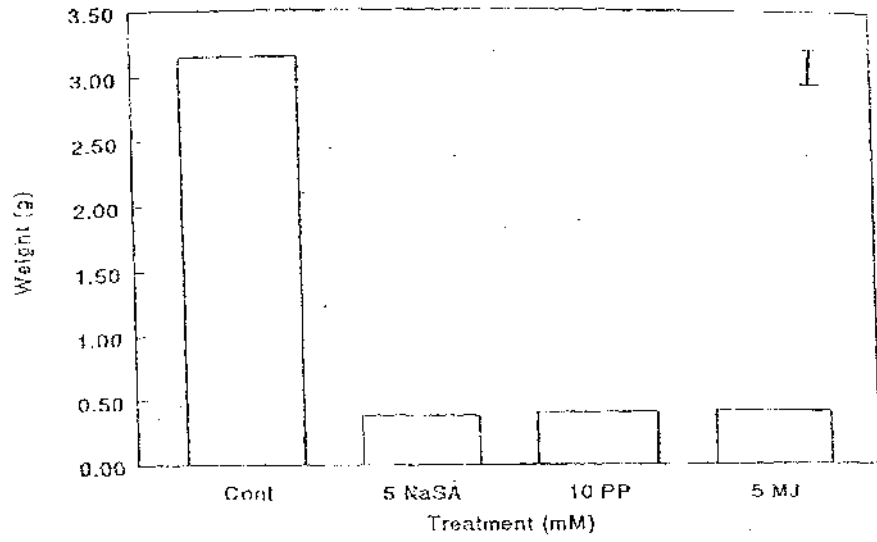


FIGURE 27. Effect of exposing the lower leaf of barley to the vapour from undiluted MJ for various time intervals on the development of SAR in the second leaf. Results are shown for the final assessment of leaves 10 days post challenge-inoculation. Figures are the means of 10 replicates. Standard error of the difference for the interaction at $P \leq 0.05$, Sed (d.f. 8, 135) ± 2.04

FIGURE 28. Effect of varying the time interval between treatment of the lower leaf with MJ vapour and subsequent challenge-inoculation of the lower leaf of barley on the development of SAR in the second leaf. Results are shown for the final assessment of the leaves 10 days post challenge-inoculation. Figures are the means of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$, Sed, interaction (d.f. 5,89) ± 1.64

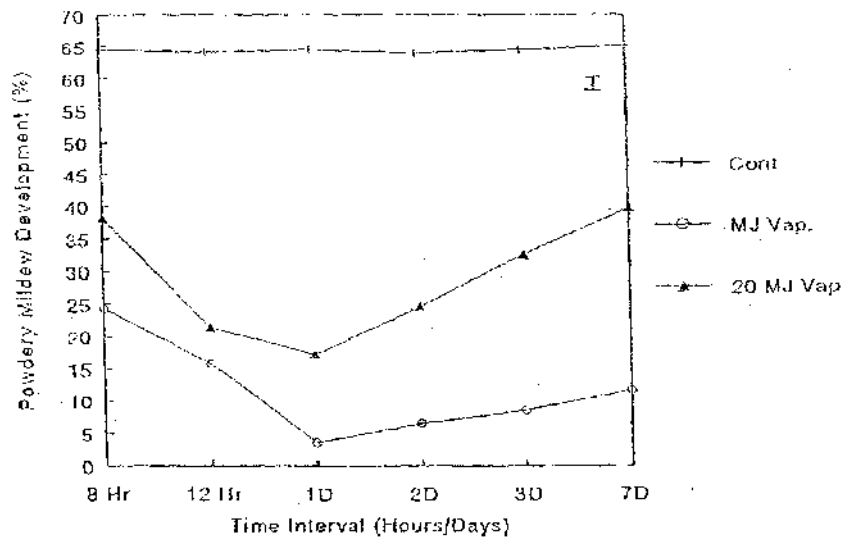
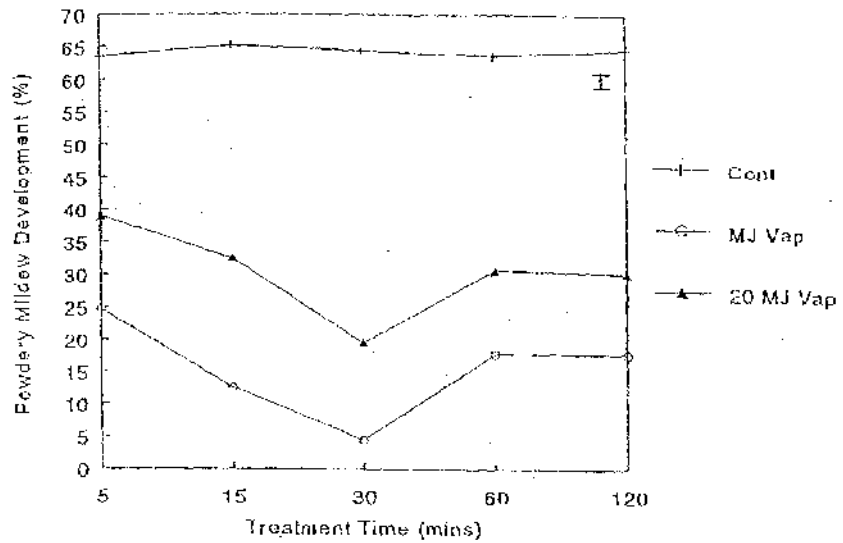


FIGURE 29. Effect of directly exposing *E. graminis* conidia on the leaf surface of barley to the vapour of either 20mM MJ solution or the vapour from undiluted MJ vapour on the development of powdery mildew. Results are shown for the final assessment of leaves 10 days after exposure to the vapour. Figures are the means of 10 replicates. Standard error of the differences from the control at $P \leq 0.05$, $Sed(d.f. = 9) \pm 1.67$

FIGURE 30. Effect of exposing *E. graminis* conidia, *in vitro*, to either vapour from a 15mM solution of MJ or from undiluted MJ, on the germination of the conidia. Results shown are for the percentage germination 24 hours after exposure to the vapour. Figures are the means of 12 replicates. Standard error of the difference from the control at $P \leq 0.05$. $Sed(d.f. = 11) \pm 1.04$

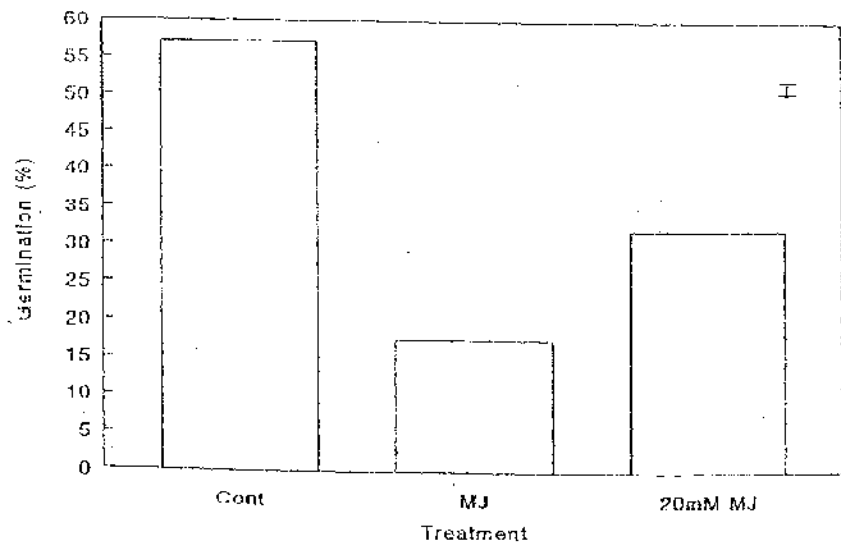
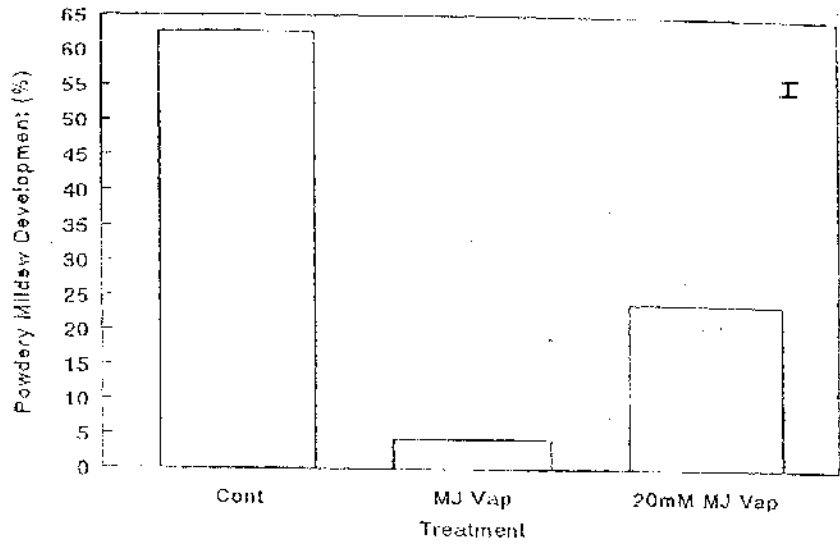


FIGURE 31. Effect of applying various concentrations of Linoleic acid, a precursor of MJ, to the lower leaf of barley on the development of SAR in the second leaf. Results shown are for the final assessment 10 days post-challenge-inoculation. Figures are the means of 10 replicates. Standard error of the differences for the interaction at $P \leq 0.05$. Sed (d.f. 6,108) ± 2.56

FIGURE 32. Effect of applying various concentrations of Linolenic acid, a precursor of MJ, on the development of SAR in the second leaf. Results are shown for the final assessment 10 days post challenge-inoculation. Figures are the means of 10 replicates. Vertical bar represents the standard error of the differences for the interaction at $P \leq 0.05$. Sed (d.f. 6,108) ± 2.56

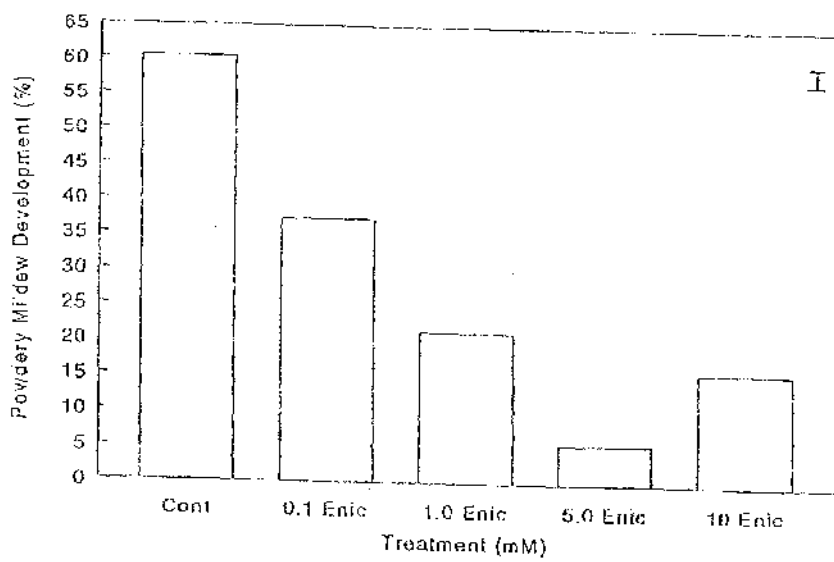
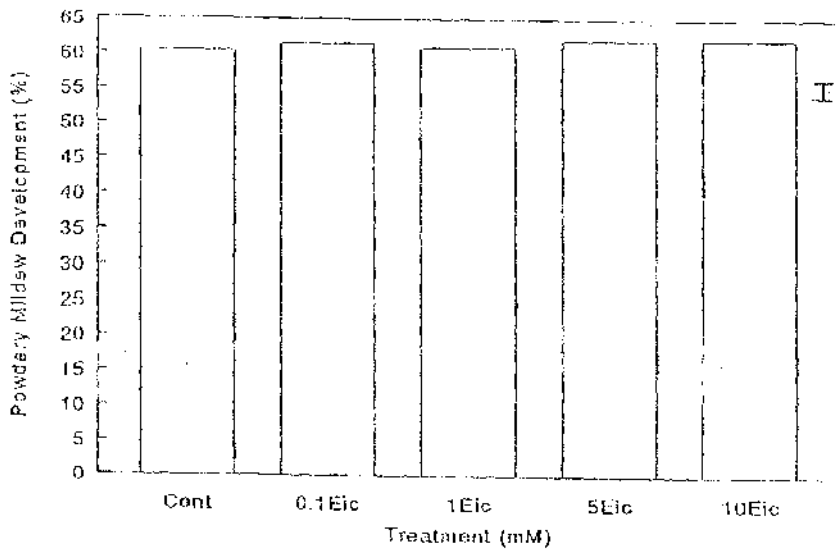


FIGURE 33. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent Linoleic acid on the radial growth, *in vitro*, of the pathogen *P.avenae*. Results shown are for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Vertical bar represents the standard error of the differences from the control at $P \leq 0.05$. Sed (d.f.8) ± 0.027

FIGURE 34. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent Linoleic acid on the radial growth, *in vitro*, of the pathogen *C. perniciosa*. Results are shown for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. Sed (d.f. 8) ± 0.027

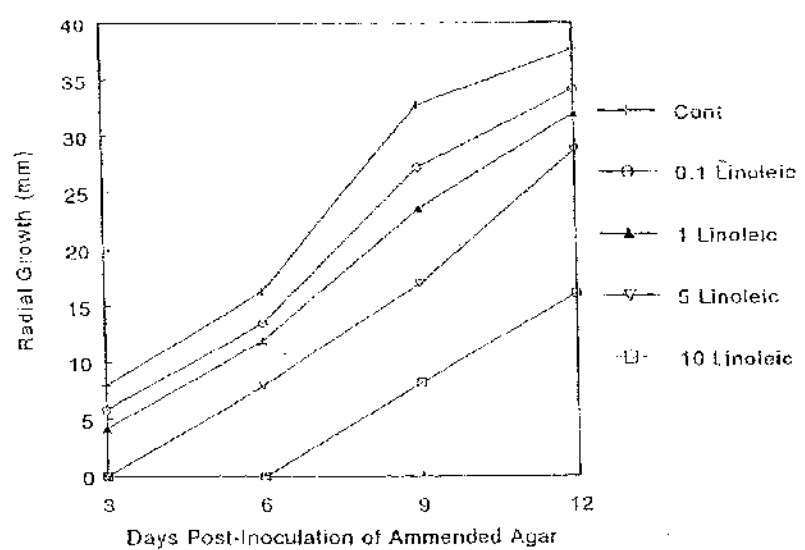
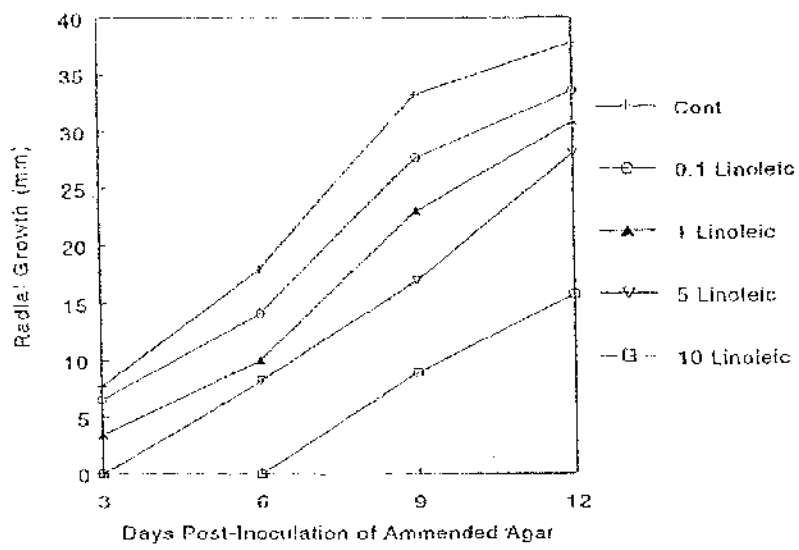


FIGURE 35. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent linolenic acid on the radial growth, *in vitro*, of the pathogen *P. avenae*. Results shown are for the radial growth measured at three day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. $Sed(d.f. = 8) \pm 0.028$

FIGURE 36. Effect of amending potato dextrose agar with various concentrations of the abiotic inducing agent linolenic acid on the radial growth, *in vitro*, of the pathogen *C. perniciosus*. Results are shown for the radial growth measured at 3 day intervals over a period of 12 days. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. $Sed(d.f. = 8) \pm 0.028$

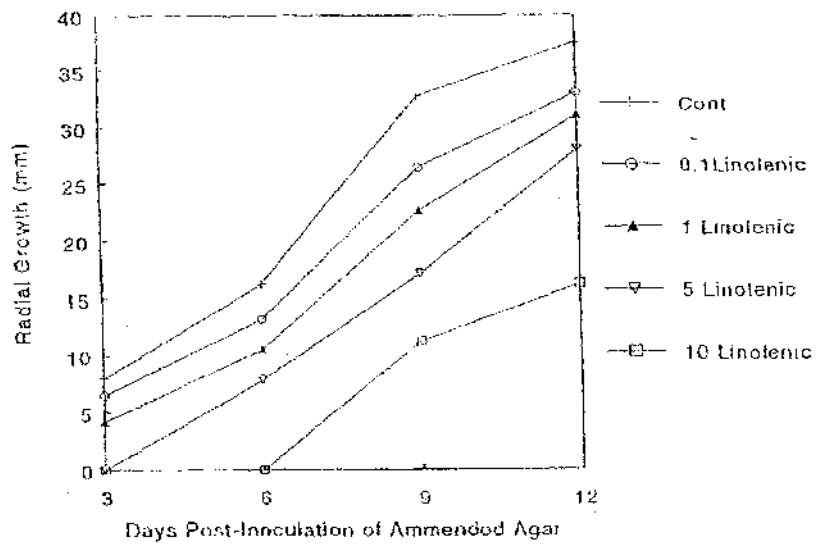
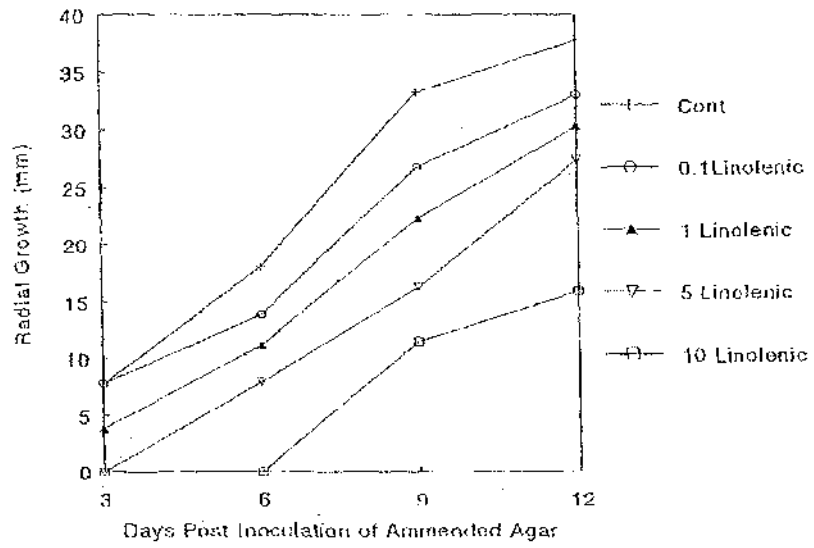


FIGURE 37. Effect of amending potato dextrose broth with various concentrations of Linoleic acid on the biomass productivity of the pathogen *P. avenae*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. $S_{cd} (d.f. = 8) \pm 0.027$

FIGURE 38. Effect of amending potato dextrose broth with various concentrations of Linoleic acid on biomass productivity of the pathogen *C. perniciosa*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. $S_{cd} (d.f. = 8) \pm 0.027$

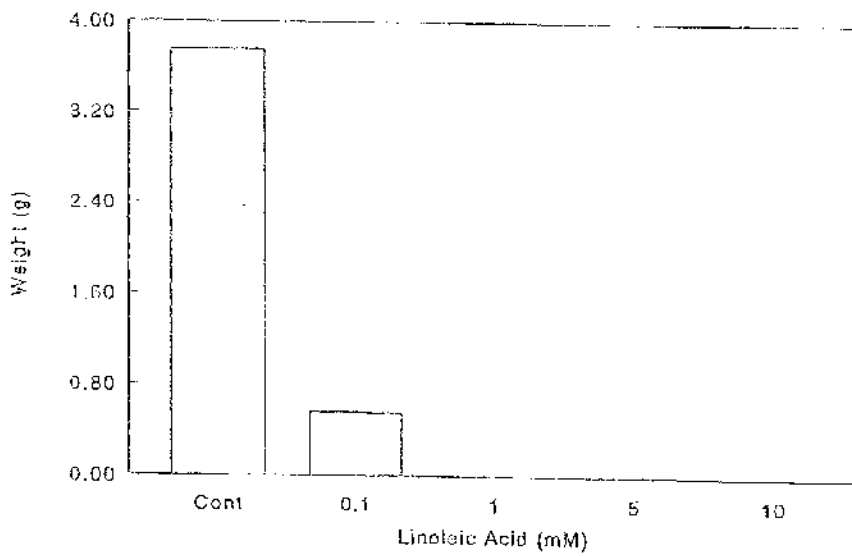
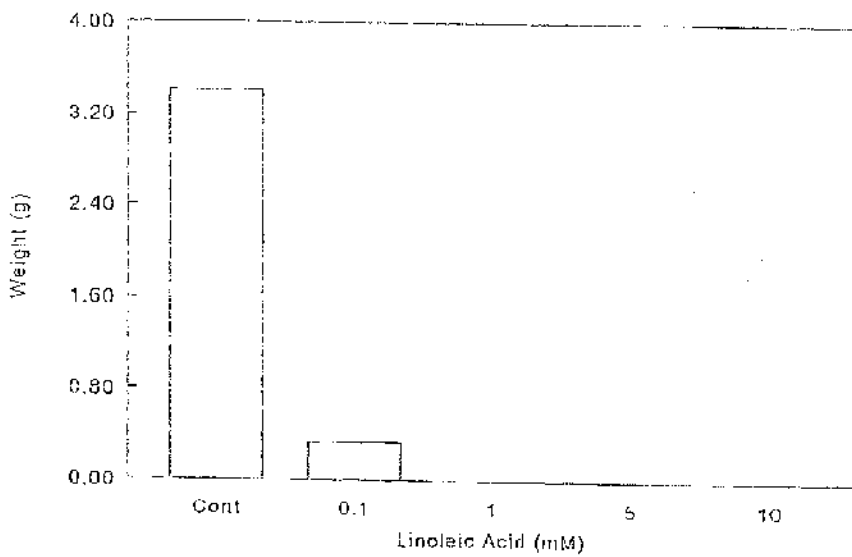
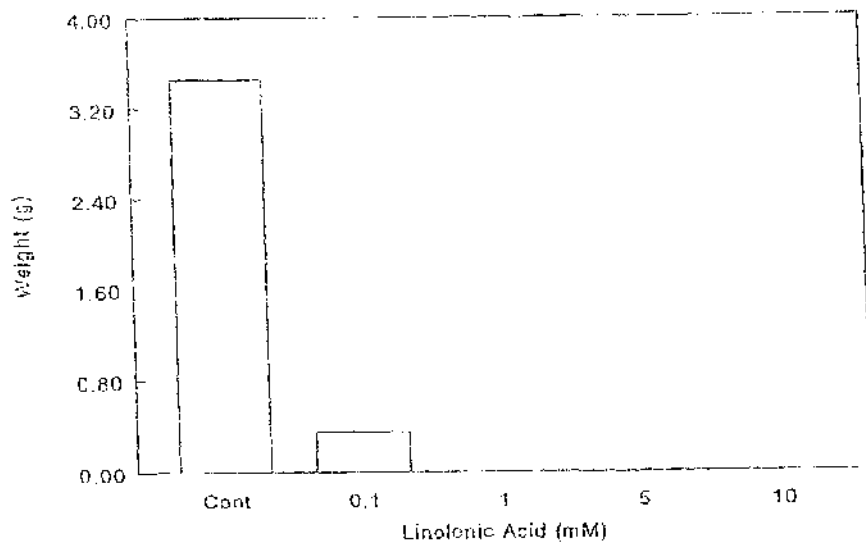
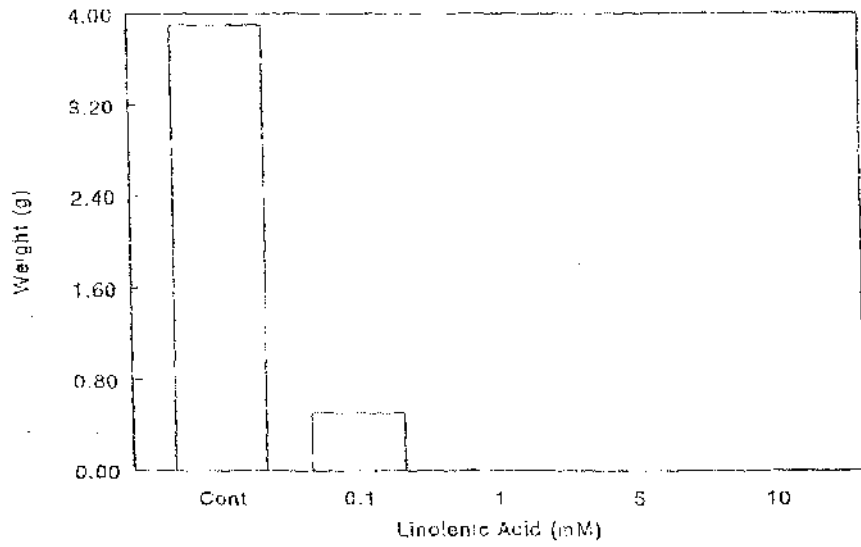


FIGURE 39. Effect of amending potato dextrose broth with various concentrations of the abiotic inducing agent Linolenic acid on the growth, *in vitro*, of the pathogen *P. avenae*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. Sed (d.f. = 8) ± 0.027

FIGURE 40. Effect of amending potato dextrose broth with various concentrations of the abiotic inducing agent Linolenic acid on the growth, *in vitro*, of the pathogen *C. perniciosus*. Results are shown for the weight of growth measured 10 days after inoculation of the broth. Figures are the means of 9 replicates. Standard error of the differences from the control at $P \leq 0.05$. Sed (d.f. = 8) ± 0.027



3.4 *Growth Analysis*

3.4.1 *Dry Weights*

Growth analysis was performed on plants treated with elicitors, and plants treated with elicitors of resistance and then challenge-inoculated with mildew, over an eight week period. In the experiment the lower leaf was treated with the elicitor and the upper leaf challenge-inoculated 2 days later. In all treatments there was a steady increase in the shoot dry weight over the experiment period (fig 41). Plants treated with 25mM PP consistently produced the highest dry weights. Plants treated with 15mM NaSA or 20mM MJ followed the growth curve of the control more closely, but were consistently heavier throughout the trial. Plants treated with elicitors of resistance and then challenge-inoculated showed a steady increase in shoot weight over the eight week period (fig 42). Plants treated with 25mM PP yielded the highest dry weight. Weeks 1 and 2 were the only points where the dry weights for the other 2 treatments, 15mM NaSA and 20mM MJ, were lower than the control (fig 42).

There was a steady increase in root dry weight for plants in all treatments over the experimental period (fig 43). With the exception of week 6, plants treated with 25mM PP had slightly higher root dry weights than the control. Root dry weights for plants treated with 15mM NaSA and 20mM MJ were little different from the control values over the 8 week period. For plants treated with elicitors and then challenge-inoculated with mildew, root dry weight increased steadily in all treatments over the experimental period. No differences were observed between the control and any of the treatments (fig 44).

3.4.2 *Leaf Area*

Analysis of growth, over an 8 week period, of plants treated with elicitors of resistance showed a steady increase in leaf area (fig 45). Leaf area of plants treated with 15mM NaSA and 25mM PP were similar to control plants over the experimental period. Leaf area of methyljasmonate treated plants was similar to the control for the first three weeks of the experiment but were substantially reduced thereafter (fig 45). Leaf area of plants treated with abiotic elicitors of resistance then challenge-inoculated showed a steady increase over the period of the study. Leaf area of plants treated with NaSA and PP were similar to the control, but after week 2, leaf area of plants treated with MJ were substantially reduced compared to the control (fig 46).

3.4.3 *Leaf Weight Ratio*

Leaf weight ratios (LWR) of plants treated with abiotic elicitors of resistance increased slightly at several points over the experimental period (fig 47). Thus, with the exception of 15mM NaSA at week 1, 25mM PP at week 1 and 3 and 20mM MJ at week 5, elicitor treated plants had higher LWRs than control plants (fig 47). LWRs of plants treated with abiotic elicitors of resistance then challenge-inoculated fluctuated a great deal throughout the period of the study (fig 48). Consistency was observed however in the LWR of plants treated with MJ and then challenge-inoculated. However, LWR was greater in treated plants compared to controls from week 2 onwards (fig 48).

3.4.4 *Leaf Area Ratio*

Leaf area Ratios (LAR) of all plants receiving elicitor treatment were reduced compared to the control (fig 49). The greatest reduction in LAR was obtained with MJ treated plants, where LAR was reduced by approximately 35% compared to the control by the end of the experiment (fig 49). In plants treated with MJ and then challenge-inoculated, LARs were considerably lower than in the controls throughout the experiment (fig 50), with smaller reductions in LAR being observed with other treatments (fig 50).

3.4.5 *Specific Leaf Area*

Specific leaf area (SLA) of all plants receiving treatments were reduced compared with the control (fig 51), although those reductions were very small in plants treated with NaSA, from week 4 onwards. Treatment with MJ led to the greatest reduction in SLA (fig 51). In plants treated with NaSA and PP, SLAs fluctuated considerably over the experimental period, where in plants treated with MJ, SLA was consistently and substantially lower than control values throughout the experiment (fig 52).

3.4.6 *Relative Growth Rate*

In all treatments relative growth rate (RGR) declined throughout the course of the experiment. Differences between the control and treatments was small and not consistent over the 8 week period (fig 53). A similar pattern was observed for plants treated with elicitors and then challenge-inoculated, although RGRs were substantially reduced between weeks 2 and 3 in plants treated with MJ and PP (fig 54).

3.4.7 *Net Assimilation Rate*

Net assimilation rate (NAR) declined in all plants over the experimental period (figs 55-56). Any differences between the control and treatments were small and inconsistant over the 8 week period (figs 55-56).

FIGURE 41. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results are shown for the dry weight of the shoots measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 42. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results are shown for the dry weight of the shoots measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

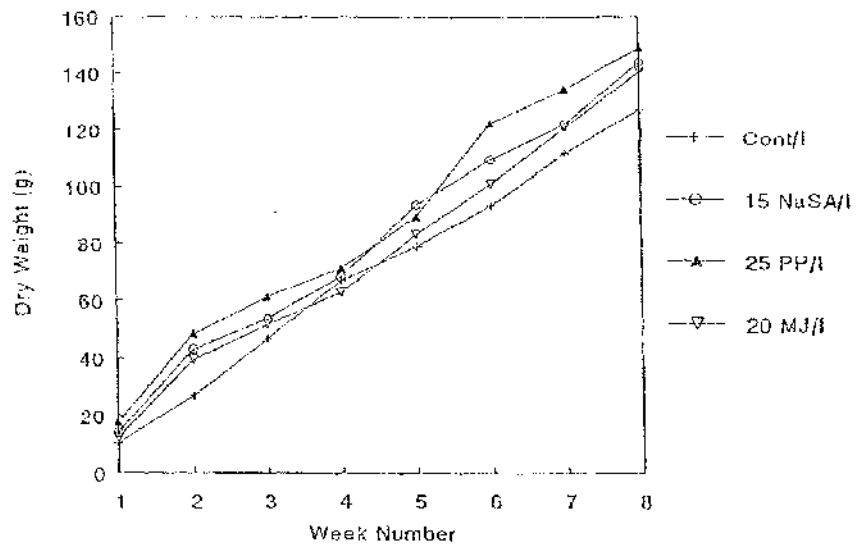
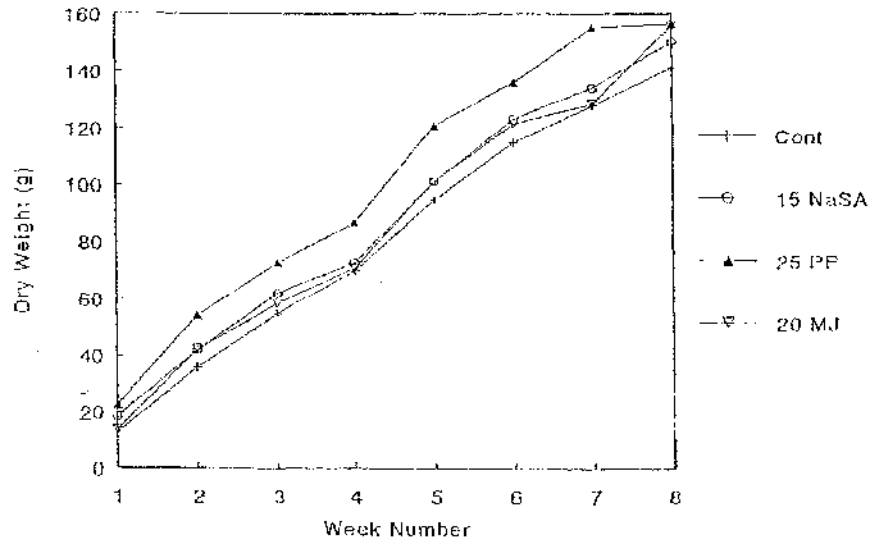


FIGURE 43. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results are shown for the dry weight of the roots measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 44. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results are shown for the dry weight of the roots measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

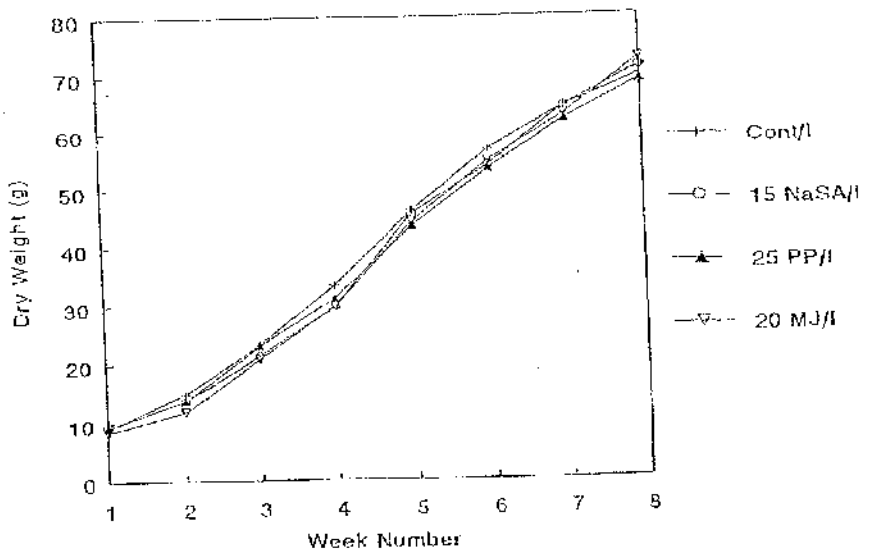
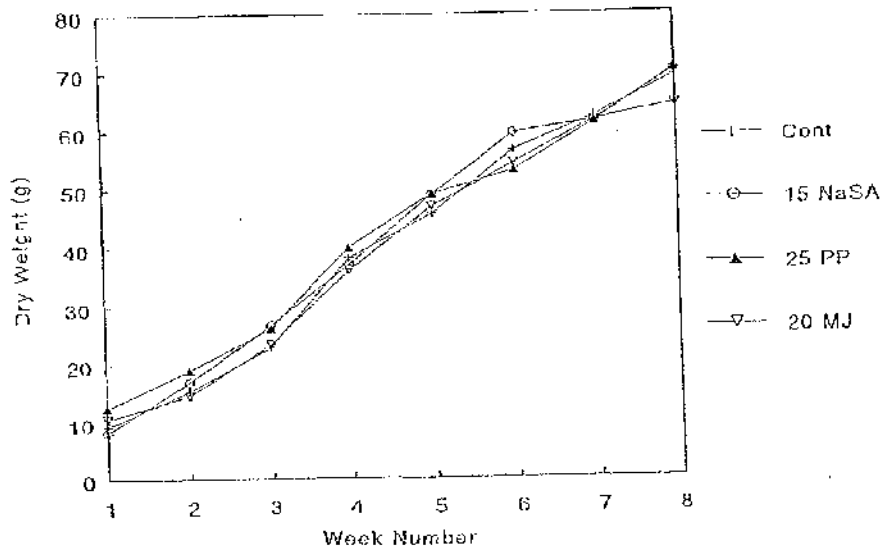


FIGURE 45. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results are shown for the leaf area (cm^{-2}) measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 46. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results are shown for the leaf area (cm^{-2}) measured at 7 day intervals over a period of 8 weeks. Figures are the means of 10 replicates.

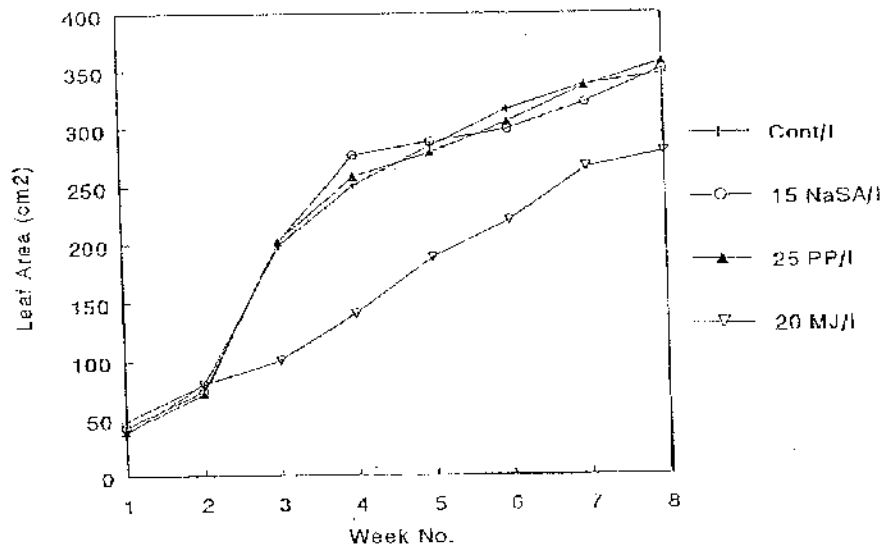
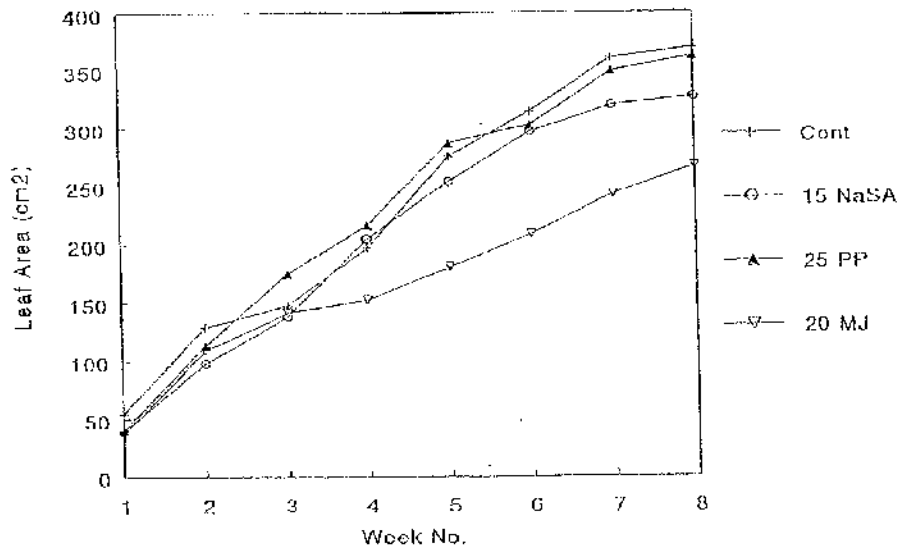


FIGURE 47. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results shown are for the leaf weight ratio measured over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 48. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results shown are for the leaf weight ratio measured over a period of 8 weeks. Figures are the means of 10 replicates.

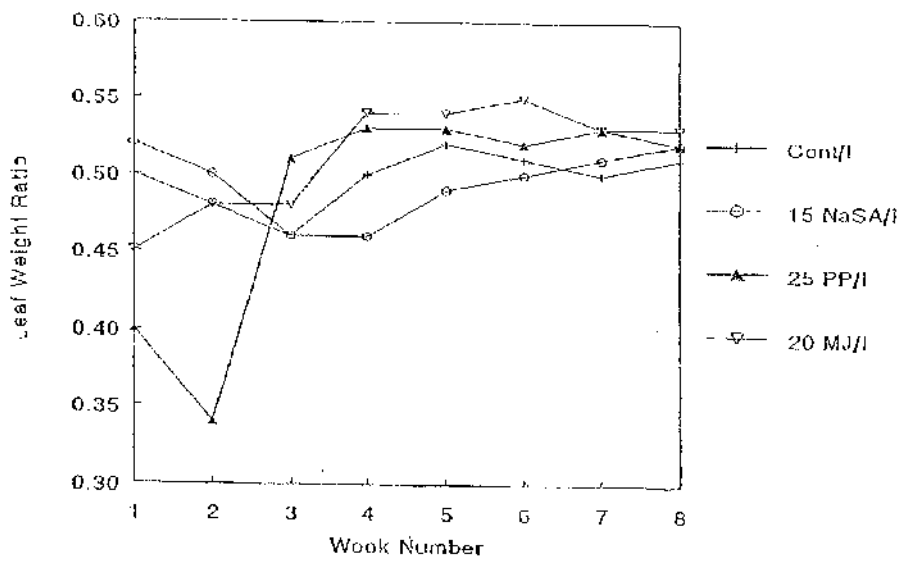
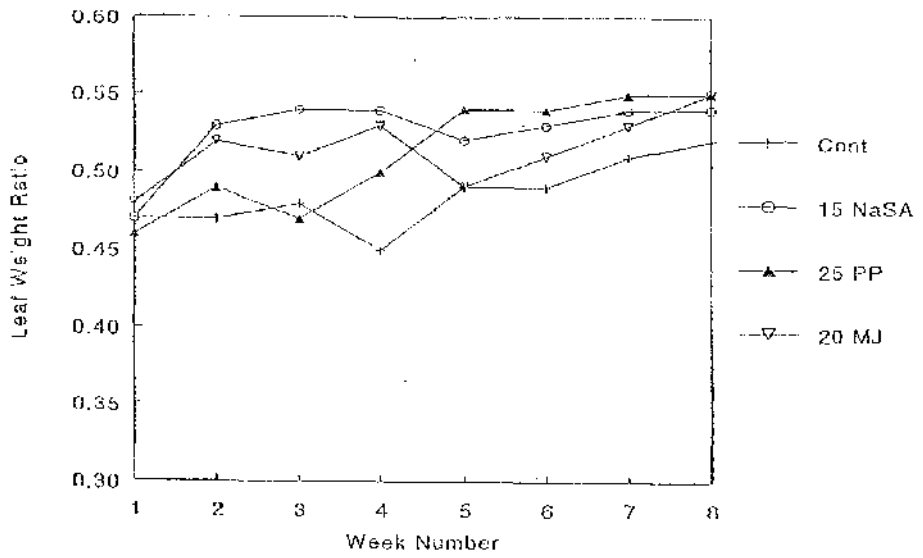


FIGURE 49. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results shown are for the leaf area ratios measured over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 50. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results shown are for the leaf area ratios measured over a period of 8 weeks. Figures are the means of 10 replicates.

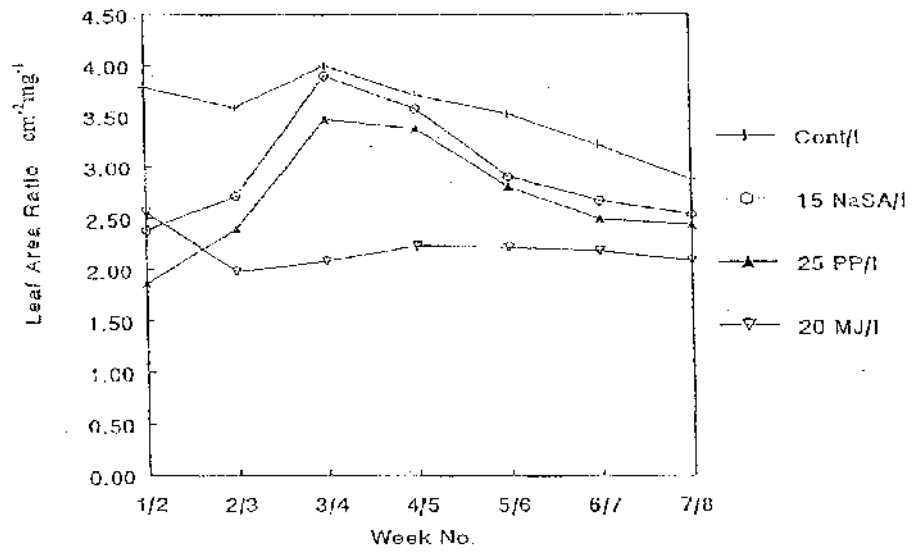
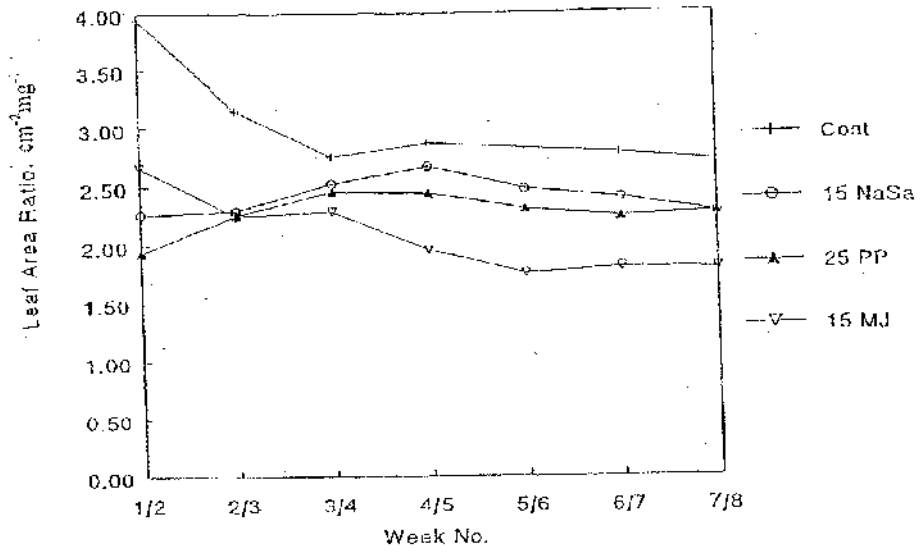


FIGURE 51. Effect of treating the lower leaf of barley with abiotic elicitors of resistance on the growth functions of the plants. Results shown are for the specific leaf area measured over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 52. Effect of treating the lower leaf of barley with abiotic elicitors of resistance, followed 2 days later by challenge-inoculation, on the growth functions of the plants. Results shown are for the specific leaf area measured over a period of 8 weeks. Figures are the means of 10 replicates.

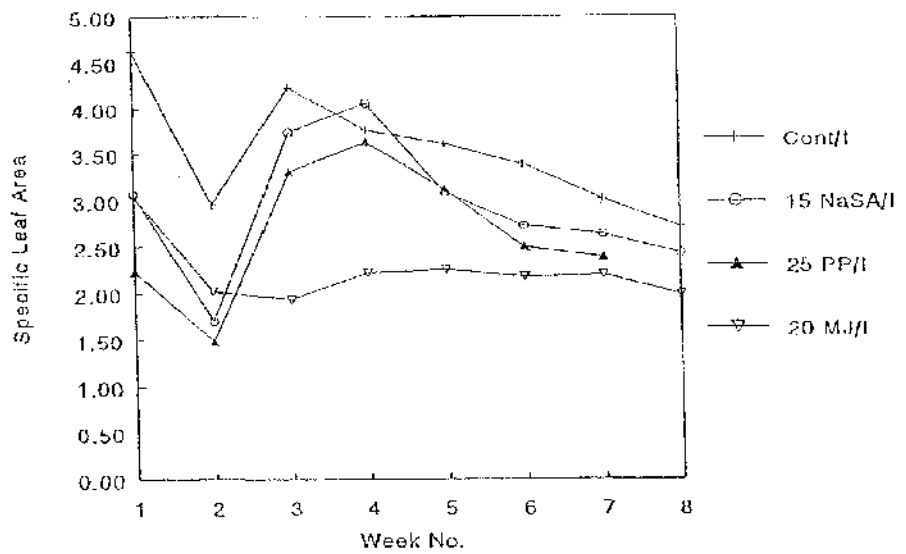
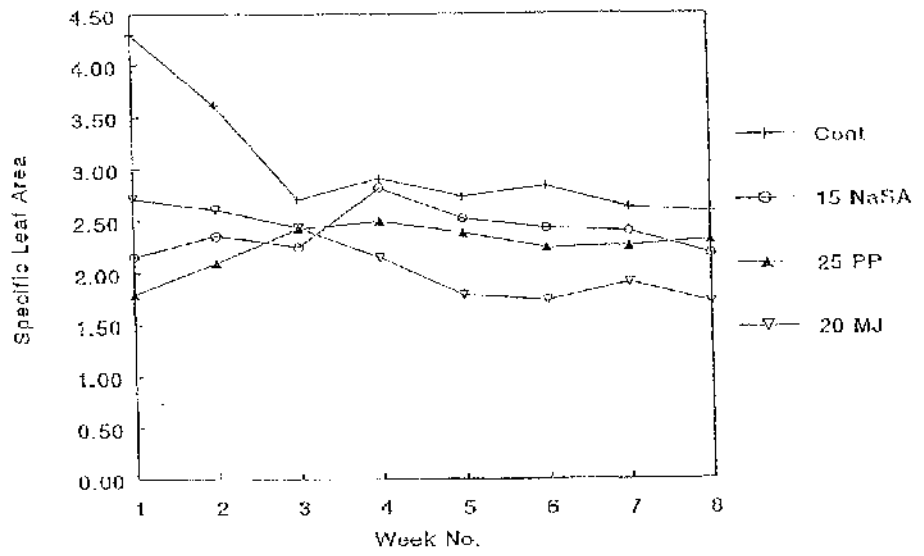


FIGURE 53. Effect of treating the lower leaf of barley with abiotic elicitors of resistance on the growth functions of the plants. Results shown are for the relative growth rate measured over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 54. Effect of treating the lower leaf of barley with abiotic elicitors of resistance, followed 2 days later by challenge-inoculation, on the growth functions of the plants. Results shown are for the relative growth rate measured over a period of 8 weeks. Figures are the means of 10 replicates.

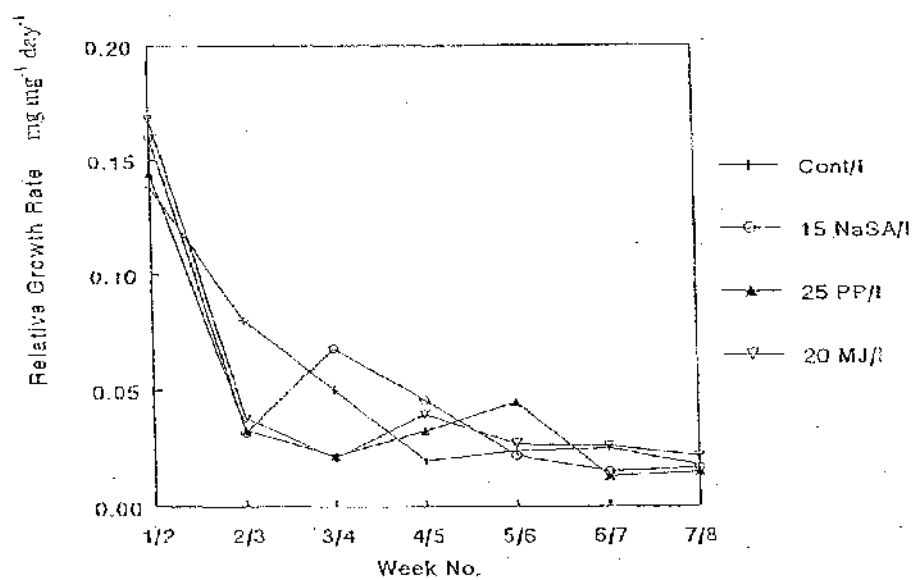
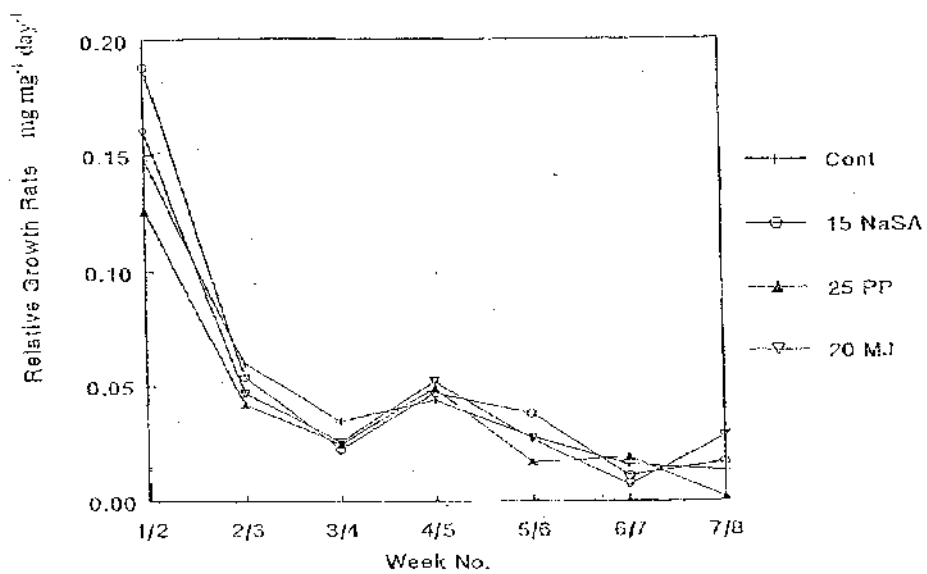
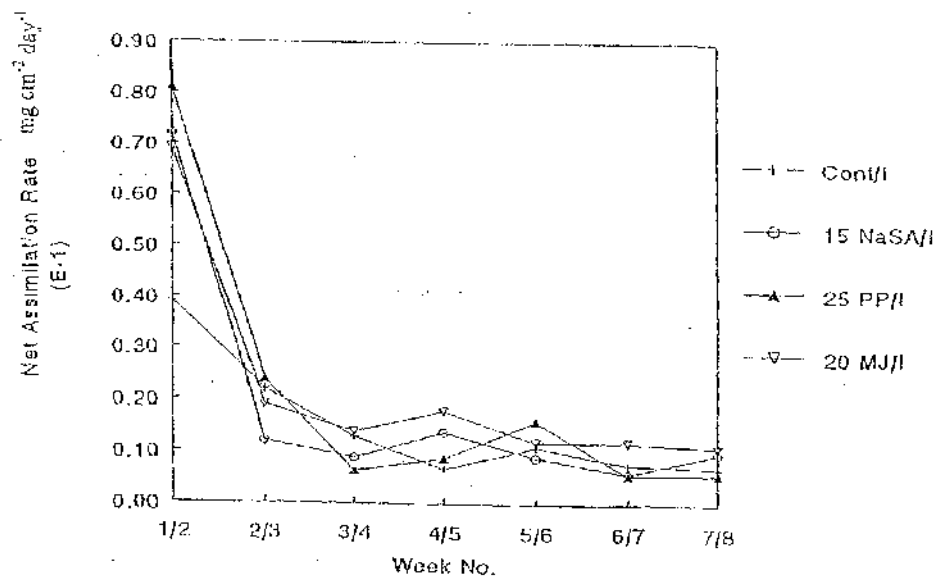
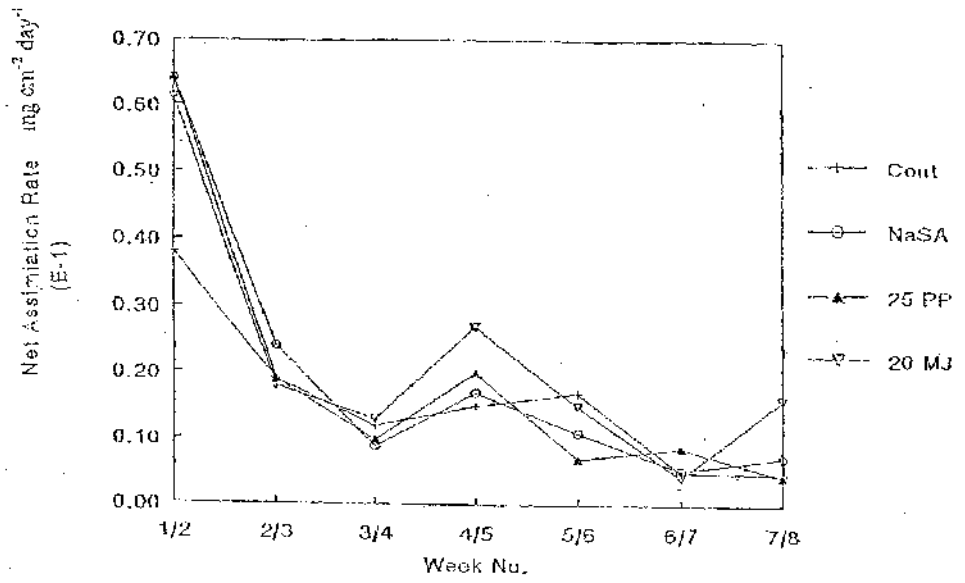


FIGURE 55. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance on the growth functions of the plants. Results shown are for the net assimilation rate measured over a period of 8 weeks. Figures are the means of 10 replicates.

FIGURE 56. Effect of treating the lower leaf of barley with abiotic elicitors of systemic resistance, followed 2 days later by challenge-inoculation of the upper leaf, on the growth functions of the plants. Results shown are for the net assimilation rate measured over a period of 8 weeks. Figures are the means of 10 replicates.



3.5 *Enzyme Activity*

3.5.1 *PAL Activity*

Treatment of the first leaves with NaSA led to a very substantial increase in PAL activity in that leaf and in the second and third leaves (Fig 57). The greatest increase in PAL activity was observed in leaf 2 just 1 day after treatment of leaf 1 with NaSA. There was a steady decline in PAL activity in this leaf with time after treatment, but by 12 days after treatment enzyme activity was still more than twice the values observed in the control (Fig 57). PAL activity in the first leaf was consistently greater in leaves 2 and 3, in treated plants, for 3 days after treatment (Fig 57). Treatment of the first leaf with NaSA followed by challenge-inoculation in the upper leaf led to even greater increase in PAL activity in all leaves compared to controls (Fig 58). Thus, 1 day after treatment PAL activity in leaf 3 was $79\mu\text{g cinnamic acid/ mg protein/ hour}$ (a 20 fold increase over the control) and 12 days after treating enzyme activity was still 5 times greater than control values (Fig 58). Very similar trends were observed following treatment with PP and MJ (Figs 59-62), although the increase in PAL activity following MJ treatment were smaller than those observed with NaSA treatment.

3.5.2 *Peroxidase Activity*

Treatment of the first leaf of barley seedlings with NaSA led to increased peroxidase activity in leaves 1, 2 and 3 (Fig 63). Following treatment with NaSA, peroxidase activity increased, reaching a peak 3 days after treatment, and although enzyme activity decreased thereafter, values were still considerably greater than controls 12 days after treatment (Fig 63). A very similar trend was observed following NaSA treatment and

subsequent inoculation on leaf 2, with mildew, except the increase in peroxidase activity was greater than those observed following NaSA treatment only (Fig 64). Treatment of leaf 1 with PP resulted in increased peroxidase activity in leaves 1, 2 and 3, although the increase observed were not as large as those observed following NaSA treatment (Fig 65). The largest increase in enzyme activity was observed in the second leaf, where 2 days following treatment, peroxidase was 5 times greater than control values (Fig 65). Challenge-inoculation of leaf 2 following treatment of leaf 1 with PP led to increased peroxidase activity in all leaves, peaking at between 3-4 days after treatment (Fig 66). Interestingly, in the inoculated second leaf of control plants (i.e. they were not treated with PP but not inoculated), peroxidase activity increased gradually with time, reaching a peak at 7 days (Fig 66). MJ treatment of the first leaf increased peroxidase activity in all leaves compared to the controls (Fig 67), but an entirely different picture was observed following subsequent challenge-inoculation of leaf 2 (Fig 68). However, peroxidase activity increased slightly over the experimental period, compared to control, where there were substantial peaks in peroxidase activity, compared to controls in leaves 2 and 3 at 4 days and 3 days respectively (Fig 68).

3.5.3 *Lipoxygenase*

In control plants, leaves 1, 2 and 3 all showed a peak in lipoxygenase activity after 2 days (Fig 69). When leaf 1 was treated with NaSA, lipoxygenase activity was little different from control values until day 3, when enzyme activity started to increase, reaching a peak in all leaves 4 days after treatment (Fig 69). In control plants with leaf 2 inoculated with powdery mildew, there was an increase in lipoxygenase activity in all leaves over the experimental period (Fig 70). In plants where leaf 1 had been treated with NaSA prior to inoculation of leaf 2, there was a more rapid and larger rise in lipoxygenase in all leaves,

reaching a peak 4 days after treatment (Fig 70). The greatest increase in lipoxygenase activity was seen in leaf 2, 4 days after treatment. Enzyme activity in treated plants was nearly double the control values. Similar trends were observed for lipoxygenase activity in plants treated with PP and MJ (Figs 71-74).

FIGURE 57. Effect of treating the lower leaf (leaf 1) of barley with NaSA₂ on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

FIGURE 58. Effect of treating the lower leaf (leaf 1) of barley with NaSA followed 2 days later by challenge-inoculation, on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

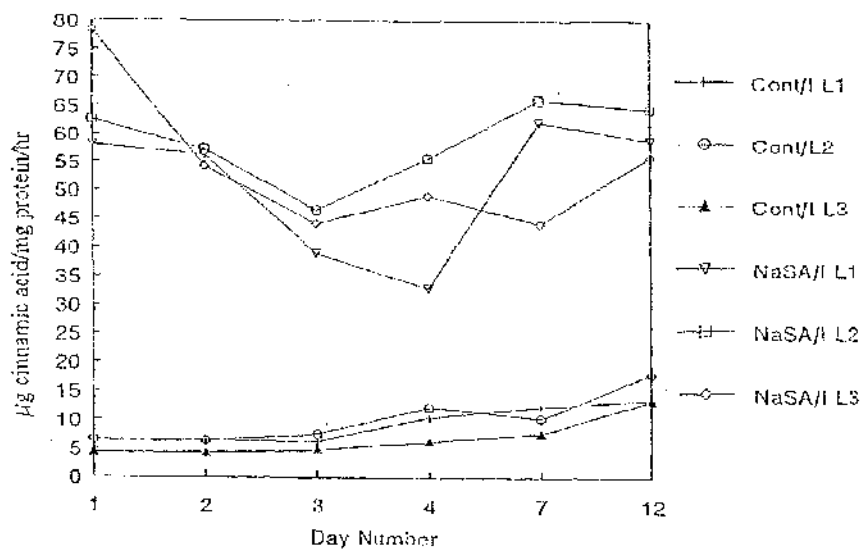
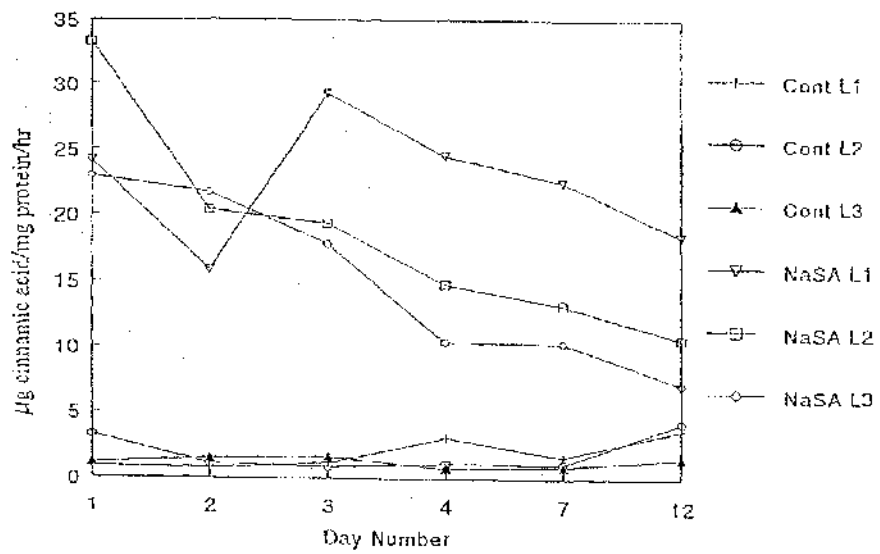


FIGURE 59 Effect of treating the lower leaf (leaf 1) of barley with PP, on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

FIGURE 60. Effect of treating the lower leaf (leaf 1) of barley with PP followed 2 days later by challenge-inoculation, on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

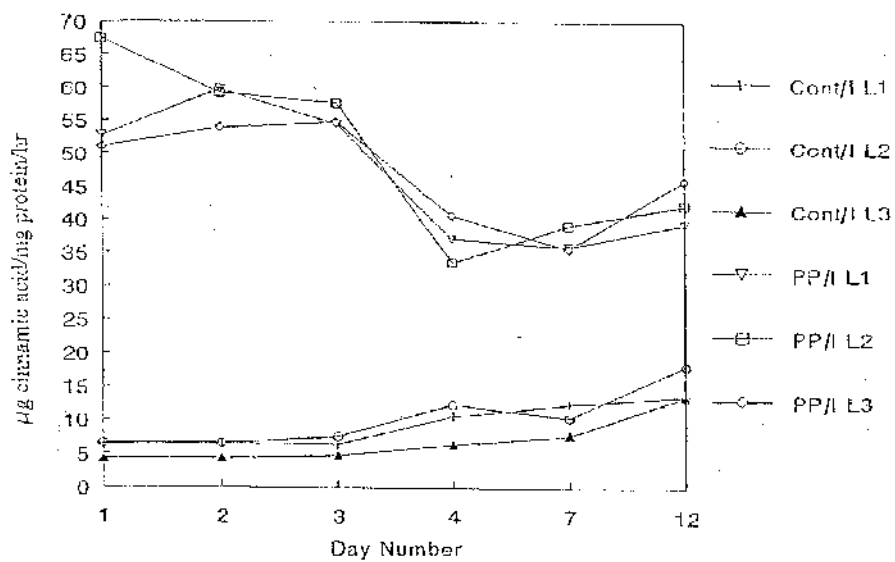
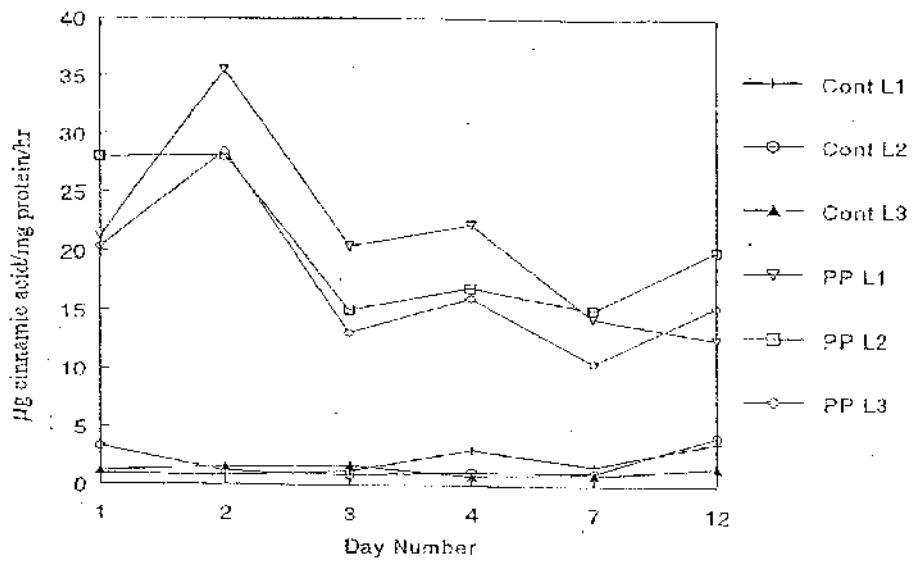


FIGURE 61 Effect of treating the lower leaf (leaf 1) of barley with MJ, on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

FIGURE 62 Effect of treating the lower leaf (leaf 1) of barley with MJ followed 2 days later by challenge-inoculation, on the activity of the enzyme PAL. Results shown is the μg cinnamic acid/mg protein/hour over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

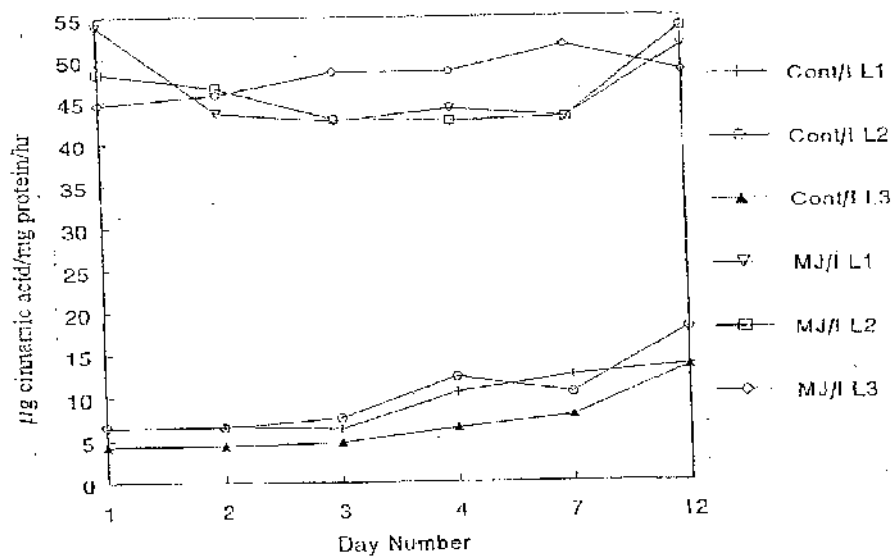
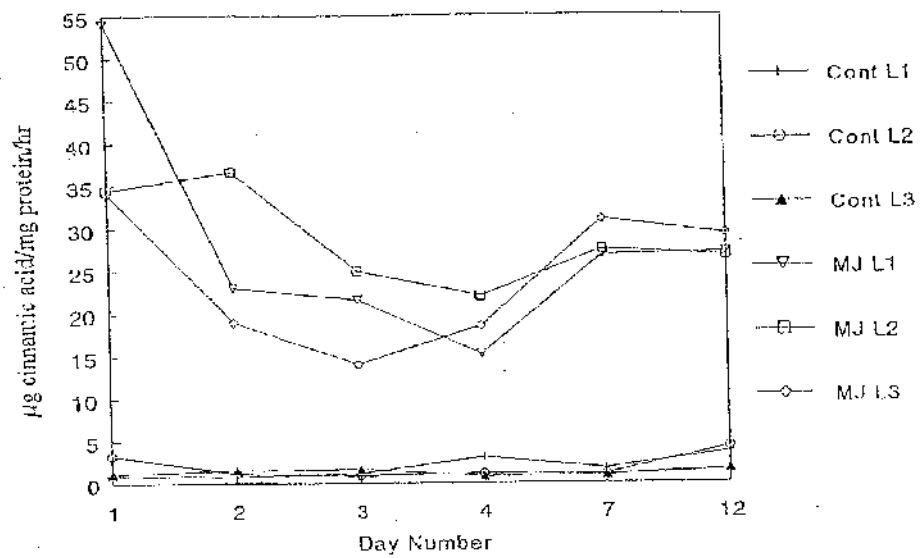


FIGURE 63. Effect of treating the lower leaf (leaf 1) of barley with NaSA on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 64. Effect of treating the lower leaf (leaf 1) of barley with NaSA, followed 2 days later by challenge- inoculation of leaf 2, on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

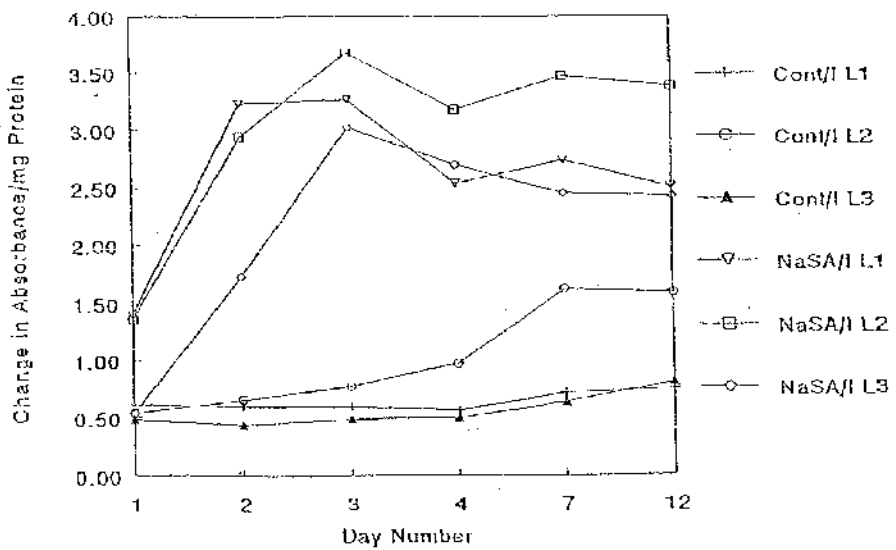
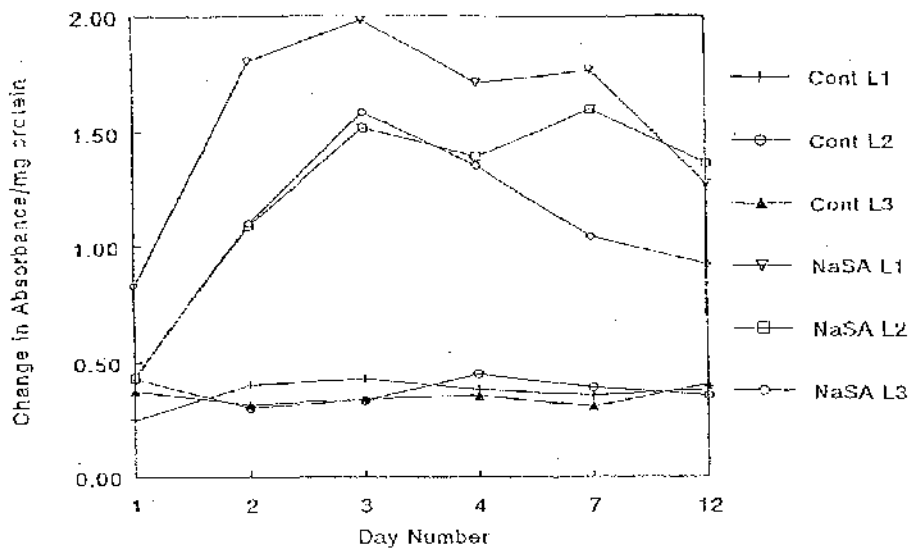


FIGURE 65. Effect of treating the lower leaf (leaf 1) of barley with PP on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 66. Effect of treating the lower leaf (leaf 1) of barley with PP, followed 2 days later by challenge- inoculation of leaf 2, on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

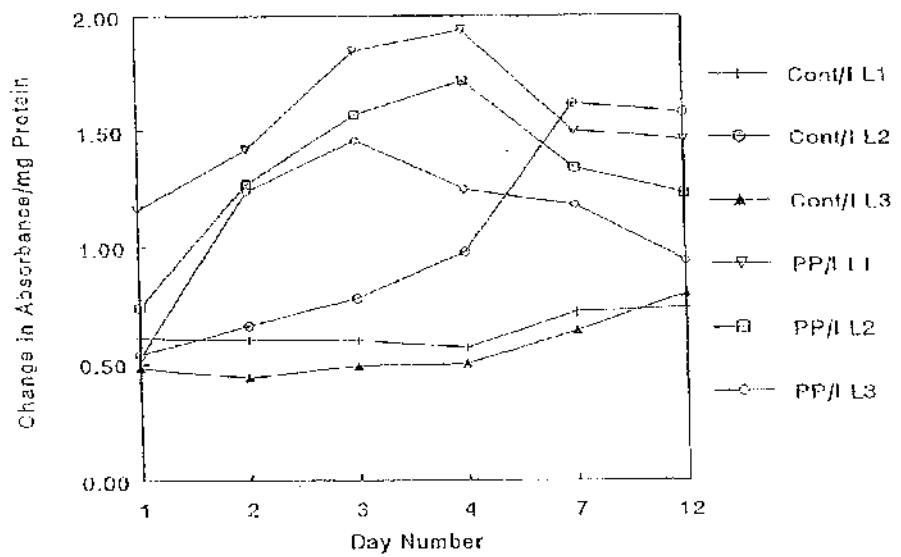
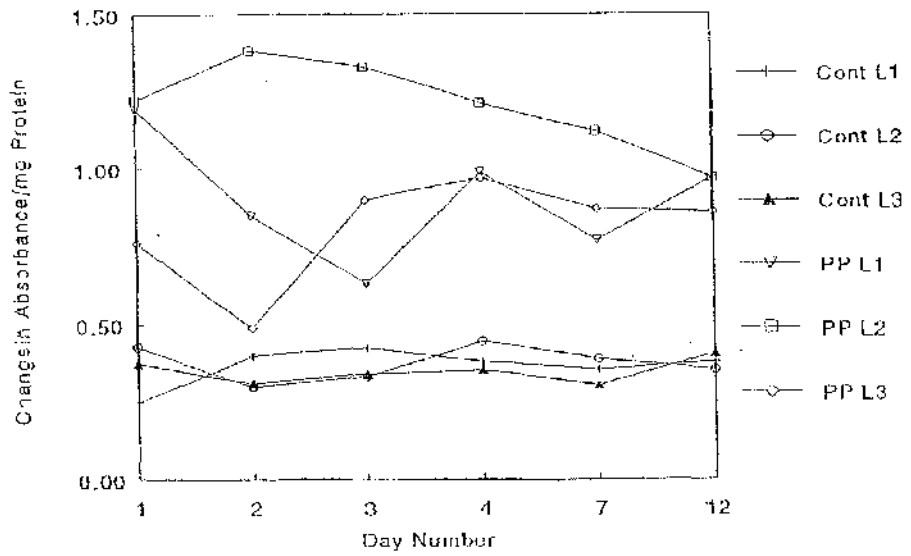


FIGURE 67. Effect of treating the lower leaf (leaf 1) of barley with MJ on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 68. Effect of treating the lower leaf (leaf 1) of barley with MJ, followed 2 days later by challenge- inoculation of leaf 2, on the activity of the enzyme peroxidase. Results are expressed as Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

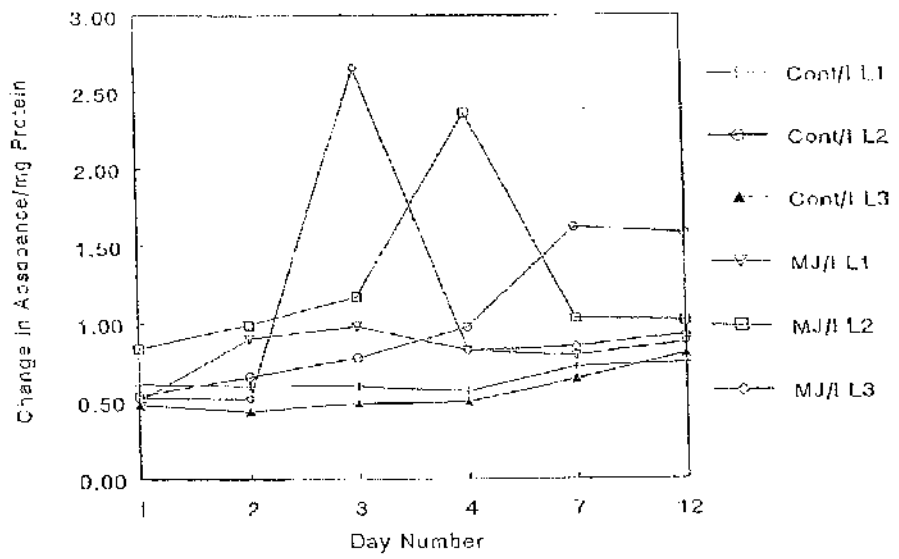
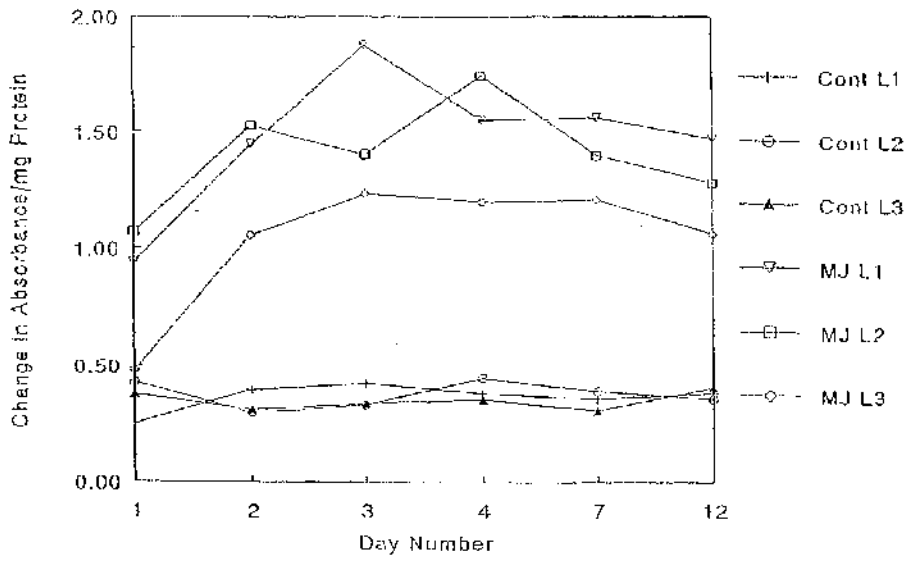


FIGURE 69. Effect of treating the lower leaf (leaf 1) of barley with NaSA on the activity of the enzyme lipoxygenase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 70. Effect of treating the lower leaf (leaf 1) of barley with NaSA, followed 2 days later by challenge- inoculation of leaf 2, on the activity of the enzyme lipoxygenase. Results are expressed as Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

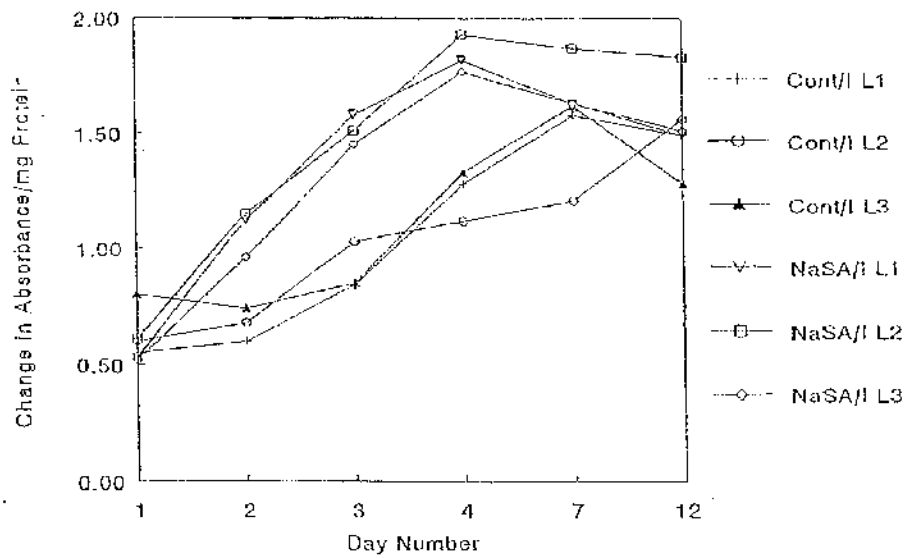
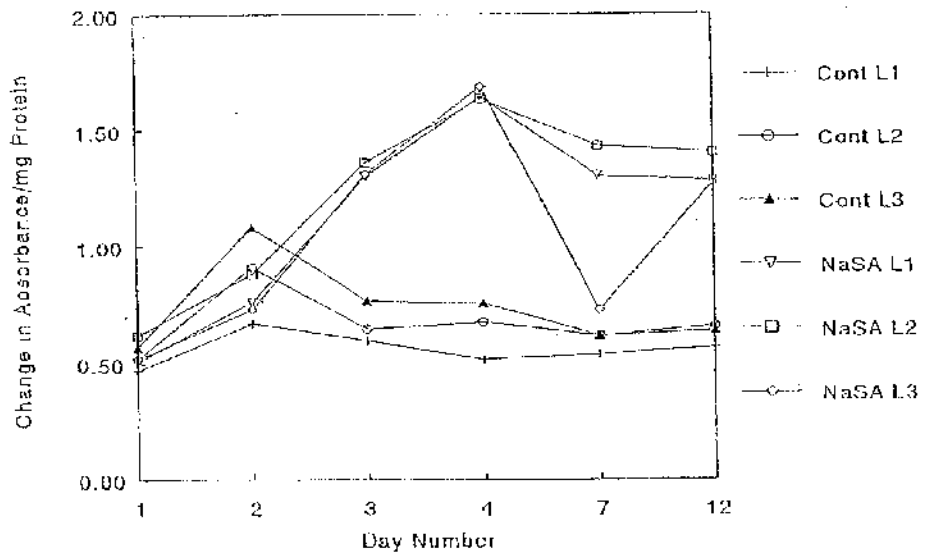


FIGURE 71. Effect of treating the lower leaf (leaf 1) of barley with PP on the activity of the enzyme lipoxygenase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 72. Effect of treating the lower leaf (leaf 1) of barley with PP, followed 2 days later by challenge- inoculation of leaf 2, on the activity of the enzyme lipoxygenase. Results are expressed as Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

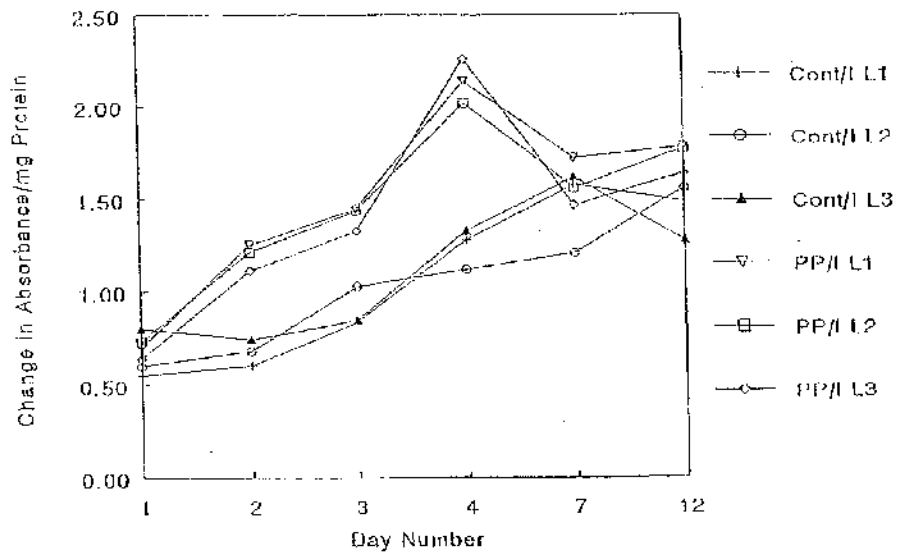
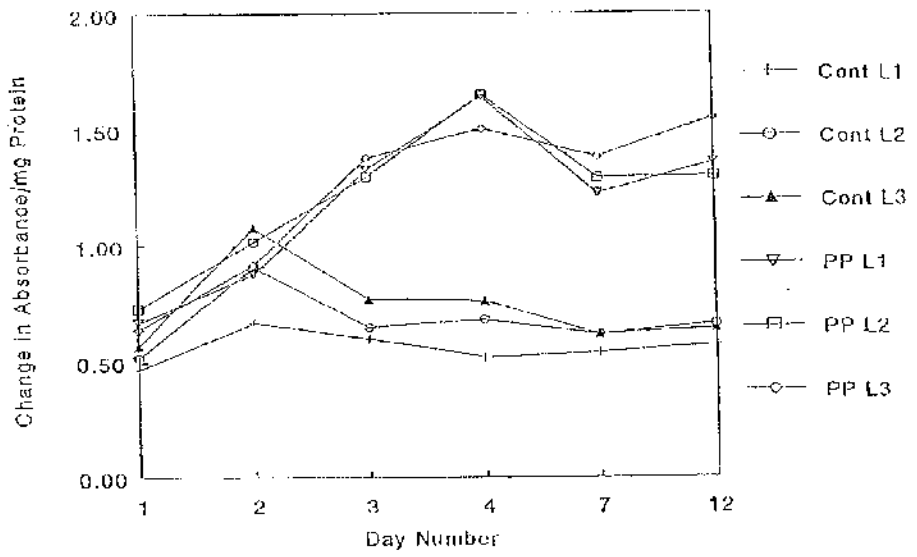


FIGURE 73. Effect of treating the lower leaf (leaf 1) of barley with MJ on the activity of the enzyme lipoxygenase. Results are expressed as Δ Abs/mg protein over a period of 12 days for leaves 1,2 and 3. Figures are the means of 3 replicates.

FIGURE 74. Effect of treating the lower leaf (leaf 1) of barley with MJ, followed 2 days later by challenge- inoculation, on the activity of the enzyme lipoxygenase. Results shown are the activity of the Δ Abs/mg protein over a 12 day period for leaves 1,2 and 3. Figures shown are the means of 3 replicates.

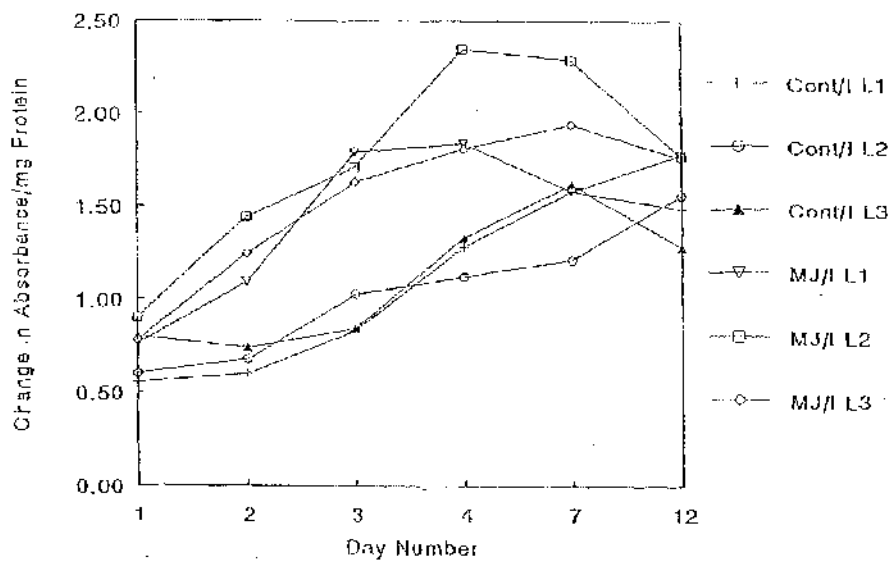
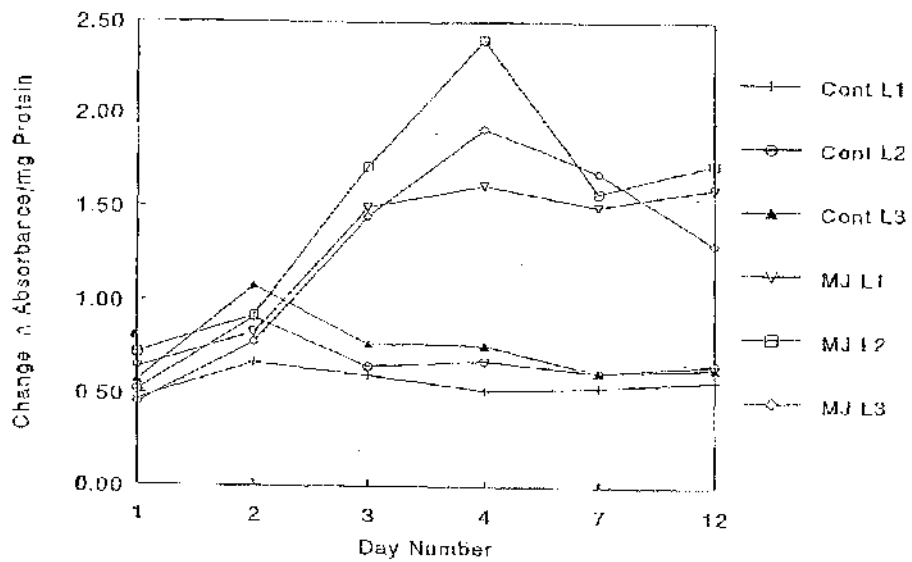


TABLE 5. Effect of applying an elicitor of resistance to the lower leaf of barley plants on P.A.L. activity in leaves 1,2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	P.A.L. Activity (μg cinnamic acid/mg protein/hr)
Control	1	3.09 ± 0.0138
	2	1.15 ± 0.0189
	3	0.79 ± 0.012
NaSA	1	22.33 ± 0.044
	2	16.96 ± 0.040
	3	16.08 ± 0.069
PP	1	24.54 ± 0.038
	2	14.84 ± 0.037
	3	10.43 ± 0.025
MJ	1	15.26 ± 0.026
	2	22.05 ± 0.039
	3	18.57 ± 0.020

TABLE 6. Effect of applying an elicitor of resistance to the lower leaf of barley plants followed by challenge-inoculation on P.A.L. activity in leaves 1,2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	P.A.L. Activity (μg cinnamic acid/mg protein/hr)
Control	1	10.56 \pm 0.118
	2	12.33 \pm 0.060
	3	6.4 \pm 0.343
NaSA	1	37.12 \pm 0.051
	2	33.59 \pm 0.060
	3	40.68 \pm 0.020
PP	1	32.99 \pm 0.075
	2	55.74 \pm 0.056
	3	49.12 \pm 0.062
MJ	1	44.08 \pm 0.050
	2	42.67 \pm 0.044
	3	48.51 \pm 0.051

TABLE 7. Effect of applying an elicitor of resistance to the lower leaf of barley plants on peroxidase activity in leaves 1,2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	Peroxidase Activity (Δ Abs/mg protein)
Control	1	0.379 \pm 0.0012
	2	0.446 \pm 0.0019
	3	0.353 \pm 0.0018
NaSA	1	1.71 \pm 0.0025
	2	1.39 \pm 0.0149
	3	1.35 \pm 0.002
PP	1	0.632 \pm 0.0187
	2	1.213 \pm 0.0021
	3	0.974 \pm 0.0014
MJ	1	1.56 \pm 0.0187
	2	1.75 \pm 0.0021
	3	1.20 \pm 0.0014

TABLE 8. Effect of applying an elicitor of resistance to the lower leaf of barley plants followed by challenge-inoculation of leaf 2 on peroxidase activity in leaves 1, 2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	Peroxidase Activity (Δ Abs/mg protein)
Control	1	0.571 \pm 0.0027
	2	0.982 \pm 0.0021
	3	0.504 \pm 0.0011
NaSA	1	2.54 \pm 0.0015
	2	3.18 \pm 0.0010
	3	2.70 \pm 0.0021
PP	1	1.95 \pm 0.0009
	2	1.71 \pm 0.0023
	3	1.26 \pm 0.0010
MJ	1	0.834 \pm 0.0022
	2	2.38 \pm 0.0008
	3	0.839 \pm 0.0009

TABLE 9. Effect of applying an elicitor of resistance to the lower leaf of barley plants on lipoxygenase activity in leaves 1,2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	Lipoxygenase Activity (Δ Abs/mg protein)
Control	1	0.523 \pm 0.0063
	2	0.681 \pm 0.0029
	3	0.771 \pm 0.0024
NaSA	1	1.651 \pm 0.0002
	2	1.642 \pm 0.0009
	3	1.698 \pm 0.0011
PP	1	1.645 \pm 0.0018
	2	1.651 \pm 0.0019
	3	1.513 \pm 0.0019
MJ	1	1.608 \pm 0.0028
	2	2.408 \pm 0.0023
	3	1.928 \pm 0.0025

TABLE 10. Effect of applying an elicitor of resistance to the lower leaf of barley plants followed by challenge-inoculation on lipoxygenase activity in leaves 1,2 and 3. Results shown are for 4 days post-elicitor treatment with standard error for 3 replicates.

Treatment	Leaf Number	Lipoxygenase Activity (Δ Abs/mg protein)
Control	1	1.284 \pm 0.0002
	2	1.129 \pm 0.0017
	3	1.336 \pm 0.0012
NaSA	1	1.824 \pm 0.0011
	2	1.929 \pm 0.0021
	3	1.769 \pm 0.0020
PP	1	2.148 \pm 0.0031
	2	2.015 \pm 0.0016
	3	2.259 \pm 0.0022
MJ	1	1.847 \pm 0.0013
	2	2.358 \pm 0.0014
	3	1.807 \pm 0.0015

3.5 *Field Trials*

3.5.1 *Disease Development*

In both 1993 and 1994 20mM NaSA, 25 mM PP, 20 mM MJ and commercial fungicides significantly reduced the level of mildew infection observed. (Table 11). The largest reductions were found in 1994 when plants were treated with 20 mM MJ. Analysis of variance highlighted significant differences between treatments and controls (Table 11).

3.5.2 *Grain Weight*

In 1993 analysis of variance showed no significant differences between elicitor treated plants and control plants (Table 12). In 1994 significant differences were observed between elicitor treated plants and controls (Table 12). No differences in grain weight were found between commercial fungicides and control plants (Table 12).

3.5.3 *Plant Height*

Analysis of variance showed no significant differences between control plants and those treated with either 15 mM NaSA, 25 mM PP or commercial fungicides (Table 13). In 1994 plants treated with 20 mM MJ were significantly smaller than control plants and other treatments (Table 13).

3.5.4 *Plant Dry Weight*

In 1993 and 1994 analysis of variance showed there were no significant differences between plant dry weight of the control and plants treated with either elicitors or commercial fungicides (Table 14).

Table 11. Effect of applying elicitors of resistance or proprietary fungicides on disease development of field grown barley. Barley powdery mildew was assessed visually using the M.A.F.F. standard area diagram. Standard deviations are shown at 95% confidence level.

Treatment	% Leaf Area	Standard Deviation	% Disease Control
1993	Infected		
Control	21.2	8.07	
NaSA (15mM)	5.1	1.38	76.0
PP (25mM)	6.42	2.04	65.0

Treatment	% Leaf Area	Standard Deviation	% Disease Control
1994	Infected		
Control	19.45	1.7	
NaSA (15mM)	7.5	3.2	61.3
MJ (20mM)	7.2	0.66	62.9
Corbel	9.9	0.16	48.9
Folicur	7.9	0.31	59.2
Radar	10.2	0.68	47.4

Table 12. Effect of applying elicitors of resistance or proprietary fungicides on the grain weight of 10 plants in field grown barley. Standard deviations are shown at 95% confidence level.

Treatment	Grain Weight	Standard Deviation	% Increase in Grain Weight
1993			
Control	86.79	7.09	
NaSa (20mM)	94.23	4.69	8.6
PP (25mM)	97.69	2.22	12.6

Treatment	Grain Weight	Standard Deviation	% Increase in Grain Weight
1994			
Control	85.82	2.71	50.2
NaSa (15mM)	128.9	3.56	47.2
MJ (20mM)	126.35	6.04	12.6
Corbel	95.97	5.54	13.9
Radar	97.71	7.07	11.9
Folicur	96.07	6.92	

Table 13. Effect of applying elicitors of resistance or proprietary fungicides on the height of field grown barley. Standard deviations are shown at 95% confidence level.

Treatment	Plant Height (cm)	Standard Deviation
1993		
Control	75.9	3.81
NaSA (15mM)	71.8	2.12
PP (25mM)	72.4	2.01

Treatment	Plant Height (cm)	Standard Deviation
1994		
Control	71.2	1.33
NaSA (15mM)	73.6	4.07
MJ (20mM)	62.1	4.84
Corbel	76.4	3.40
Folicur	75.5	2.42
Radar	77.0	2.1

Table 14. Effect of applying elicitors of resistance or proprietary fungicides on plant dry weight of field grown barley. Standard deviations at 95% confidence level are shown in the table.

Treatment	Plant Dry Weight (g)	Standard Deviation
1993		
Control	20.8	2.06
NaSA (15mM)	19.4	1.27
PP (25mM)	20.3	0.54

Treatment	Plant Dry Weight	Standard Deviation
1994		
Control	21.3	0.32
NaSA (15mM)	19.9	1.29
MJ (20mM)	20.2	0.84
Corbel	21.1	0.37
Radar	26.6	6.61
Folicur	22.2	3.65

4. DISCUSSION

It is now recognised that plants susceptible to pathogens contain the same genetic information for defence as resistant plants. What distinguishes the two is the speed and magnitude to which the latent genetic information is expressed. As long ago as 1933, Chester observed it was possible to enhance the expression of this genetic information by prior inoculation with plant pathogens. Since then it has become apparent that a great number of 'agents' have the ability to enhance the expression of genetic information carried by susceptible plants. Abiotic chemicals are amongst the agents which have been shown to successfully prime plants, enabling them to react more rapidly when faced with pathogen attack. It has been shown in many cases that the final level of mRNA coding for defence factors is higher in susceptible plants than in resistant ones. However, the synthesis and accumulation of defence related chemicals takes place too late in susceptible plants. The pathogen by this stage has successfully established infection.

A study of abiotic chemicals was undertaken in an attempt to gain a deeper insight into the environmental and physical requirements for maximal induction of SAR in barley. The study also attempted to study some of the defence mechanisms induced following treatment with abiotic elicitors.

4.1 General Screening of Abiotic Agents

In order to determine which chemical agents and which concentrations effected greatest induced resistance, a screening trial of 28 combinations of chemicals and concentrations was undertaken. All four concentrations of the seven potential elicitors screened gave significant reductions in mildew infection. The degree of protection conferred varied with the chemical and the concentration used. Thus, although Chitosan, ASA and INA gave

reductions in mildew infection of upper leaves which were statistically significant, the magnitude of these effects were not considered sufficient to warrant further study.

NaSA, MJ, and PP all gave reductions in mildew development of at least 19 %. The three optimal concentrations of these compounds were 15mM NaSA, 25mM PP and 15mM MJ. These concentrations may be considered relatively high, but this may be related to the method of induction. In this study it was important to consider field application methods in assessing SAR as an alternative to conventional chemical treatment. It is possible the concentration of the elicitor used could have been greatly reduced if the inducing treatment had been in the form of stem or leaf injection. It is therefore conceivable that higher concentrations are required due to the problems associated with the uptake of exogenously applied compounds. Only a tiny proportion of what is applied will be taken up by the plant. It is possible that only by the use of relatively high concentrations is sufficient chemical absorbed to induce the chain of reactions required for the induction of SAR. It has been reported that K_2HPO_4 is an effective foliar fertiliser (Reuveni et al., 1992), and so it is possible that a portion of the PP applied is utilised in enhancing plant growth, thus restricting the level of PP available for initiating the cascade of events leading to the expression of induced resistance. In 1991 Ward et al. acknowledged that salicylates may be conjugated during transport in the plant. This again may influence the availability of NaSA for initiation of SAR. MJ may also have problems associated with its uptake due to its volatile nature. Low applied concentrations may evaporate before sufficient has been taken up by the leaf to induce systemic resistance.

It is now agreed that it is not a specific component of the abiotic elicitor which is responsible for the induction of SAR, but rather a persistent low level of metabolic

perturbation leading to stress in the host (Doubrava et al., 1988). This acceptance has arisen because of the diversity of chemicals capable of inducing the response. In many cases of SAR, plant cell death is visible and associated with the expression of SAR. In this trial the chemicals chosen for further study showed no visible symptoms on the foliage. It is likely however, that concentrations applied were sufficient to produce the low level stress required for the expression of SAR, but without producing foliar necrosis. Evidence suggests, that SAR induced by application of PO_4^{2-} results in the sequestration of Ca^{2+} from host tissues (Gottstein and Kúic, 1991). Indeed, application of Ca^{2+} was shown to produce a complete reversal of SAR (Walters and Murray, 1992). It is possible that loss of Ca^{2+} after phosphate application affects cell membranes, destroys cell compartmentalisation and causes the release or synthesis of hydrolytic enzymes. Due to the loss of Ca^{2+} the hydrolytic enzymes can act, on the now susceptible, pectic substances in the plant cell wall forming oligosaccharides or oligogalacturonates. These may then cause the release of the alarm signal, the exact nature of which, as yet, remains unidentified.

4.1.1 *Optimal Lag Period Between Elicitor Treatment and Challenge-Inoculation*

The effectiveness of the chemical agents in inducing resistance is affected by the time interval between treatment of the plant tissue with the inducing agent and challenge-inoculation with the pathogen. A range of time intervals, from 24 hours up to 12 days were studied. Although a number of the time intervals studied led to significant reductions in the level of powdery mildew infection, the most effective interval between inducing treatment and challenge-inoculation was found to be, with all three chemicals, 48 hours.

In all cases of induced resistance a lag period is observed between inducer treatment and detection of systemic acquired resistance. The delay corresponds to a delay in the production or release of a signal from the inducer leaf and not, as is the popular misconception, its transport to distal leaves and the initiation of resistance mechanisms, which from time course studies are known to be very rapid (Dean and Kuc, 1986). At time intervals less than 48 hours it is possible that insufficient signal has been produced or released from the inducer leaf for SAR to be fully induced. At time intervals greater than 48 hours the potency of the signal from the inducer leaf may have begun to decline, again resulting in a weaker induction of SAR.

4.1.2 *Effect of Environmental Factors on the Expression of Systemic Resistance*

Daylength and temperature are two environmental factors thought to influence the successful establishment of SAR. Both of these factors were assessed, independently, to determine a set of environmental conditions which allow optimal systemic protection to be initiated. A set of four potential photoperiod and four potential temperature regimes were examined.

At the shorter photoperiods of 7 hr and 12 hr reductions of 24 % and 38 % in mildew development respectively were observed. The greatest reduction (78 %) was observed with a 16 hr daylength, while the longest photoperiod (20 hrs) lead to a much smaller decrease in mildew infection. Activation of host defence mechanisms is an energy demanding process, the energy must ultimately be generated by photosynthesis. It is possible that with the photoperiods of 7 hr and 12 hr the energy demands placed on the plant by pathogen infection and activation of resistance mechanisms are, in combination, too great a burden for the metabolism of the plant. It would appear therefore that the

shorter photoperiods are unable to meet the photosynthetic demands of the plant for metabolites to fuel the activation of defense mechanisms required to limit the spread of mildew. Exactly why systemic protection against mildew should have been less in plants grown in a 20 hour photoperiod compared to plants grown under a 16 hour photoperiod is not clear. It is known that in a variety of plants increasing the photosynthesis leads to a decreased rate of starch accumulation and an increased rate of sucrose production and export (Wardlaw, 1990). It is possible throughout that an increased export of sucrose under the longer photoperiod may have left less available in the leaf for use in financing SAR. It is worth noting that it is unlikely that sucrose could have been imported into the systemically protected leaf from other leaves, since there is no evidence of assimilate import into mature monocot leaves (Farrar, 1992).

In light of the work of Klement and Goodman (1968) on the effect of temperature on the development of induced resistance, four temperature regimes were examined in the present study. The two lower temperature regimes of 10°C and 17°C both led to significant reductions in powdery mildew development when systemic protection was induced with the three chemicals. However, it is interesting to consider that the temperature at which greatest pressure by *E. graminis* would be expected i.e. at the optimal temperature for growth of *E. graminis* was in fact the temperature at which greatest induced resistance was observed. Thus, at 22°C a reduction in powdery mildew infection of 81% was observed. This decrease in mildew development is unlikely to be the result of a direct effect of temperature on the development of SAR, and more likely to be due to the effect of temperature on the photosynthetic activity of the plant. Rates of photosynthesis was probably limited at 10°C and 17°C and so the energy to induce and maintain SAR would also have been limited. A temperature of 22°C is unlikely to have been a limiting factor in photosynthesis, and the plants would have been able to provide the energy required for the

expression of induced resistance. At the highest temperature regime of 35°C there was an overall reduction in the development of powdery mildew, both in the chemically treated plants and in the controls. No significant differences were observed between treatments and controls. A temperature of 35°C is well above the optimum for photosynthesis in cereals (approx. 22°C) and, in addition to reduced rates of photosynthesis, rates of respiration would have been considerably increased. It is not surprising that SAR expression is suppressed at high temperatures. In addition growth of *E. graminis* would have been reduced at this temperature, since it is at the upper limit for growth of powdery mildew (Akai, 1952).

4.1.3 *Direction of Signal Movement*

The generally accepted perception of SAR is that a lower leaf is treated with an inducer which leads to the development of resistance at some distal point above the inducer leaf. Nevertheless SAR has been reported to occur at points below the inducer leaf. The hypothesis that it is possible to induce SAR below the inducer leaf was tested using the three abiotic treatments, 15mM NaSA, 25mM PP and 20mM MJ. Small reductions in mildew infection in the lower three leaves were observed following treatment of the upper three leaves with all three chemicals. All three chemicals gave significant reductions in mildew in leaves 1 and 2. However, only 20mM MJ gave significant reductions in leaf 3. Despite the reductions in infection being statistically significant, they were not of the magnitude obtained when SAR was induced in leaves above the inducer leaf. Therefore, it is clear that the signal for SAR in barley moves more strongly in an upward direction. The results for 15mM NaSA and 25mM PP were not as expected. If it was possible to induce SAR below the inducer leaf it would be expected that the strongest induction would be in the leaf immediately below the inducer leaf. However, both these chemicals showed no significant reductions in leaf 3, while significant reductions were observed in

leaves 1 and 2. At this point no explanation can be offered for these results. Application of 20mM MJ yielded the expected with the largest reductions in mildew observed in leaf 3 just below the inducer leaf.

4.1.4 *Duration of Effectiveness of a Single Elicitor Treatment*

An important factor in considering SAR as a potential means of disease control in the field is how long a single application of elicitor can effect disease control. The study was carried out using 15mM NaSA, 25mM PP and 20mM MJ as elicitors of resistance. As expected, the largest reductions were observed in the earlier stages of the trial. Even as long as 7 weeks after challenge-inoculation significant reductions in mildew were still being observed. At 10 weeks post challenge-inoculation significant reductions were observed with 25mM PP and 15mM MJ, when no reductions were observed with 15mM NaSA. As the time from challenge-inoculation increased the efficacy of the systemic protection declined. It is possible that a second or booster treatment would be required to maintain the level of resistance expression observed in the early stages of the experiment. The use of booster treatments was examined in the field trial situation and will be discussed at a later stage.

A further consideration in assessing the potential of induced resistance as a viable alternative to propriety chemicals for the control of powdery mildew is the evaluation of how increasing the time interval between elicitor treatment and challenge-inoculation affects the level of disease control achieved. It was established in an earlier experiment that optimal resistance was expressed when plants were challenge-inoculated 48 hours after elicitor treatment. However, in a field situation disease pressure cannot be manipulated to ensure it occurs when the expression of induced resistance is maximal. The time interval between elicitor treatment and challenge-inoculation was increased in 7

day intervals and the effect on disease development assessed. Significant reductions in mildew development were observed, for all 3 chemicals tested, even if challenge-inoculation was delayed by as much as 42 days for the 3 chemicals examined, there was a similar tailing off in the level of protection observed as the time interval between elicitor treatment and challenge-inoculation increased. It is possible this is directly linked to a decrease in either signal production or signal potency from the inducer leaf.

Present chemical control techniques require at least 2 or more applications in the growing season, with timing of the application being a critical factor. For certain chemicals i.e. those with preventative action, initial applications must be made prior to the first symptoms being observed. This requires careful crop monitoring, to ensure the pathogen is not established. However, infection can spread rapidly even in the space of a few days. If induced resistance is to be used in the field, timing of application would be less critical since the plants defences appear to stay primed for a considerable period of time. The point at which induced resistance crosses the economic threshold of no longer being economically viable would require input from professional economists. But, it appears that the use of induced resistance to protect crops in the field would require no more physical input than that required for a conventional proprietary fungicide.

4.1.5 *Alternative Methods of Inducing Resistance*

Although the methods used to induce resistance so far in this project are no more difficult to use than the current methods of pesticide application, in the field, the simplest method of applying the inducer would obviously be the most advantageous. Potentially the simplest method of inducing SAR would be to treat the seed. This method, if successful, would be carried out by seedhouses, thus freeing farmers from the need to apply any crop protection treatments. The three agents known to induce resistance in plants when applied

aerially i.e. 15mM NaSA, 25mM PP and 20mM MJ, were applied to seeds of a barley cultivar which is susceptible to powdery mildew. Results from these seed treatments were poor. Although statistically significant reductions were observed for the two shorter seed soaking times of 5 minutes and 15 minutes, mildew control was not sufficient to warrant further investigation. It seems unlikely that the reductions in mildew development were the result of SAR although further research will be required to clarify this point. It is interesting to observe, however, that after treatment with the chemicals for either 3 or 6 hrs, all cases resulted in an increase in the levels of powdery mildew in the germinated seedlings relative to the controls. It is possible that following such prolonged exposure of the seeds to the chemicals, there was physiological damage to the embryo. On germination, these seeds then produced a plant which was actually more susceptible to infection by powdery mildew and possibly other plant pathogens.

The possibility that resistance could be induced via the root system, by means of a root drench, was also investigated. This hypothesis was tested with three chemicals at various stages pre-inoculation. All time intervals tested yielded statistically significant reductions in mildew development. The chemicals tested did not, however, give reductions which would make induction of resistance via a root drench an economically viable method of disease control. It would appear that the induction of resistance via the root system is weak. There are a number of possible explanations for this. Thus, it is possible that the concentration of the chemical inducing agent was not high enough to activate resistance via this route. The chemical may be broken down and metabolised by the plant, and so the level entering the shoot was not sufficient to trigger the generation of the signal for SAR. It must also be considered that soil organisms may break down or metabolise the chemical agents before they are taken up by the plant, or perhaps the agents applied to the rooting medium were sequestered making less available for uptake by the root.

Recently this area of investigation has been given further consideration. De Cal et al., (1997) found it was possible induce systemic resistance in tomatoes to *Fusarium oxysporum* f.sp. *lycopersici* by applying a conidial suspension of *Penicillium oxalicum* to the substrate tomato seedlings were to be grown in. Miyazawa et al., (1998) induced systemic resistance to tomato wilt disease *in vitro* by treating tomato roots with either 2-furoic acid, 4- hydrobenzoic acid or salicylic acid.

4.1.6 *Direct Antifungal Effects of Elicitor Chemicals in vivo*

Although the chemicals screened did not come into direct contact with the mildew and the effects were observed at some point distant from the inducer treatment, it is, nevertheless, essential to determine the direct effects of the chemicals on pathogens. Some controversy has arisen around which chemicals can and cannot be classed as elicitors of resistance. Two schools of thought exist. One school maintains that for a chemical to be considered an elicitor of systemic resistance it should have no antifungal properties, while the other school suggests that the antifungal properties of the chemicals are irrelevant since the resistance is expressed at some point distant from the initial treatment.

The direct effect of 15mM NaSA, 25mM PP and 20mM MJ on pathogen growth was examined *in vivo*. Plants which had the chemical applied and allowed to dry for 4 hours before inoculation all exhibited significant reductions in the level of mildew infection observed. The second treatment where plants were inoculated prior to spraying also exhibited significant reduction in mildew development. The reductions observed in this instance for 15mM NaSA and 25mM PP were much smaller than for the first treatment.

Treatment with 20mM MJ caused reductions in the second trial of the same magnitude as those observed in the first trial.

Plants which exhibited a reduction in mildew development following the spray/dry/inoculate treatment were likely to be exhibiting some degree of local induced resistance in addition to possible fungitoxic effects. The 4 hour delay would have been sufficient for localised resistance to be induced, which can, at least partially, contain the development of the pathogen. Under the second set of conditions, i.e. spray/inoculate, the reduction in mildew development obtained with NaSA and PP, was unlikely to be due to any fungitoxic effects. It is more probable that the reduction is directly related to the effect of liquid on the germination of mildew conidia. Very large reductions in mildew development were observed on those plants sprayed with 15mM MJ, post inoculation. The 83% reduction observed is unlikely to be solely due to the effect of liquid on the germination of mildew conidia. It appears 15mM MJ has fungitoxic properties.

4.1.7 Direct Antifungal Effects of the Elicitor Chemicals *in vitro*

The antifungal effects of the three chemical elicitors, 15mM NaSA, 25mM PP and 20mM MJ, were investigated further *in vitro*, although since *E. graminis* is an obligate biotroph, alternative plant pathogens had to be used. With both *P. avenae* and *C. pernicioso* the rate of radial growth decreased as the concentration of the amendment increased. This was true for all 3 chemicals examined. When a core was taken from the leading edge and transferred to a PDA plate with no amendments, similar rates of growth were achieved with all samples. Statistical analysis showed no significant difference between the control and the treatments (results not shown). From these results alone it would be easy to assume that the chemicals were not fungitoxic, but fungistatic. It must be considered,

however, that if the chemical is not uniformly distributed within the medium, the pathogen may avoid direct physical contact with the chemicals. It is likely that the pathogen biomass on amended agar plates was significantly less than on control plates, since mycelial growth was probably very thin on treated plates.

To further clarify the effects of the elicitor chemicals on pathogen growth *in vitro*, *P. avenae* and *C. perniciososa* were grown in amended PDB. The effect, in all cases, was a very marked reduction in growth. Only the lowest concentration of each chemical amendment was capable of supporting pathogen growth. This was true for both *P. avenae* and *C. perniciososa*. This data suggests that the 3 chemicals used throughout this project, 15mM NaSA, 25mM PP and 20mM MJ, all have fungitoxic properties *in vitro*.

It is important to remember that the work on the direct effect of the chemicals on mildew and the effect on pathogen growth in culture are not directly compared. Thus, with mildew, the effects of the chemicals were studied on developing germlings on the leaf surface, as compared to mycelial growth in culture. It is highly likely that the developing germling and mycelium exhibit markedly different sensitivity to inhibition/ chemicals, as noted in other studies (Walters, 1995).

In the 1994 review by Kessman he outlined a set of general criteria which had to be satisfied before a compound could be classified as an elicitor of SAR (See section 1.3.5). One condition is that the compound must not possess any antifungal activity, a claim which is disputed by Kúc (1995). From this work it is clear all compounds screened in this trial exhibited antifungal properties. Therefore whether the response observed can be classified as systemic acquired resistance is unresolved. Nevertheless, although these chemicals possess antifungal properties, they are also capable of inducing systemic protection to powdery mildew. The mechanism for the expression of this resistance is

likely therefore be systemic. Since it is unlikely that sufficient chemical could have been transported out of the inducer leaf to the upper leaf and then been taken up by the developing mildew. Indeed Walters et al. (1993) found that treatment of the lower leaf of barley with ^{14}C -salicylate led to the accumulation of small amounts of the compound in the upper leaf. Although this small concentration of salicylate ($\cong 1\mu\text{mol g}^{-1}$) would have been sufficient to induce SAR, it is unlikely to have been sufficient to exert direct antifungal effects on the mildew. Similar work is required for the three compounds used in this study.

4.2 *Effects of Methyljasmonate Vapour*

4.2.1 *General Screening of Methyljasmonate Vapour*

Methyljasmonate liquid, in earlier screening trials, was shown to be a potent elicitor of systemic resistance. It was therefore considered useful to determine whether MJ vapour exhibited any potential as an elicitor of resistance. When the lower leaf of barley was exposed to undiluted MJ vapour significant reductions in mildew were observed. This was also true for the vapour from 15mM MJ. However, it is interesting to note that the undiluted MJ showed optimal induction of resistance after a 30 minute exposure, whereas with the vapour from 15mM MJ, a 2 hr exposure time was required. The uptake of MJ vapour will almost certainly be via the stomata. One acknowledged effect of MJ in plants is stomatal closure (Curtis, 1984). It is quite possible therefore that for undiluted MJ and 15mM MJ, 30 mins and 2 hrs represents the time taken for stomatal closure to occur. The 30 mins and 2 hrs taken for undiluted and 20 mM MJ to effect stomatal closure would have allowed an increased uptake of MJ, leading to increased elicitation of systemic resistance. Upon stomatal closure, uptake of MJ would be reduced and so no

further increase in the elicitation of systemic resistance would occur. Indeed after stomatal closure the level of MJ within the leaf might decrease due to metabolism, leading to a possible fall in the elicitation of systemic resistance.

4.2.2 *Optimal Lag Period for Methyljasmonate Vapour*

As with the liquid elicitors of resistance to ensure optimal resistance was induced it was important to establish the optimal lag period between elicitor treatment and challenge-inoculation. As in section 2.2.2 a range of lag times were examined. The optimal lag period was found to be 24 hours. This was 24 hours less than the time interval which was found to be optimal in plants treated with liquid elicitors. It is possible that the lag period required for vapour treated plants was reduced because of the method of uptake into the plants. The MJ vapour, if taken up via the stomata, would penetrate the plant more rapidly than if absorbed as a liquid through the leaf epidermis. Therefore the elicitor in vapour state may more rapidly initiate the chain of reactions leading to the expression of induced resistance. It is also possible that the vapour misses out some of the earlier stages in the cascade of events leading to the induction of SAR, since MJ has been suggested as a possible signalling molecule in SAR.

4.2.3 *Effects of Methyljasmonate Vapour on Fungal Growth and Development in vivo and in vitro*

Following on from this work, the direct effect of MJ on fungal pathogens was examined both *in vivo* and *in vitro*. When the leaf was inoculated and then exposed to vapour from either undiluted or 20mM MJ, solution, a significant reduction in mildew development

was observed. As expected exposure to undiluted MJ resulted in the largest reduction in powdery mildew development. To further investigate the direct effect of MJ on the germination and development of *E. graminis* conidia, tap water agar was inoculated with mildew conidia and then exposed to the vapour from either undiluted or 20mM MJ. It is acknowledged that because *E. graminis* is an obligate parasite, the germination of the conidium and germ tube production are the only 2 stages to occur on agar. During the experiment, if germination had occurred but germ tube development had failed or was distorted this was classed as a fail, since *in vivo* this would translate into a failed penetration attempt and hence restricted pathogen growth. On examination it appeared that germination of the conidia was not affected by the presence of MJ vapour, either in its diluted or undiluted form, but germ tube development was severely disturbed. The germ tubes observed after exposure to MJ vapour of any sort were severely stunted and distorted. Despite the short exposure time of 30 minutes, it would appear that the vapour present after the cotton tipped applicators had been removed was sufficient, with both undiluted and 20mM MJ solution, to severely restrict germ tube development. It is possible that in the *in vivo* trial the delay between inoculation and exposure to MJ vapour allowed the germination process to begin, thus the tip of the germ tube may have been present as the plant was exposed to the vapour. As in the case of liquid elicitors localised resistance may have had some part to play in the restriction of pathogen growth. Another possible though less likely explanation is that the vapour left behind some form of residue on the leaf surface which had a direct antifungal effect on the pathogen.

4.3 *Effects of Two Jasmonic Acid Precursors*

Two fatty acid precursors of jasmonic acid were assessed for their ability to induce systemic resistance. All concentrations of linolenic acid showed significant reductions in

mildew development, with the largest reductions obtained using the 5mM concentration. Linoleic acid, however, gave no reduction in disease development at any of the concentrations tested. In fact slight increases in mildew development were observed with all concentrations of linoleic acid tested. Farmer and Ryan (1992) examined the ability of jasmonic acid precursors to induce the accumulation of proteinase inhibitors, defense related compounds induced as part of a SAR type response to insect feeding. They found that linolenic acid strongly induced their accumulation, while linoleic acid had little effect. Although different sets of genes are involved here, the principle remains the same. It appears that linolenic acid, which is closer in structure to jasmonic acid than linoleic acid, has the ability to mimic the action of jasmonates.

4.4 Growth Analysis

Treatment of the lower three leaves of young barley plants with the elicitors led to increases in plant dry weight. Although the dry weight increases following treatment with NaSA and MJ were small, those obtained following application of PP were larger. Challenge-inoculation of the upper leaves with mildew reduced dry weight slightly in both control plants and in treated plants, although the dry weights of the treated plants were still greater than controls. Interestingly, Reuveni et al. (1993) showed that treatment of the first leaf of cucumber seedlings with 100 mM K_2PO_4 led not only to systemic protection of the upper leaves against the powdery mildew fungus *Sphaerotheca fuliginea*, but it also resulted in increased plant growth. In the present study, the leaf area of the plants treated with NaSA and PP were little different from the controls, whereas for the MJ-treated plants, leaf areas were substantially reduced compared to controls. The results for PP treatment suggest the greater dry weight of these plants must be due to increased rates of photosynthesis. Certainly, the calculated leaf area ratios, leaf

weight ratios and specific leaf areas of these plants confirm that leaves in PP treated plants may not be larger in terms of area, but they are heavier i.e. they contain more dry weight. The calculated values for net assimilation rate for the PP treated plants fluctuate over the experimental period, with no consistent differences between the controls and the treated plants. Although this might appear to suggest that increases in photosynthesis in PP treated plants are unlikely, it must be remembered that NAR is a calculated measure of the rates of net photosynthesis and respiration for all leaves on the plant (Hunt, 1975). Given that infection increases rates of dark respiration in plants (Walters, 1985), and we know nothing of the rates of respiration in elicitor treated plants, it would seem prudent for future studies to examine CO₂ exchanges in such plants.

Treatment with MJ led to substantial reductions in leaf area, with LARs and SLAs indicating that these plants invested much less energy in leaf area than the controls. Since there were small increases in shoot dry weight with MJ treatment, it seems plausible to suggest that rates of photosynthesis were increased. Indeed, LWRs point to these leaves being heavier than control leaves. But exogenously applied jasmonates have been shown to reduce rates of photosynthesis in treated tissues (Popova et al., 1989), and what effect MJ treatment of lower leaves has on photosynthesis in upper leaves is unknown. Jasmonates are also known to inhibit expression of genes encoding proteins involved in photosynthesis, and to stimulate the accumulation of vegetative storage and other proteins (Creelman and Mullet, 1997). If this is so, then perhaps we should not be looking towards a change in photosynthesis as a mechanism for the increased dry weight in MJ treated plants, but towards an increased accumulation of, for example, storage proteins. This is certainly deserving of further investigation.

The effects of NaSA treatment on barley were small. Plant dry weight was increased slightly and leaf areas were unaffected. Although there are reports that exogenous SA can increase plant growth, it is more commonly reported to be phytotoxic (Pierpoint, 1994). SA is known to close stomata in some species (Pierpoint, 1994), but it is not known whether it does so in barley. Even if the NaSA treatments in this study led to decreased stomatal aperture, this effect has been shown to be transient in other species (Pierpoint, 1994). In any event it seems clear from the results presented here that NaSA is unlikely to perturb the rates of photosynthesis.

4.5 *Enzyme Activity*

It is interesting to observe that when control plants were challenge-inoculated, enzyme activity at the end of the experiment was often as high as, if not higher than that observed in elicitor treated plants. Therefore, susceptible plants do respond to pathogen attack, but it is the speed of the response which is the critical factor in determining the success or failure of pathogen containment. The responses observed in control plants would be too slow to contain or restrict pathogen growth. The pathogen would have successfully established itself before enzyme activity was sufficient to limit fungal growth.

In many cases leaf 2 i.e. the leaf which was challenge-inoculated, showed the largest increases in enzyme activity. This would be expected since it was under the greatest pathogen pressure and hence the magnitude of the response should be greatest. This was true for both control plants and elicitor treated plants. In challenge-inoculated control plants, enzyme activity in leaves 1 and 3 barely rose above the values recorded at the beginning of the experiment. Uninoculated control plants showed little fluctuation in enzyme activity, probably because of the absence of disease pressure.

From this study it was not possible to conclude which of the enzymes monitored, if any, were responsible for the restriction of pathogen growth observed. It is unlikely that any of the observed increases in enzyme activity were responsible for the reductions in powdery mildew development, since they occurred too late to account for the reductions in mildew levels observed. More probably the observed increases were part of a secondary response, responsible for the long term protection of the plant. The possibility exists that there already had been a burst in enzymic activity in the early stages of pathogen attack, but that was not monitored in this study. It is also possible that the enzymes monitored are not those responsible for the primary responses.

Reglinski et al., (1997) reported similar findings for PAL when Salicylic acid was applied. 2mM Salicylic acid applied to the leaves of Kiwi fruit resulted in a 10 fold increase in PAL activity after 2 days. This is consistent with reports for other plant species. (Kauss et al., 1993). Anfoka and Buchenaur (1997) found significant increases in both PAL and peroxidase levels in tomato plants treated with TNV which were expressing SAR.

Both Heitz et al., (1997) and Bohland et al., (1997) found that different elicitors appear to activate different lipoxygenase species. When chitin oligosaccharides or methyljasmonate were applied to wheat the increase in lipoxygenase activity was more rapid than when germ tube extract was used. In addition different lipoxygenase species were induced, supporting the theory that different types of exogenously applied elicitor exert their effects via different reaction cascades, and that previously inconsistent results may at least partially be explained by this (Bohland et al., 1997).

4.6 Field Trials

In the 1993 field trial, levels of powdery mildew observed on control plants were significantly higher than the levels on plants treated with elicitors of resistance. Although both elicitors of resistance exhibited significant reductions in mildew development, it appears that 15mM NaSA performed marginally better than 25mM PP.

There were no significant differences in plant height or plant dry weight between control plants and those treated with elicitors in 1993. This was also observed in 1994. Grain weight in elicitor treated plants was significantly increased in 1994, though not in 1993.

There are three possible reasons for the increase in grain weight:

- A- A decrease in the level of disease in the flag leaf means that more assimilates were available for grain fill
- B- An increase in photosynthesis to compensate for SAR
- C- A combination of the A and B

The second application of elicitors coincided with the development of the flag leaf. As the flag leaf emerged, the crop was sprayed. As a result, the flag leaf was systemically protected against pathogen attack. A decrease in disease in the flag leaf is known to be directly linked to an increase in grain fill, since the flag leaf is responsible for the synthesis of assimilates for grain fill, resulting in an increase in yield (Hay and Walker, 1989). It is possible therefore that a similar situation occurs in barley, and with the increased photosynthesis, SAR would not occur at the expense of plant yield. An increase in photosynthesis in systemically protected leaves would also compensate for the reduction in photosynthesis in mildewed leaves. It is unlikely that either A or B are solely responsible for the increase in yield and it is more probable that a combination of

the two brought about the observed results. It is recognised that infection by biotrophs results in a decline in the rate of net photosynthesis in the host (Walters, 1985). Bearing this in mind, a heavy infection of the flag leaf would result in a decrease in assimilates required for grain fill, and hence a reduction in yield. This is likely to be the reason for the observed reduction in yield of control plants.

In 1994, in addition to the two elicitors of resistance, 15mM NaSA and 20mM MJ, 3 commercial fungicides were included in the field trial. The leaf area infected by powdery mildew was decreased with all treatments. Results were mixed however, and at some assessments the commercial fungicides gave better results than the elicitors of resistance and vice versa. However, by assessment 4, 20mM MJ had outperformed any of the commercial fungicides included in the trial. Powdery mildew control provided by NaSA was only slightly inferior to that provided by Folicur.

Plant height in 1994 was unaffected by treatment with either elicitor or commercial fungicide, with the exception of MJ treated plants. MJ treated plants were significantly smaller than both control plant and other treatments. It is possible this is a reflection of the influence on plant physiology that MJ has. Treatment of plants with MJ has been associated with a number of plant responses including inhibition of growth, early senescence, stomatal closure and loss of chlorophyll (Anderson, 1989; Horton, 1991; Herrmann et al., 1989).

All elicitor and fungicide treated plants exhibited an increase in grain weight. Elicitor treated plants exhibited a significantly higher grain weights than fungicide treated plants. In fungicide treated plants it is probable that it was the reduction in disease on the flag leaf which was responsible for the observed increase in grain weight. With elicitor treated

plants it is possible that increased photosynthesis in systemically protected tissue, if this indeed occurred in the field trial, would, in combination with the reduction in mildew on the flag leaf, account for the increase in grain weight.

However, it must be noted that environmental factors outwith our control may have influenced the results observed. It is possible that they exacerbated or masked the magnitude of some of the results observed. To evaluate the suitability of SAR for field scale use, a long term study would be required in order to eliminate variability brought about by environment.

It is important to note that the reductions in mildew development following the application of the inducer may not have been solely the result of SAR induction. To consider the field trial as purely an exercise in SAR, it would have been necessary to paint individual leaves with the elicitor. This would have been impractical and not viable on a field scale. Hence, the elicitors were treated in the same way as any other fungicide and evaluated on the same basis. It is likely that the reductions observed in mildew infection were due to a combination of 3 factors:

- 1- Direct effect of the chemicals on the pathogen. It was shown in earlier experiments that all chemicals exhibit some degree of antifungal activity
- 2- Local acquired resistance
- 3- Systemic acquired resistance

It is virtually impossible therefore to determine what proportion of the disease control observed could be attributed to the development of SAR. Whatever the mode of action of the chemicals screened they appear to offer considerable potential as a means of disease control.

4.7 Conclusions

The definition of disease resistance is highly subjective and is more often classified by economic restrictions imposed upon a crop than by the level of disease the plant is naturally capable of fighting. It is these financial and economic considerations which place growers and farmers in an increasingly difficult position when choosing cultivars for production. It is a popular misconception that apparently susceptible plants lack the genetic information and hence the mechanisms for fighting disease. However, it is now apparent that it is the fine control of these defense mechanisms and genetic information, rather than a complete lack of them *per se* which determines resistance under a given set of environmental conditions.

More emphasis has been placed, historically, on the discovery of new resistance genes than the use of potential which already exists within the plant. Although the use of induced resistance in the field is still in its infancy, indications are that it is an effective means of controlling disease. At present there are no indications that induced resistance produces metabolites or toxins which taint food. The commercially available elicitors of resistance eg the Novartis produced product Bion® operate via the production of PR-proteins (Lawton et al., 1996), which represent no problems regarding food safety.

Despite the fact that the mode of action of the elicitor is to induce naturally occurring defence mechanisms which are latent in the plant, they are still subject to the same legislation which applies to 'normal' pesticides i.e. Plant Protection Products Regulations 1995 (European legislation which governs pesticides with new active ingredients). Despite many abiotic elicitors being commercially available products e.g. Acetylsalicylic acid or Potassium phosphate, they would still require full safety and toxicological data

packages to be submitted to the Pesticide Safety Directorate prior to an approval for their use as a pesticide being granted. These data packages may be cost prohibitive. Indeed for this reason Bion® in Germany is marketed as a plant tonic and not as a pesticide (Lyon and Newton, 1997), thus abolishing the requirement to go through the lengthy and expensive process of pesticide registration. Indeed this would appear to be an increasingly common route to launching new products on the market. The phosphate based product Phosphyte® is marketed as a readily available form of phosphate used primarily in strawberries. However, it is commonly recognised that one of the breakdown products of this 'fertiliser' is phosphorus acid- a highly effective treatment for the control of red core of strawberries. Indeed phosphorus acid is the active ingredient in another highly successful pesticide used for the control of red core i.e. Alliette.

Because of the variability which can sometimes be detected in elicitors as a result of seasonality or environment, they may appear less attractive than the current range of commercially available pesticides. It is likely that a number of the field scale trials reported have used unformulated elicitors. The formulation of elicitors may decrease the variability of the observed effects, this however is an area which still requires a significant amount of research.

The nature of elicitors of resistance is to act by priming plant defence mechanisms to respond more rapidly to pathogen attack. To be useful, therefore, it has to be applied prior to pathogen attack, unlike some commercial products which have curative as well as protectant properties. It would appear feasible that elicitors of resistance have a place, in the future, in integrated crop management. There is at present an acute awareness of the possibility of pathogens developing resistance to fungicides. It may be possible at a later date to combine elicitors of resistance with commercial fungicides to produce a broad

spectrum product with multi-site activity, which will lessen the possibility of pesticide resistance developing.

The phenomenon of SAR presents plant pathologists with an exciting system of disease control which is not open to breakdown due to the development of resistant strains of pathogen. Kuc (1987) has already outlined the merits and demerits of SAR as a potential form of disease control in the field. Amongst the more obvious is the fact it protects plants from a wide range of plant pathogens including viral, bacterial and fungal diseases; it depends on more than one mode of action, therefore it is more stable than other systemic fungicides; it is persistent; it is systemic and it utilises the potential for disease resistance which is present in all plants, hence it may be considered natural, depending on the elicitor used, and so safe for man and the environment. The disadvantages are predominantly associated with insufficient scientific data to support the observation in the field, economic feasibility and social attitudes towards the phenomenon. SAR has provided the possibility of developing a form of disease control which relies on the expression of latent genetic information for the expression of disease resistance in high quality and high yielding plants.

5. FUTURE WORK

5 FUTURE WORK

In continuing the work undertaken for this thesis, there are a number of areas where further investigation would provide information useful in determining the viability of SAR as a potential means of disease control in the field.

In discussing the results of the growth analysis experiments and the field trial, it seems possible that changes in photosynthesis and assimilate partitioning were important. Future work should study the direct influence of SAR on photosynthesis and respiration since this would provide a more accurate impression of how growth is altered in plants expressing SAR.

A more detailed study of defence related enzyme activity in SAR plants would broaden the picture and provide useful information on the defence reactions initiated in barley plants. Many studies to date have reported increases in mRNAs of defence related compounds. However, many of these studies have not gone on to determine whether they are subsequently transcribed and synthesised. Future studies should include an examination of lignification and papilla formation, since they are important in resistance of barley to powdery mildew and since both PAL and peroxidase are involved in lignin formation (Carver et al., 1995).

Further consideration should also be given to further detailed studies on MJ vapour. It would be useful to carry out a comparative study of the defence mechanisms expressed by treatment with MJ solutions and those expressed following treatment with MJ vapour. MJ vapour would appear to be a potent elicitor of SAR, and therefore it would be

interesting to consider whether the mode of action and the defences induced are the same as those induced by liquid MJ.

A preliminary study (results not shown) into the direct effects of MJ vapour on the development of *E. graminis* germination on the leaf surface indicated that certain stages were more susceptible to MJ vapour damage than others. The early indications are that the formation of the appressorial initials and development of elongating secondary hyphae are more seriously affected by exposure to MJ vapour, than conidial germination or maturation of appressoria. These data need to be confirmed by further detailed examination.

This study highlighted how two of the most fundamental environmental factors, temperature and photoperiod, influenced the development of SAR. Before giving SAR further consideration as a potential means of disease control in the field, it would be essential to determine how other environmental factors, such as light intensity, nutritional status of the soil and water stress, influence the ability of the plant to express SAR and maintain normal growth. Though seemingly unimportant, these environmental factors are the key to our ability to use SAR in the field as an alternative means of disease control. Factors such as temperature would influence where in the world SAR could be used, photoperiod and light intensity would be affected by the seasons thus influencing when in the year it could be used most effectively.

6. BIBLIOGRAPHY

6 BIBLIOGRAPHY

- Adam, A., Farkas, T., Somlyai, G., Havesi, T., Kiraly, Z. (1998). Consequence of O₂⁻ generation during bacterially induced hypersensitive reaction of tobacco: determination of membrane lipids. *Physiological and Molecular Plant Pathology* 50:234-239.
- Aist, J.R., Bushnell, W.R. (1991). Invasion of plant hosts by powdery mildew fungi and cellular mechanisms of resistance. In: *The Fungal Spore and Disease Interaction in Plants and Animals*. Cole, G.T., Hoch, H.C. Plenum Press. New York.
- Akai, S. (1952). *Agri. Hort. Tokoyo* 27: 11-35.
- Anderson, J.M. (1985). Evidence for phloem transport of jasmonic acid. *Plant Physiology* 77 S. 75.
- Anderson, J.M. (1989) Membrane derived fatty acids as precursors to second messengers In: *Second messengers in plant growth and development*. Boss, W.E. and Moore, D. (eds.) pp181-212 Alan R. Liss, Inc. N.Y.
- Anfoka, G., Buchanauer, H. (1997). Systemic acquired resistance in tomato against *Phytophthora infestans* by pre-inoculation with tobacco necrosis virus. *Physiological and Molecular Plant Pathology* 50: 85-101.
- Aydiushko, S.A; Ye, X.S.; Hudebrand, D.F. and Kúc, J. (1993). Induction of lipoxygenase activity in immunised cucumber plants. *Physiological and Molecular Plant Pathology* 42: 83-95.
- Ayres, P.G. (1979). CO₂ exchange in plants infected by obligately biotrophic pathogens In: *Photosynthesis and plant development*, Marcelle, H.; Clysters, H and Nan Paucke, M.N. (eds.) pp343-354 W. Junk, The Hague.
- Baardseth, P., Russwurm, H. Jr. (1978). Content of some organic acids in Cloudberry (*Rubus chamaemorus* L.) *Food Chemistry* 3: 43-46.
- Bailey, J. (1982). Mechanisms of phytoalexin accumulation. In: *Phytoalexins* (Bailey, J. and Mansfield, J. eds.) pp289-318 Blackie and Sons, Glasgow.

- Bainbridge, A., Legg, B.J. (1976).** Release of Barley- Mildew conidia from shaken leaves. *Transactions of the British Mycological Society*, 66: 495-498.
- Bartnicki-Garcia, S. (1969).** Cell wall chemistry morphogenesis and taxonomy of fungi. *Annual Review of Microbiology*. 22: 87-108.
- Bayles, C.J., Ghemcawat, M.S., Aist, J.R., (1990).** Inhibition by 2-deoxy-D-glucose of callose formation papillae deposition and resistance to powdery mildew in an ml-o barley mutant. *Physiological and Molecular Plant Pathology* 36: 63-72.
- Beardmore, J., Ride, J.R., Granger, J.W., (1983).** Cellular lignification of a factor in the hypersensitive resistance of wheat to stem rust. *Physiological and Molecular Plant Pathology*. 22: 209-220.
- Bohland, C., Balkenhol, Th., Loers, G., Feussner, I., Grambow, H.J. (1997).** Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharides, chitosan and methyljasmonate. *Plant Physiology*. 114: 679-685.
- Bol, J.F., Linthorst, W.J.M., Cornelissen, B.J.C. (1990).** Plant pathogenesis related proteins induced by virus infection. *Annual Review of Phytopathology*. 28:113-138.
- Boller, T. (1987).** Hydrolytic enzymes in plant disease resistance. In: Plant- microbe interactions and genetic perspectives. Vol. 2 (Kosuge, T. and Nester, E.W., Eds) pp 385-413 MacMillan, New York.
- Boller, T., Metraux, J.P. (1988).** Extracellular localisation of chitinase in cucumber. *Physiological and Molecular Plant Pathology*. 33: 11-16.
- Bougehey, A.S. (1949).** The ecology of fungi which cause economic plant diseases. *Transactions of the British Mycological Society*. 32: 179-189.
- Bower, C.A., Fireman, M. (1957).** Saline and alkali soils. In: Soils U.S.D.A. Year book of agriculture.

- Bowles, D.J. (1990).** Defense related proteins induced by virus infection. *Annual Review of Biochemistry*. 59:873-907.
- Boyd, L.A., Smith, P.H., Green, R.M., Brown, J.K.M. (1984).** The relationship between the expression of defense-related genes and powdery mildew development in barley. *Molecular Plant-Microbe Interactions*. 7: 401-410.
- Bracker, C.A. (1968).** Ultrastructure of the haustorial apparatus of *Erysiphe graminis* and its relationship to the epidermal cell of barley. *Phytopathology* 58: 12-30.
- Brown, N.A. (1923).** Experiments with paris daisy and rose to produce resistance to crown gall. *Phytopathology* 13: 87-99.
- Carleton, M.A. (1916).** The small grains. *The MacMillan Company, New York*
- Caruso, F., Kúc, J. (1977).** Protection of watermelon and musk melon against *Colletotrichum lagenarium* against *Colletotrichum lagenarium*. *Phytopathology*. 67: 1285-1289.
- Caruso, F., Kúc, J. (1979).** Induced resistance of cucumber to anthracnose and angular leaf spot by *Pseudomonas lachrymans* and *Colletotrichum lagenarium*. *Physiological Plant Pathology*. 14:191-201.
- Carver T.L., Carr, A.J. (1987).** Effect of host resistance on the development of haustoria and colonies of oat mildew. *Annals of Applied Biology*. 88: 171- 178.
- Carver, T.L.W., Zeyen, R.J., Lyngkjaer, M.F. (1995).** Plant cell defences to powdery mildew of *Gramineae*. *Aspects of Applied Biology. Physiological Responses of Plants to Pathogens*. 42:257-266.
- Castresana, C., de Carvalho, F., Gheysien, G., Habetz, M., Inze, D., van Montague, M. (1990).** Tissue- specific and pathogen- induced regulation of a *Nicotiana plumbaginifolia* β -1,3-glucose gene. *Plant Cell*. 2: 1131-1143.
- Chada, K.C., Brown, S.A. (1974)** Biosynthesis of phenolic acids in tobacco plants with *Agrobacterium tumefaciens*. *Canadian Journal of Botany*. 52: 2041-2046.

- Chen, J., Klessig, D. (1991).** Identification of a salicylic acid-binding protein that may function in signal transduction in the plant disease- resistance response. *Proceedings of the National Academy of Science. U.S.A.* 88: 8179-8183.
- Chen, Z., Silva, H., Klessig, D. (1994).** Involvement of active oxygen species in the induction in the induction of systemic acquired resistance by salicylic acid in plants. *Science* 242: 1883-1886.
- Cherf, M., Benhamou, N., Menzies, J.G., Belander, R.R. (1992).** Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiological Molecular Plant Pathology.* 41: 411-425.
- Cherwick, W.J. (1944).** Canadian Journal of Research. 22: 52-86.
- Chester, K.S. (1933).** The problem of acquired physiological immunity in plants. *Quarterly Review of Biology.* 8: 275-324.
- Clark, T.A., Zeyen, R.J., Carver, T.L.W., Smith, A.G., Bushnell, W.R. (1995).** Epidermal cell cytoplasmic events and response gene transcript accumulation during *Erysiphe graminis* attack in isogenic lines differing at the ml-o locus. *Physiological and Molecular Plant Pathology.* 46: 1-16.
- Clark, T.A., Zeyen, R.J., Smith, A.G., Bushnell, W.R., Szabo, I.J., Vance, C.P. (1993).** Host response gene transcript accumulation in relation to visible cytological events during *Erysiphe graminis* attack in isogenic barley lines differing at the ml-a locus. *Physiological and Molecular Plant Pathology.* 43: 283-298.
- Clark, T.A., Zeyen, R.J., Smith, A.G., Carver, T.L.W., Vance, C.P. (1994).** Phenylalanine ammonia lyase mRNA accumulation enzyme activity and cytoplasmic responses in barley isolines differing at ml-a and ml-o loci attacked by *Erysiphe graminis* f.sp. *hordei*. *Physiological and Molecular Plant Pathology.* 44: 171-185.
- Cleland, C.F. (1974).** Isolation of flower inducing and flower inhibiting factors from aphid honeydew. *Plant Physiology.* 54: 899-903.

- Cohen, Y., Gisi, U., Mossinger, E., (1991).** Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. *Physiological and Molecular Plant Pathology*. 38: 255-263.
- Cooper, J.B., Varner, J.E. (1984).** Cross-linking of soluble extensin in isolated cell walls. *Plant Physiology*. 76: 414.
- Coxon, D. (1982).** Phytoalexins from other families. In: Phytoalexins (Bailey, J., Mansfield, J. Eds.) pp106-132. Blackie and Sons, Glasgow.
- Creelman, R.A., Mullet, J.E. (1997).** Biosynthesis and activation of Jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*. 118: 355-381.
- Croft, K.P.C., Voisey, C.R., Slusarenko, A.J. (1990).** Mechanisms of hypersensitive cell collapse: correlation of increased lipoxygenase activity with membrane damage in leaves of *Phaseolus vulgaris* (L) inoculated with avirulent races of *Pseudomonas syringae* pv. *phaseolicola*. *Physiological and Molecular Plant Physiology*. 36: 49-62.
- Cruickshank, I.A.M., Maudryk, M. (1960).** The effect of stem infection of tobacco with *Peronospora tabacina* Adam on foliage reaction to the mould. *Journal of Australian Institute of Agriculture Science*. 26: 369-372.
- Cruickshank, I.A.M., Perrin, D.R. (1965).** Studies of phytoalexins. VIII. The effect of some further factors on the formation, stability and localisation of pisatin *in vivo*. *Australian Journal of Biological Science*. 18: 817-828.
- Curtis, R.W. (1984).** Abscission-inducing properties of methyljasmonate, ABA and ABA-methylester and their interactions with ethephon, Ag-NO₃ and Malformin. *Journal of Plant Regulation*. 3: 157-158.

- Dalisay, R.F., Kúc, J. (1995). Persistence of induced resistance and enhanced peroxidase and chitinase activities in cucumber plants. *Physiological and Molecular Plant Pathology*. 47: 347-357
- Daly, J.M. (1976). The carbon balance of diseased plants: Changes in respiration, photosynthesis and translocation. In: *Physiological Plant Pathology*. (Heitfuss, R., Williams, P.H. Eds.) pp450 Springer-Verlag, Berlin.
- Darpoux, H., Faivre-Amiot, A. (1952). *Phytopharm 1: 21-24.*
- Dathe, W., Ransom, H., Preiss, A., Schode, W., Sembdner, G., Schriber, K. (1981). Endogenous plant hormones of the broad bean *Vicia faba*, L., (-) jasmonic acids, a plant growth inhibitor in pericarp. *Planta 153: 530-535.*
- Datta, K.S., Nanda, K.K. (1985). Effect of some phenolic compounds and gibberellic acid on growth and development of cheena millet (*Panicum millaceum* L.). *Indian Journal of Plant Physiology*. 28: 298-302.
- Davies, D., Chait, L., Rothrock, J.w., Deaks, J., Halmos, S., Garber, J.D. (1960). Chemotherapy of cereal rusts with a new antibiotic. *Plant Physiology*. 50: 841-843.
- De Cal, A., Pascual, S., Melgarejo, P. (1997). Involvement of resistance induction by *Penicillium oxalicum* in the biocontrol of tomato wilt. *Plant Pathology*. 46: 72-79.
- Dean, R.A., Kúc, J. (1986). Induced systemic protection in cucumber: Time of production and movement of the signal. *Phytopathology*. 76: 966- 970.
- Delaney, T., Ulncs, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessman, H., Ward, Ryals. (1994). A central role for salicylic acid in plant disease resistance. *Science*. 266: 1247-1250.
- Demole, E., Lederer, E., Mercier, D. (1962). Isolation and determination of the structure of the methyljasmonate component of the essential oil of jasmine. *Heb. Chem. Acta*. 45: 675-685.
- Dickson, J.G. (1956). Diseases of field crops. McGraw-Hill. New York.

- Dickinson, S. (1960).** The mechanical ability to breach the host barriers. In: *Plant pathology, an advanced treatise*. Vol. II. (Horsfall, J.G., Diamond, A.E. Eds.) pp203-232. Academic Press. New York.
- Dixon, R.A. (1986).** The phytoalexin response- elicitation, signalling and control of host gene expression. *Biological Reviews of the Cambridge Philosophical Society.* 61: 239-291.
- Dixon, R.A., Harrison, M.J., Lamb, C.J. (1994).** Early events in the activation of plant defense responses. *Annual Review of Phytopathology.* 32: 479-501.
- Doodson, J.K., Saunders, P.J.W. (1970).** *Annual Review of Plant Pathology.* 66: 361-374.
- Doubrava, N.S., Dean, R.A., Kúc, J. (1988).** Induction of systemic resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves. *Physiological and Molecular Plant Pathology.* 33: 69-79.
- Edwards, H.H. (1970).** A basic staining material associated with the penetration process in resistant and susceptible powdery mildewed barley. *New Phytologists.* 69: 299-301.
- Einhellig, F.A. (1986).** Mechanisms and modes of action of allelochemicals. In: *The science of allelopathy.* (Putman, A.R., Tang, S.S. Eds.). John Wiley and Sons, New York. 317pp
- Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, I. (1992)** Localisation, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Proceedings of the National Academy of Science, U.S.A.* 89:2480-2484.
- Esquerré-Tugayé, M.T., Lafitte, C., Mazau, D., Tappau, S., Touze, A. (1979).** Cell surfaces in plant- microorganism interactions. *Plant Physiology,* 64: 320-326.

- Ewart, G., Raitt, J.S. (1969).** Water soluble polyphosphate and methods of preparation. *U.S. Patent.* 3: 432, 261.
- Falkenstein, E., Groth, B., Mithöfer, A., Weiler, E. (1991).** Methyljasmonate and linoleic acid are potent inducers of tendrill coiling. *Planta.* 185: 316-322
- Falkhof, A. (1990).** Einfluss induzierter Resistenz auf den Mehltaubefall und die Leistung von Gerste bei verschiedenen Umweltbedingungen, Diss. Univ., Hanover, 1990. Cited in Kehlenbeck, H., Krone, C., Oerke, C., Schonbeck, F. (1994). The effectiveness of induced resistance on yeild of mildewed barley. *Journal of Plant Diseases and Protection.* 101: 11-21.
- Farmer, E.E., Johnson, R.R., Ryan, C.A. (1992).** Regulation of expression of proteinase inhibitor genes by methyljasmonate and jasmonic acid. *Plant Pathology.* 98: 995-1002.
- Farmer, E.E., Ryan, C.A. (1990).** Interplant communication: Air borne methyljasmonate induces synthesis of proteinase inhibitors in plant leaves. Proceedings of the National Academy of Science. *U.S.A.* 87: 7713-7716.
- Farmer, E.E., Ryan, C.A. (1992).** Octodecanoid precursors of jasmonic acid activates the synthesis of wound-induced proteinase inhibitors. *Plant Cell.* 4: 129-134.
- Farrar, J.F. (1992).** Beyond photosynthesis: the translocation and respiration of diseased leaves. In: Pests and Pathogens Ed. P.G. Ayres. Oxford Press.
- Ferraris, L., Abbaltisa Gentile, I., Matta, A. (1987).** Activation of glycosides as a consequence of infection stress in Fusarium wilt of tomato. *Journal of Phytopathology.* 118:317-325.
- Gaffney, T., Fredrich, L., Vernooj, B., Negretto, D., Nye, G., Uknes, S., Ward, E., Kessmann, HJ., Ryals, J. (1993).** Requirement of salicylic acid for the induction of systemic acquired resistance. *Science.* 261: 754-756.

- Gair, R.J., Jenkins, E.E., Lester, E. (Eds.) (1987). General pests and diseases (4th Edition). Page Brothers (Norwich) Ltd., England.
- Gessler, C., Kúc, J. (1982). Induction of resistance to *Fusarium* wilt in cucumber by root and foliar pathogens. *Phytopathology*. 72: 1439-1441.
- Glass, A.D.M. (1973). Influence of phenolic acids on uptake: 1. Inhibition of phosphate uptake. *Plant Physiology*. 51: 1037-1041.
- Goodman, R.N., Kiraly, Z., Zaitlin, M. (1967). The biochemistry and physiology of infections. D. Van Nostrand Co. Princeton, New Jersey. 345p
- Gottstein, H.D., Kúc, J. (1980). Induction of systemic resistance to anthracnose by phosphates. *Phytopathology*. 79: 176-179.
- Graham, T.L., Sequira, L., Huang, T.S.R. (1977). Bacterial lipopolysaccharides as inducers of disease resistance in tobacco. *Applied Environmental Biology*. 34: 424-432.
- Graham, T.L., Sequira, L., Haug, T.R. (1977). *Physiological Plant Pathology*. 14: 19-30.
- Grebenchuck, E.A. (1965). Vest. Khar. Kov. Institute of Biology. Set 1: 53-56.
- Green, N.E., Hadwiger, L.A., Graham, S.O. (1975) Phenylalanine ammonia lyase, tyrosine ammonia lyase and lignin in wheat inoculated with *Erysiphe graminis* f.sp. *tritici*. *Phytopathology*. 65: 1071-1074
- Grimes, H.D., Koetje, D.S., Franceschi, V.P. (1992). Expression, activity and cellular accumulation of methyljasmonate responsive lipoxygenase in soybean seedlings. *Plant Physiology*. 100: 433-443.
- Gross, G.G. (1981). Phenolic acids. In: the biochemistry of plants. Vol. 7: 301-316. Academic Press, New York.
- Guedes, M.E., Richmond, S., Kúc, J. (1980). Induced systemic resistance to anthracnose in cucumbers is influenced by the location of the inducer inoculation with

Colletotrichum lagenarium and onset of flowering and fruiting. *Physiological Plant Pathology*. 17: 229-233.

Guitard, A.A. (1960). The influence of variety, temperature and stage of growth on the response of spring barley to photoperiod. *Canadian Journal of Plant Science*. 40: 65-80.

Gundlach, H., Müller, M.J., Kutschau, T.M., Zenk, M.H. (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceedings of the National Academy of Science. U.S.A.* 89: 2389-2393.

Hammett, K.R.W., Manners, J.G. (1973). Conidium liberation in *Erysiphe graminis* II. Conidial chain and pustule structure *Transactions of the British Mycological Society*. 61: 121-133.

Hammett, K.R.W. Manners, J.G. (1974). Conidium liberation in *Erysiphe graminis* III. Wind tunnel studies. *Transactions of the British Mycological Society*. 62: 267-282.

Hammerschmidt, R., Acres, S., Kúc, J. (1976). Protection of cucumber against *Colletotrichum lagenarium* and *Cladosporium cucumerinum*. *Phytopathology*. 66: 790-793.

Hammerschmidt, R., Kúc, J. (1980). Enhanced peroxidase activity and lignification in the induced systemic protection of cucumbers. *Phytopathology*. 70: 689.

Hammerschmidt, R., Kúc, J. (1982). Lignification as a mechanism for induced systemic resistance in cucumber. *Physiological Plant Pathology*. 20: 61-71.

Hammerschmidt, R., Nuckles, E.M., Kúc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumbers to *Colletotrichum lagenarium*. *Plant Pathology*. 20: 73-82.

Hansch, C., Anderson, S. (1967). The effect of intramolecular hydrogen bonding on partition coefficients. *Journal of Organic Chemistry*. 32: 2583-2586.

- Haralan, J.R. (1968).** The origin of barley. U.S.D.A. Agriculture handbook. 338pp
127.
- Haralan, J.R. (1976).** In : Evolution of crop plants. (Simmons, N.W. Ed.). pp93-98.
Longman, London and New York.
- Haralan, J.R., Zohary, D. (1966).** Distribution of wild wheats and barley. *Science*.
153: 1074-1080.
- Harper, J.R., Balke, N.E. (1981).** Characterization of the inhibition of K⁺ absorption in
oat roots by salicylic acid. *Plant Physiology*. 68: 1349-1353.
- Haslam, E. (1974).** In: The Shikimate pathway. Butterworth, London. 316pp.
- Hay, R.K.M., Walker, A.J. (1984).** An introduction to the physiology of crop yield.
Longman.
- Hecht, E.J., Bateman, D.F. (1964).** Non-specific acquired resistance to pathogens
resulting from localised infection by *Thielaviopsis basicola* or viruses in tobacco leaves.
Phytopathology. 54: 523-530.
- Heitz, T, Bergey, D.R., Ryan, C.A. (1997).** A gene encoding a chloroplast-targeted
lipoxygenase in tomato leaves is transiently induced by wounding, systemin and methyl
jasmonate. *Plant Physiology*. 114: 1085-1093.
- Helbaek, H. (1966).** Commentary on the diseases of triticum and hordeum. *Economic
Botany*. 20: 350-360.
- Hermann, G., Lehmanf, J., Peterson, A., Sembdner, G., Weidhase, R.A., Parthier,
B. (1989).** Species and tissue specificity of jasmonate-induced abundant proteins.
Journal of Plant Physiology. 134:703-709.
- Hermansen, J.E., Wilberg, A. (1972).** *Fresia*. 10: 30-34. Cited in The powdery
mildews. (Spencer, d.m. Ed.)

- Ho, L.C. (1988). Metabolism and compartmentalisation of imported sugars in sink organs in relation to sink strength. *Annual Review of Plant Physiology and Plant Molecular Biology*. 39: 355-378.
- Horton, R.F. (1991). Methyljasmonate and transpiration in barley. *Plant Physiology*. 96:1376-1378.
- Hsu, F., Kleier, D.M. (1990). Phloem mobility of xenobiotics. III. Sensitivity of unified model to plant parameters and application to patented chemical hybridizing agents. *Weed Science*. 38:315-323.
- Hunt, R. (1975). Further observations on root-shoot equilibria in perennial ryegrass (*Lolium perenne* L.) *Annals of Botany*. 39: 745-755.
- Ingham, J. (1982). Phytoalexins from the leguminosae. In: Phytoalexins (Bailey, J., Mansfield, J. Eds.) pp21-80. Blackie and Sons, Glasgow.
- Irving, H.R., Kúc, J. (1990). Localised and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2PH_4 . *Physiological and Molecular Plant Pathology*. 37:355-366.
- Jenns, A.E., Caruso, F.L., Kúc, J. (1979). *Phytopathologia Mediterranae* 18:129-134.
- Jenns, A.E., Kúc, J. (1977). Localised infection with tobacco necrosis virus protects cucumber against *Colletotrichum lagenarium*. *Physiological Plant Pathology*. 11: 207-212.
- Jenns, A.E., Kúc, J. (1979). Graft transmission in systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium*. *Phytopathology*. 69:753-756.
- Jenns, A.E., Kúc, J. (1980). Characterisation of anthracnose resistance induced by localised infection with tobacco necrosis virus. *Physiological Plant Pathology*. 17: 81-91.

- Ji, C., Kúc, J. (1997). Non-host resistance to *Colletotrichum lagenarium* in pumpkin and squash is not primarily associated with β -1,3-glucanase and chitinase activities. *Physiological and Molecular Plant Pathology*. 50: 361-370.
- Jones, D.G., Clifford, B.C. (1975). Cereal diseases: their pathology and control. B.A.S.F. Pervian Press Ltd., England.
- Joonsten, M.H.A.J., De Wit, P.J.G.M. (1989). Identification of several pathogenesis related proteins in tomato leaves inoculated with *Cladosporium fulvum* (syn. *Fulvia fulva*) as 1,3- β -glucanases and chitinases. *Plant Physiology*. 89: 945-951.
- Kaunan, S. (1986). Foliar nutrition: Its place in crop production. *Journal of Fertiliser Issues (U.S.A.)*. 3: 244-252.
- Kauffman, S., Legrand, M., Geofroy, P., Frittig, B. (1987). Biological function of pathogenesis related proteins: four PR proteins have 1,3- β -glucanase activity. *E.M.B.O. Journal*. 6: 3209-3212.
- Kauss, H., Franke, R., Krause, K., Conrath, U., Jeblick, W., Grimmig, B., Matern, U. (1993). Conditioning of parsley (*Petroselinum crispum* L.) suspension cells increase elicitor-induced incorporation of cell wall phenolics. *Plant Physiology*. 102: 459-466.
- Kauss, H., Theisinger- Hinkel, E., Mendersmann, R., Conrath, U. (1992). Dichloroisonicotinic and salicylic acid inducers of systemic acquired resistance enhance fungal elicitor responses in parsley cells. *Plant Journal*. 2:655-660.
- Keen, N.J., Yoshikaura, M. (1983). β -1,3- Endoglucanase from soybean releases elicitor active carbohydrates from fungus cell walls. *Plant Physiology*. 71: 460-465.
- Keen, N.J., Yoshikaura, M., Wong, M.C. (1983). Phytoalexin elicitor activity of carbohydrates from *Phytophthora megasperma* f.sp. *glycine* and other sources. *Plant Physiology*. 71: 466-471.

- Kehlenbeck, h., Krone, C., Schönbeck, F. (1993).** Induced resistance in barley towards powdery mildew and the influence on sink- source relationships. In: Bull. OIL B/SROP proceedings workshop on 'Biological control of foliar post harvest diseases' in Wageningen.
- Keppler, L.D., Atkinson, M.M., Baker, C.J. (1988).** Plasma membrane alteration during bacteria-induced hypersensitivity reaction in tobacco suspension cells as monitored by intracellular accumulation of fluorescein. *Physiological and Molecular Plant Pathology*. 32: 209-210.
- Kessman, H., Staub, T., Hoffmann, C., Naitzke, Th., Herzog, J. (1994).** Induction of acquired resistance in plants by chemicals. *Annual Review of Phytopathology*. 32: 439-459.
- King, J.E. (1972).** Surveys of foliar diseases of spring barley in England and Wales 1967- 1970. *Plant Pathology*. 21: 23-25.
- Klages, K.H.W. (1942).** Ecological crop geography. MacMillan Company. New York.
- Kleier, D.M (1988).** Phoen mobility of xenobiotics. I Mathematic model unifying the weak acid and intermediate permeability theories. *Plant Physiology*. 86: 803-810.
- Klement, Z., Goodman, R.M. (1968).** The hypersensitive reactions to infection by bacterial plant pathogens. *Annual Review of Phytopathology*. 5: 17-44.
- Kombrink, E., Schroeder, M., Halbrock, K. (1988).** Several 'pathogenesis-related' proteins in potatoes are 1,3- β -glucanases and chitinases. *Proceedings of the National Academy of Science U.S.A.* 85:782-786.
- Komochi, S.J., Goto, T., Ashima, N. (1966).** Studies on the control of plant virus diseases by vaccination of attenuated viruses. III Reduction in fruit setting resulting from infection of tomato plants with TMV. *Journal of the Japanese Society for Horticultural Science*. 35: 269-276.

- Kúc, J. (1977).** Activated coordinated chemical defence resistance in plants. In: *Host Resistance To Pests.* (Hedin, P. Ed.). pp78-89. American Chemical Society Press., Washington D.C.
- Kúc, J. (1977).** Plant protection by activation of latent mechanisms for resistance. *Netherlands Journal of Plant Pathology.* 83 (Supplement 1): 463-477.
- Kúc, J. (1981).** Plant Immunisation- mechanisms and practical implication. Journal paper No. 80-11-188. Kentucky Agricultural Experimental Station, Lexington, Kentucky. U.S.A.
- Kúc, J. (1982).** Phytoalexins from the Solanaceae. In: *Phytoalexins.* (Bailey, J., Mansfield, J. Eds.). pp81-105. Blackie and Sons. Glasgow.
- Kúc, J. (1982).** Induced immunity to plant disease. *Bioscience.* 32: 854-860.
- Kúc, J. (1983).** Induced systemic resistance in plants to diseases caused by fungi and bacteria. In: *The Dynamics of Host Defence.* Academic Press, Australia.
- Kúc, J. (1984).** Phytoalexins and disease resistance from a perspective of evolution and adaptation. In: *Origins and Development of Adaptation.* pp 100-118. Ciba Foundation Symposium 102. Pitman. London.
- Kúc, J. (1985).** Expression of latent genetic information for disease resistance in plants. In: *Cellular and Molecular Biology and Development of Adaptation.* Ciba Foundation.
- Kúc, J. (1995).** Phytoalexins, stress metabolism and disease resistance in plants. *Annual Review of Phytopathology.* 33:275-297.
- Kúc, J. (1995).** Systemic Induced Resistance. *Aspects of Applied Biology. Physiological Responses of Plants to Pathogens.* 45: 235-242.
- Kúc, J., Caruso, F. (1977).** Activated coordinated chemical defense against disease in plants. In: *Host resistance to pests.* (Hedin, P. Ed.) American Chemical Society Press. Washington D.C. 78-89.

- Kúć, J., Presig, C. (1984). Fungal regulation of disease resistance mechanisms in plants. *Mycologia*. 76(5); 767-784.
- Kúć, J., Richmond, S. (1977). Aspects of the protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. *Phytopathology*. 67: 533-536.
- Kúć, J., Rush, J. (1985a). Phytoalexins. *Archives of Biochemistry and Biophysics*. 236: 379-389
- Kúć, J., Rush, J. (1985b). Phytoalexins. *Archives of Biochemistry and Biophysics*. 236: 455-472.
- Kúć, J., Shockley, G., Kearney, K. (1975). Protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. *Physiological Plant Pathology*. 7: 195-199.
- Kúć, J., Tuzan, S. (1983). Immunization for disease resistance in tobacco. *Recent Advances in Tobacco Science*. 9: 179-213.
- Lamport, D.T.A. (1986). Roles of peroxidases in cell wall genesis. In: Molecular and Physiological Aspects of Plant Peroxidases. (Groppin, H., Penel, C., Graspar, T. Eds.). pp 199-207. University of Geneva Press. Geneva.
- Larque-Saavedra, A. (1978). The anti-transparent effect of acetylsalicylic acid on *Phaseolus vulgaris* L. *Physiological Plant Pathology* 43: 126-128.
- Larque-Saavedra, A. (1979). Stomatal closure in response to acetylsalicylic acid. *Plant Physiology*. 43: 126-128.
- Lawton, K., Weyman, K., Friedrich, L., Hunt, M., Neuenschwander, U., Steiner, H.Y., Malek, K., Unkes, S., Ryals, J. (1996). Systemic acquired resistance signal transduction. In: *Proceedings of the Brighton Crop Protection Conference*. Number 3:967-972.
- Leinon, S., Kurkela, . (1969). *Ann. Bot. Fenn.* 6:340-343.

- Lester, E. (1971). *Proceedings of the 6th insect and fungi conference*. 3: 643-647.
- Li, W.X., Kodama, O., Akatsuha, P. (1991). Role of oxygenated fatty acids in rice phytoalexin production. *Agricultural and Biological Chemistry*. 55(4): 1041-1047.
- Lowry, D.H., Rosebrough, N.J., Farr, A.L., Randall, B.B. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biochemical Chemistry*. 193: 265-275.
- Lozano, J.C., Sequiera, L. (1970). Prevention of the hypersensitive reaction in tobacco leaves by heat-killed bacterial cells. *Phytopathology*. 60: 875-879.
- Lupu, R., Grassman, S., Cohen, Y. (1980). The inducement of lipoxygenase and antioxidants in pathogenesis in powdery mildew on tobacco plants. *Physiological Plant Pathology*. 16: 241-248.
- Lyon, G.D., Newton, A.C. (1997). Do resistance elicitors offer new opportunities in integrated disease control strategies. *Plant Pathology*. 46:636-641.
- Lyon, G.D., Reglinski, T., Forrest, R.S., Newton, A.C (1995). The use of resistance elicitors to control plant disease. Aspects of Applied Biology. *Physiological Responses of Plants to Pathogens*. 45: 227-234.
- McKeen, W.E., Rimmer, S.R. (1973). Initial penetration process in powdery mildew infection of susceptible barley leaves. *Phytopathology*. 63: 1049-1052.
- McKeen, W.E., Smith, R., Bhallacharya, P.K. (1969). Alterations of the host wall surrounding the infection peg of powdery mildew fungi. *Canadian Journal of Botany*. 47: 701-706.
- Malamy, J., Carr, J.P., Klessig, D., Raskin, I. (1990). Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*. 250: 1002-1004.

- Mandryk, M. (1963).** Acquired systemic resistance to tobacco mosaic virus in *Nicotiana tabacina* evoked by stem injection with *Pseudomonas tabacina* adam. *Australian Journal of Agricultural Research*. 14: 315-318.
- Manners, J.G., Hossain, S.M.M. (1963).** Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. *Transactions of the British Mycological Society*. 46: 225-234.
- Matta, A., Garibaldi, A. (1977).** Control of Verticillium wilt of tomato by preinoculation with avirulent fungi. *Netherlands Journal of Plant Pathology*. 83: 457-462.
- Mauch, F., Stahlin, L.A. (1989).** Functional implications of the subcellular localization of ethylene-induced chitinase and β -1,3-glucanase in bean leaves. *Plant Cell*. 1: 447-457.
- Mauch, F., Mauch-Mani, B., Boller, Th. (1988).** Antifungal hydrolases in pea tissue. II Inhibition of fungal growth by combinations of chitinases and β -1,3-glucanases. *Plant Physiology*. 88: 936-942.
- Maunt, M.S., Ellingboe, A.H. (1968).** Effects of UV radiation on the establishment of *E. graminis* f.sp. *tritici* on wheat. *Phytopathology*. 58:1171- 1175.
- Mayer, M.G., Zeigler, E. (1988).** An elicitor from *Phytophthora megasperma* f.sp. *glycinae* influences the membrane potential of soybean cotyledonary cells. *Physiological and Molecular Plant Pathology*. 33: 397-407.
- Mazzuchi, U., Bazzi, C., Pupillo, P. (1979).** The inhibition of susceptible and hypersensitive reactions by protein lipopolysaccharide complexes from phytopathogenic pseudomonads: Relationship to polysaccharide antigenic determinants. *Physiological Plant Pathology* 14:19-30.

- Mazzuchi, U., Pupillo, P. (1976). Prevention of confluent hypersensitive necrosis in tobacco leaves by a bacterial protein-lipopolysaccharide complex. *Physiological Plant Pathology*. 9: 101-112.
- Meins, F., Ahl, P. (1989). Induction of chitinase and β -1,3-glucanase in tobacco plants with *Pseudomonas tabaci* and *Phytophthora parasitica* var. *nicotianae*. *Plant Science*. 61: 151-161.
- Mettraux, J.P., Boller, Th. (1986). Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal infections. *Physiological and Molecular Plant Pathology*. 28: 161-169.
- Mettraux, J.P., Signer, H., Ryals, H., Ward, E., Wyss-Benz, A. ((1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science*. 250: 1004-1006.
- Mettraux, J.P., Streit, L., Staub, T. (1988). A pathogenesis-related protein in cucumber is a chitinase. *Physiological and Molecular Plant Pathology*. 33: 1-10
- Millard, A., Scott, K. (1956). Host pathogen relations in powdery mildew of barley. II. Changes in respiratory pattern. *Australian Journal of Biological Science*. 9: 37-44.
- Minnick, L., Kilpatrick, M. (1993) Acid base equilibria in aqueous and nonaqueous solutions. *Journal of Physical Chemistry*. 43: 259-268.
- Miyazawa, J., Kawabata, T., Ogasawara, N. (1998). Induction of an acidic isozyme of peroxidase and acquired resistance to wilt disease in response to treatment of tomato roots with 2-furoic acid, 4-hydroxybenzoic hydrazide or salicylic hydrazide. *Physiological and Molecular Plant Pathology*. 52: 115-126.
- Moleyar, V., Narasimham, P. (1986). Antifungal activity of some essential oil components. *Food Microbiology*. 3(4): 331-336. (Abstract).
- Moore, W.C., Moore, F.J. (1961). M.A.F.F. Bulletin No 129. Cereal Diseases. H.M.S.O. London.

- Morgan, M.F., Gourley, J.H., Albeiter, J., K. (1938).** The soil requirements of economic crops. In: U.S.D.A. Agricultural Yearbook. pp753-776.
- Moseman, J.G., Greeley, L.W. (1966).** Effects of UV light on *E. graminis* f.sp. *hordei*. *Phytopathology*. 56: 1375-1380.
- Moseman, J.G., Powers, H.R. (1957).** Function and longevity of cleistothecia of *Erysiphe graminis* f. sp. *hordei*. *Phytopathology*. 47: 53-56.
- Murray, J. (1970).** The first European agriculture. Edinburgh.
- Murray, D.C., Walters, D.R. (1992).** Increased photosynthesis and resistance to rust infection in upper uninfected leaves of rusted leaves of broad beans (*Vicia faba* L.). *New Phytologist*. 120: 235-242
- Naldony, L., Sequiera, L. (1980).** *Plant Physiology*. 16: 1.
- Namai, T., Kato, T., Jamaguch, J., Togashi, J. (1990).** Time-course alteration of lipoxygenase activity in blast infected rice leaves. *Annals of the Phytopathological Society of Japan*. 56: 26-32.
- Neunenschwander, U., Freidrich, L., Delancy, T., Vernooil, B., Kessman, H., Ryals. (1995).** Activation of plant disease resistance. Aspects of Applied Biology. *Physiological Responses of Plants to Pathogens*. 45:217-225.
- Nilan, R.A. (Ed.) (1971).** Barley Genetics (II). Proceeding of the 2nd. International Barley Genetics Symposium. Pullman. Washington. pp622.
- Northcote, D.H. (1985).** Control of cell wall formation during growth. In: Biochemisrty of plant cell walls. Ed. Brett, C.T. and Hillman, J.R. Cambridge University Press. Cambridge.
- Nuttson, M.Y. (1957).** Barley climate relationships and the use of phenology in ascertaining the thermal and photothermal requirements for barley. Washington: American Institute of Crop Ecology.

- O'Connell, R.J., Brown, I.R., Mansfield, J.W., Bailey, J.A., Mazau, D., Russwurm, D., Esquerré-Tugayé, M.T. (1990). Immunocytochemical localisation of hydroxyproline rich glycoproteins accumulating in melon and bean at sites of resistance to bacteria and fungi. *Molecular Plant-Microbe Interactions* 3:33-40.
- Ocampo, C.A., Moersbacher, B., Grambo, H.J. (1986). Increased lipoxygenase activity is involved in the hypersensitive response of barley to powdery mildew using salicylates and various phenolic acids. *Annals of Applied Biology*. 122: 451-456.
- Ohata, H., Shida, K., Peng, Y.L., Furasanta, I., Shishyuama, J., Aibara, S., Monta, Y. (1990). A lipoxygenase pathway is activated in rice after infection with rice blast fungus *Magnaporthe grisea*. *Plant Physiology*. 90: 285-290.
- Oota, Y., (1972). The response of *Lemna gibba* G3 to a single long day in the presence of E.D.T.A. *Plant Cell Physiology*. 18: 575-580.
- Oota, Y., (1975). Short- day flowering of *Lemna gibba* G3 as induced by salicylic acid. *Plant Cell Physiology*. 16: 1131-1135.
- Pan, S.Q., Ye, X.S., Kúc, J. (1989). Direct detection of β -1,3- glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing gels. *Analytical Biochemistry*. 182: 136-140.
- Pan, S.Q., Ye, X.S., Kúc, J. (1991). Association of β -1,3- glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. *Plant Pathology*. 39: 25-39.
- Pan, S.Q., Ye, X.S., Kúc, J. (1992). Induction of chitinase in tobacco plants systemically protected against blue mould by *Peronospora tabacina* or tobacco mosaic virus. *Phytopathology*. 82: 119-123.
- Pearce, R.B., Ride, J.P. (1980). Specificity of the induction of the lignification response in wounded wheat leaves. *Physiological Plant Pathology*. 16: 197-204.

- Pegg, G.F., Young, D.H. (1982).** Purification and characterisation of chitinase enzymes from healthy and *Verticillium albo-atrum* infected plants and from *V. albo-atrum*. *Physiological Plant Pathology*. 2: 389-409.
- Pierpoint, W.S. (1994).** Salicylic acid and its derivatives in plants : medicines, metabolites and messenger molecules. *Advances in Botanical Research*. 20: 164-235.
- Pielcerse, A.H., Müller, L.J. (1977).** Induction of flowering in *Lemna gibba* G3 under short day conditions. *Plant Cell Physiology*. 18:45-53.
- Polley and King. (1973).** A preliminary proposal for the detection of barley mildew infection periods. *Plant Pathology*. 22:11-16.
- Popova, L. Maslenkova, L., Zanev, Yu., Ivanov, A., Cristor, A. (1989).** Stomatal and non-stomatal response of photosynthesis in barley leaves after treatment with ABA and JA. *Colloquia of Pflanzenphysiologie*. 13, *Stomata 89, Berlin*.
- Popova, L.P., Tsoner, T.D., Valkinova, S.G. (1987).** A possible role for abscisic acid in regulation of photosynthetic and photorespiratory carbon metabolism. *Plant Physiology*. 83:824-828.
- Procter, W. (1943).** Observations on the volatile oil of *Betula lenta* on gaultherin. *Annual Journal of Experimental Botany*. 37: 129-134.
- Raskin, I., Skubatz, H., Tangi, W., Mecuse, B.J.D. (1990).** Salicylic acid levels in thermogenic and nonthermogenic plants. *Annals of Botany*. 66: 373-376.
- Rast, A.T.B. (1975).** Agricultural research report No. 8343. Wageningen: Pudoc. 76pp
- Rathmell, W.G. (1982).** In: *Active Defense Mechanisms*. (Wood, R.K.S. Ed.) pp 347-348. Plenum Press. New York.
- Reglinski, T., Lyon, G.D., Newton, A.C. (1994a).** Induction of resistance mechanisms in barley by yeast-derived elicitors. *Annals of Applied Biology*. 124:509-517.

- Reglinski, T., Newton, A.C., Lyon, G.D. (1994b).** Assessment of the ability of yeast derived elicitors to control barley powdery mildew in the field. *Journal of Plant Disease*. 101:1-10.
- Reglinski, T., Poole, P.R., Whitaker, G., Hoyte, S.M. (1997).** Induced resistance against *Sclerotinia sclerotinium* in kiwifruit leaves. *Plant Pathology*. 46: 716-721.
- Renfrew, J.M. (1969).** The archaeological evidence for the domestication of plants, methods and problems. In: The Domestication and exploitation of plants and animals. (Ucko, P.J., Dimbleby, G.W. Eds.) pp149-172. Chicago.
- Reuveni, M., Agapov, V., Reuveni, R. (1992).** Local and systemic resistance against powdery mildew and growth increase induced by phosphate salts. *Phytopathology*. 82: 1179 (abstract).
- Reuveni, R., Agapov, V., Reuveni, R. (1992).** Foliar spray of phosphates induces growth increase and systemic resistance to *Puccinia sorghi* in maize. *Plant Pathology*. 43: 245-250.
- Reuveni, M., Agapov, V., Reuveni, R. (1993).** Induction of systemic resistance to powdery mildew and growth increase in cucumber by phosphates. *Biological Agriculture and Horticulture*. 9:305-315.
- Reuveni, R., Agapov, V., Reuveni, M. (1994).** Foliar spray of phosphates induces growth increase and systemic resistance to *Puccinia sorghi* in maize. *Plant Pathology*. 43:245-250.
- Rhodes, A., Frantes, K.H., Boothroyd, B, McGonagle, M.P., Crasse, R. (1961).** *Nature Land*. 192: 952-954.
- Richmond, S., Kúc, J., Elliston, J. (1979).** Penetration of cucumber leaves by *Colletotrichum lagenarium* is reduced in plants systemically protected with the pathogen. *Physiological Plant Pathology*. 14:329-338.

- Ride, J.P. (1978). The role of cell wall alteration in resistance to fungi. *Annals of Applied Biology*. 89: 302-306.
- Ride, J.P., Barber, M.S. (1990). Purification and characterisation of multiple forms of endochitinase from wheat leaves. *Plant Science*. 71:185-197.
- Rodgers, D.P. (1959). *Mycologia*. 51: 96-98.
- Ross, A.F. (1961 A). Localised acquired resistance to plant virus infection in hypersensitive roots. *Virology*. 14: 329-339.
- Ross, A.F. (1961 B). Systemic acquired resistance induced by localised virus infections in plants. *Virology*. 14: 340-358.
- Ross, A.F., (1964). Systemic resistance induced by localised infections in beans and cowpea. *Phytopathology*. 50: 436.
- Ross, A.F., Bozarth, R.F. (1960). Resistance induced in one plant part as a result of virus infection in another part. *Phytopathology*. 50: 652.
- Ryan, C.A. (1978). *Trends in Biochemical Science*. 3: 148-150.
- Ryan, C.A. (1982). In : Impact of quality by herbivorous insects. (Deuno, R.F., McClure, M.S. Eds.). Academic Press. New York.
- Salt, S.D., Tuzan, S., Kúc, J. (1986). Effects of β -inoc and abscisic acid on the growth of tobacco and resistance to blue mould. *Physiological and Molecular Plant Pathology*. 28: 287-297.
- Saniewski, M., Czapski, J. (1985). Stimulatory effect of methyljasmonate on ethylene production of tomato fruits. *Experientia*. 41: 256-257.
- Sauer, S.M. (1957). Non-enzymatic macromolecules as matrices in biological synthesis: the role of polysaccharides in peroxidase- catalysed lignin polymer formation from eugenol. *Journal of the American Chemical Society*. 79: 1628-1632.
- Schlaubaum, A., Mauch, F., Vogeli, V., Boller Th. (1986). Plant chitinases are potent inhibitors of fungal growth. *Nature, London*. 324: 365-367.

- Schönbeck, F., Dehne, H.W., Beicht, W. (1980). *Z. Pflanzenkrankh. Pflanzenschutz*. 87: 654-666.
- Schneider, S., Ullrich, W.R. (1994). Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiological and Molecular Plant Pathology*. 45: 291-304.
- 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.
- Seguchi, K., Kurotaki, M., Sekido, S., Yanaguchi, I. (1992). Action mechanisms of N-cyanamethyl-2-chloroisonicotinic-amide in controlling rice blast disease. *Journal of Pesticide Science*. 17: 107-113.
- Seigel, S.M. (1957). Non enzymatic macromolecules as matrices in biological synthesis: The role of polysaccharides in peroxidase-catalysed lignin polymer formation from eugenol. *Journal of the American Chemistry Society*. 79: 1628-1632.
- Sempio, C., Castori, M. (1952). *Review of Biology*. 42: 287-293.
- Sequeira, L. (1979). In: Recognition and specificity in plant host-parasite interactions. (Daly, J.M., Uritani, I. Eds.) pp 231-251. Japan Science Society. Tokyo.
- Sethi, P.N., Nenkataraman, R., Maheshwari, S.C. (1970). Studies on the ground and flowering of a short-day plant, *Wolffia microscopia* II. Role of metal ions and chelates. *Planta*. 90: 349-359.
- Sharma, M., Mase, S., Iwata, M. (1983). Anti-conidial germination factors induced in the presence of probenazole in infected host leaves. III- Structural elucidation of substances A and C. *Agricultural and Biological Chemistry*. 47: 1983-1989.
- Sharp, E.L., Schmitt, C.G., Stately, J.M., Kongsolver, C.N. (1958). Some critical factors involved in establishment of *Puccinia graminis* var. *tritici*. *Phytopathology*. 48: 469-474.

- Shettle, N.L., Balke, N.E. (1983).** Plant growth response to several allelopathic chemicals. *Weed Science*. 31: 293-298.
- Shimura, M., Mase, S., Iwata, M. (1983).** Anti-conidial germination factors induced in the presence of probenazole in infected host leaves. III. Structural elucidation of substances A and C. *Agricultural and Biological Chemistry*. 47: 1983-1989.
- Singh, G., Kaiv, M. (1980).** Effect of growth regulators on podding and yield of mung beans, *Vigna radiata* (L.) Wilezek. *Indian Journal of Plant Physiology*. 23: 366-370.
- Slykius, J.T. (1967).** Virus diseases of cereals. *Review of Applied Mycology*. 46: 401-429.
- Smedegaard-Petersen, V. (1982).** The effect of defense reactions in energy balance and yield of resistant plants. In: Active Defense Mechanisms in Plants. (Wood, R.K.S. Ed.) pp299-315. New York. London.
- Smedegaard-Petersen, V., Stølen, O. (1981).** Effect of energy requiring defense reaction on yield and grain quality in a powdery mildew resistant cultivar. *Phytopathology*. 71; 396-399.
- Smith, E.F., Brown, N.A., Townsend, C.O. (1911).** Crown gall of plants: Its cause and remedy. *United States Department of Agriculture Plant Industries Bulletin*. 213.
- Smith, J.A., Hammerschmidt, R. (1988).** Comparative study of acidic peroxidases associated with induced resistance in cucumber, musk melon and water melon. *Physiological and Molecular Plant Pathology*. 33: 255-261.
- Southerton, S.G., Deverall, B.J. (1990).** Changes in phenylalanine ammonia lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf rust fungus. *Plant Pathology*. (1990) 39: 223-230.
- Stafford, H.A. (1974).** The metabolism of aromatic compounds. *Annual Review of Plant Physiology*, 25; 459-486.

- Staswick, P.E. (1992). Jasmonates genes and fragrant signals. *Plant Physiology*. 99: 804-807.
- Staub, T., Ahl-Goy, P., Kessman, H. (1993). Chemically induced disease resistance in plants. In: Proceedings of the 10th international symposium on systemic fungicides. (Lyr, H., Polter, C. Eds.). pp 239-249. Stuttgart: Ulmer.
- Staub, T., Kúc, J. (1980). Systemic protection of cucumber plants against disease caused by *Cladosporium cucumerinum* and *Colletotrichum lagenarium* by prior inoculation with either fungus. *Physiological Plant Pathology*. 17: 389-393.
- Takahashi, R. (1955). The origin and evolution of cultivated barley. *Advances in Genetics*. 7: 227-266.
- Tuzan, S, Kúc, J. (1985). A modified technique for inducing systemic resistance to blue mould and increasing growth of tobacco. *Phytopathology*. 75: 1127-1129.
- Ueda, J., Kato, J. (1980). Induction and identification of a senescence- promoting substance from wormwood (*Artemisia celsinhiun*). *Plant Physiology*. 66: 246- 249.
- Ueda, J., Kato, J. (1982). Identification of cytokinin- induced plant growth by jasmonic acid and its methyl ester. *Physiological Plant Pathology*. 54: 249-252.
- Van der Plank, J.E. (1963). Plant Diseases: Epidemics and Control. Academic Press. London.
- Vernooij, B., Friedrich, L., Ahl-Goy, P., Staub, T., Kessmann, H., Ryals, J. (1995). 2,6-dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Molecular Plant-Microbe Interactions*. 8: 228-234.
- Vernooj, B., Freidrich, L., Morse, A., Reist, Kolditz- Jonar, R., Ward, E., Ukness, S., Kessmann, H., Ryals, J. (1994). Salicylic acid is not the translocated signal for inducing systemic acquired resistance. *Plant Cell*. 3: 1085- 1094.
- Vick, B.A., Zimmerman, D. (1984). Biosynthesis of jasmonic acid by several plant species. *Plant Physiology*. 75: 458- 461.

- Vick, B.A., Zimmerman, D. (1986).** Characterisation of 12- oxo- phytodienoic acid reductase in corn. *Plant Physiology*. 80: 202- 205.
- Vogeli, U., Meins, F. Jr., Boller, B. (1988).** Coordinated regulation of chitinase and β -1,3- glucanase in bean leaves. *Planta*. 174: 364- 372.
- Walters, D.R. (1985).** Shoot:root interrelationship: the effect of obligately biotrophic fungal pathogens. *Biological Reviews*. 60: 47-79.
- Walters, D.R. (1995).** Inhibition of polyamine biosynthesis in fungi. *Mycological Research*. 99:129-139.
- Walters, D.R., Ayres, P.G. (1983).** Changes in nitrogen utilisation and enzyme activities associated with CO₂ exchanges in healthy leaves of powdery mildew. *Physiological Plant Pathology*. 23: 447- 459.
- Walters, D.R., Mitchell, A.F., Hampson, J., McPherson, A. (1993).** The induction of systemic resistance in barley to powdery mildew using salicylates and various phenolic acids. *Annals of Applied Biology*. 122: 451-460.
- Walters, D.R., Murray, D.C. (1992).** Induction of systemic resistance to rust in broad beans by phosphate and E.D.T.A. and its reversal by calcium. *Plant Pathology*. 41: 444- 448.
- Ward, S.N., Manners, J.G., (1974).** Environmental effects on the quality and viability of conidia produced by *Erysiphe graminis*. *Transactions of the British Mycological Society*. 62: 119-128.
- Ward, E.R., Ukness, S.J., Williams, S.C., Dincher, S.S. (1991).** Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell*. 3: 1085-1094.
- Ward, E.R., Ukness, S.J., Willims, S.C., Dincher, S.S., Weilderhold, L., Ahl-Goy, P., Metraux, J.P., Ryals. (1991).** Coordinate gene activity in tobacco against agents that induce acquired resistance. *Plant Cell*. 25; 381- 386.

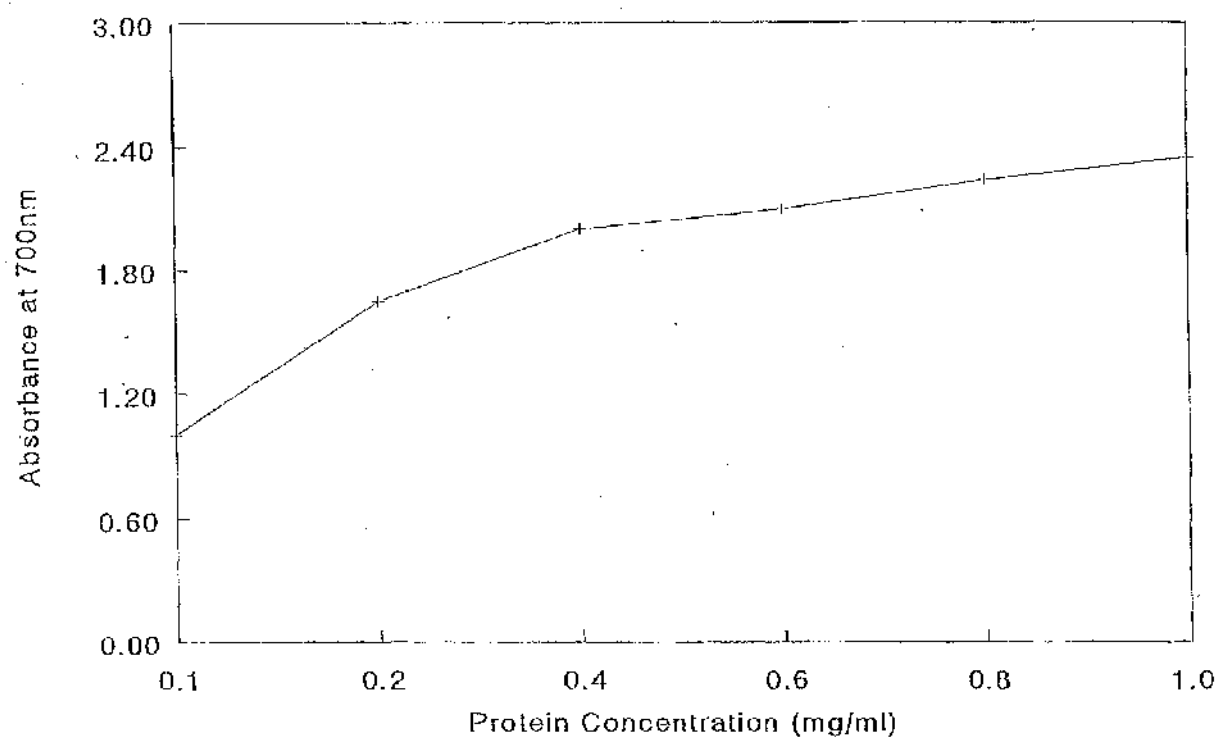
- Wardlaw, I.F. (1990). The control of carbon partitioning in plants. *New Phytologist*. 116:341-381.
- Weaver, J.B. (1950). American Barley Production. Burgess Press. Minneapolis.
- Weidhase, R.A., Kramell, H.M., Lehmann, J., Leibsch, H.W., Leries, W., Pathier, B. (1987). Methyljasmonate- induced changes in the polypeptide pattern of senescing barley leaf segments. *Plant Science*. 51: 177- 186.
- Whipps, J.M., Lewis, D.W. (1981). Patterns of translocation storage and interconversion of carbohydrates. In: Effects of Disease in the Growing Plant. (Ayres, P.G. Ed.). Cambridge University Press. London. New York.
- Wyatt, S.E., Pan, S.Q., Kúc, J. (1991). β -1,3-glucanase, chitinase and peroxidase activities in tobacco tissues resistant and susceptible to blue mould as related to flowering, age and sucker development. *Physiological and Molecular Plant Pathology*. 39: 430-433.
- Xie, C., Kúc, J. (1997). Induction of resistance to *Peronospora tabacina* in tobacco leaf discs by leaf discs with induced resistance. 51:279-286.
- Yamane, H., Tagaki, H., Abe, H., Yokata, T., Takahashi, N. (1981). Identification of jasmonic acid in three species of higher plant and its biological activities. *Plant Cell Physiology*. 22: 689- 697.
- Yarham, D.J., Bacon, E.T.G., Haywood, C.F. (1971). *Proceedings of the 6th British Insect and Fungi Conference*. 1: 15-25.
- Yarwood, C.E. (1956). Cross protection with two rust fungi. *Phytopathology*. 46: 540- 544.
- Yarwood, C.E. (1957). *Botanical Reviews*. 23: 235-301.
- Young, D.H., Pegg, G.F. (1982). The action of tomato and *Verticillium albo-atrum* glycerases on the hyphal wall of *V. albo-atrum*. *Plant Pathology*. 21: 411- 423.

Zadoks, J.C., Chang, T.T., Konzale, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, 14: 415-421.

Zeyen, R.J., Bushnell, W.R., Carver, T.L.W., Vance, C.P., Robbins, M.P., Clark, T.A. (1995). Inhibiting phenylalanine ammonia-lyase and cinnamyl-alcohol dehydrogenase suppresses mla 1 (HR) but not mlo-5 (non-HR) barley powdery mildew resistances. *Physiological and Molecular Plant Pathology*, 47: 119-140.

APPENDIX 1.

Protein Calibration Curve



Cinnamic Acid Standard Curve

