STUDIES IN SYSTEMIC INDUCED DISEASE RESISTANCE

by

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For my Father.

ABSTRACT

Systemic induced resistance by pre-inoculation with live pathogens has been shown to occur in several host-parasite combinations and has been proposed as a general phenomenon. The present work has shown that this may not be so as protection could not be achieved in grape vines pre-inoculating with Plasmopara viticola, or in French beans against Colletotrichum lindemuthianum, pre-inoculating with the same fungus, Uromyces phaseoli or tobacco necrosis virus. Nor were broad beans immunized against Uromyces fabae infection by pre-inoculating with the same fungus. Systemic resistance was induced in broad beans by pre-inoculating with Botrytis cinerea or Botrytis fabae and challenging with Botrytis fabae although this was somewhat variable.

Elicitors such as germination fluids, heat-killed cells, culture filtrates and cell wall extracts did not induce systemic resistance in any of the host-parasite combinations tested.

Many growth regulating substances have been shown to induce localized resistance. Their ability to induce systemic resistance was investigated but no significant resistance was induced.

Polyacrylic acid, gentisic acid and acetylsalicylic acid are well-documented inducers of systemic resistance against virus diseases. Systemic protection was achieved against Botrytis fabae in broad bean but not against Colletotrichum lindemuthianum in French bean.

In commercial screening for potential fungicides a significant correlation has been shown between chemicals that cause phytotoxicity and those that induce resistance. A range of phytotoxic compounds was tested for ability to induce systemic resistance and some positive results were obtained.

Exposure to ultra-violet irradiation was shown to induce localized but not systemic resistance to Plasmopara viticola in grape vine. Resistance was due to the accumulation of phytoalexins.

LIST OF ABBREVIATIONS

A^{o} - Angstrom

c. - about

C^{o} - Centrigrade degrees

cv. - cultivated variety

cm - centimeter(s)

g - gram(s)

h - hour(s)

l - litre(s)

mg - milligram(s)

ml - millilitre(s)

mm - millimeter(s)

M - molar

MW - molecular weight

N - normal

nm - nanometer(s)

ppm - parts per million

p.s.i.- pounds per square inch

Rf - rate of flow

rpm - revolutions per minute

tlc - thin layer chromatography

UV - ultra violet (irradiation)

µl - microlitre(s)

µm - micrometer(s)

% - percentage

V - volume

w - weight

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I. INTRODUCTION

Kuc et al. (1975) have shown that prior inoculation of the lower leaves of cucumber with Colletotrichum lagenarium induces resistance to subsequent infection of the upper leaves with the same fungus. Further publications have shown this phenomenon to occur in other members of the Cucurbitaceae and to be induced by certain other fungi as well as some bacteria and viruses (Caruso and Kuc, 1977; Staub and Kuc, 1980; Jenns and Kuc, 1977; Caruso and Kuc, 1979). Sutton (1979) has also reported systemic induced resistance after infecting French beans with Colletotrichum lindemuthianum and more recently Gessler and Heller (1981) have observed induced reactions in tomatoes infected with Colletotrichum phomoides. Caruso and Kuc (1977) suggested that systemic protection induced by pre-inoculation may be common in plant-parasite interactions. The work described in this Thesis was designed to investigate this possibility in other host-parasite systems and the report of its occurrence in French beans.

Various substances notably fungal cell wall and culture filtrate extracts have been shown to induce localized resistance often through elicitation of a phytoalexin response (Albersheim and Anderson-Prouty, 1975). Several 'elicitors' also were investigated for any systemic effects.

Langcake et al. (1977) have suggested that certain fungicides may elicit their effect on plant pathogens by initiating an induced resistance reaction in the host rather than by direct fungitoxicity. Rathmell (1981) has postulated a correlation between substances which cause phytotoxicity and those that induce systemic resistance. Several groups of chemicals including potential fungicide compounds were assayed for systemic effects on host resistance.

II. LITERATURE REVIEW

Kuc (1981) has described immunity as "a condition of being able to resist a particular disease especially through preventing development of a pathogenic micro-organism or by counteracting the effects of its products". He thus uses the term "immunization" in preference to "induced systemic resistance", "induced systemic protection", "acquired systemic resistance" and "activated systemic resistance" to describe the phenomenon whereby pathogens which cause disease can be used to protect plants against disease. Deverall (1977), who has also defined the phenomenon, uses the term "cross-protection" whereby "a plant is protected from infection by earlier or simultaneous exposure to another organism". He clearly defines two possible modes of cross-protection, direct antagonism between pathogens and activation of physiological change within the host so that it becomes resistant. Chester reviewed the topic as early as 1933 and recognised the importance of plant immunization as a means of disease control. Johnson (1978) more recently emphasised the practical significance of induced resistance. He considered that there were four important factors involved:- i) the timing of the induction, ii) its specificity; iii) the possibility of induced susceptibility and iv) whether effects were local or systemic. Ouchi et al. (1976) discussed induced susceptibility and induced accessibility in the light of their own and other research. However, such effects are only of a localized nature. The difference between localized effects of elicitors and direct antagonists, and systemic effects where physiological change is induced in the host, has been defined by Touze and Rossignol (1980).

Systemic acquired resistance induced by localized virus infections in plants was described by Ross (1961). Virus induced resistance has since been shown to occur in many local lesion hosts and has been

thoroughly reviewed by Loebenstein (1972) and Wagih (1981).

Systemic protection by virulent pathogens

Undoubtably the most dramatic example of systemic induced resistance discovered to date exists in cucumbers and other members of the cucurbitaceae. Much of this work has involved Kuc who has recently published a comprehensive review of the subject (1981). The following points are drawn from this review:-

i) limited infection with Colletotrichum lagenarium or tobacco necrosis virus protects cucumber against disease caused by a wide range of pathogens including obligate and facultative fungi, local lesion and systemic viruses, fungi and bacteria that cause wilts and others that attack both foliage and fruit;

ii) Necrosis, macroscopic or microscopic, has so far been a common factor in immunization occurring in cucurbits;

iii) Removal of the inducer·leaf 72-96 hours after infection does not reduce the immunization. Once tissues have been conditioned they do not require the presence of the inducer leaf for immunization;

iv) The signal for immunization is graft transmissable;

v) Girdling the petriole of the inducer leaf prevents immunization above or below the inducer leaf;

vi) A single induction immunizes for 4-6 weeks;

vii) Cucumbers cannot be immunized once they have started to flower and set fruit;

viii) The concentration of inoculum used for induction and the number of lesions produced on the inducer leaf are directly related to the extent of immunization until a saturation point of inoculum or lesions is attained;

ix) The mode of action of immunization seems to involve a reduction in penetration, germination of conidia on plant surfaces is unaffected;

x) Associated with immunization is a three-fold increase in peroxidase activity;

xi) In immunized plants lignification in response to infection is more rapid, intense and non-diffuse than in non-immunized plants;

xii) Recent work has produced extracts from inducer leaves infected with Colletotrichum lagenarium or Pseudomonas lachrymans that when infiltrated into cotyledons or the first true leaf of cucumbers induce resistance to Colletotrichum lagenarium infection in the upper leaves;

xiii) It has been postulated that the genetic basis for plant immunization may be a fundamental, primitive response to stress.

Induced resistance by pre-inoculation with a pathogen has also been demonstrated in other systems. Cruickshank and Mandryk (1960) reported reduced infection by Peronospora tabacina on the leaves of tobacco after stem infestation by the same fungus. Thielaviopsis basicola and virus infection in tobacco have been shown to be inducers of systemic resistance against Thielaviopsis basicola and Phytophthora parasitica var. nicotianae (Hecht and Bateman, 1964; McIntyre and Dodds, 1979). Systemic protection over shorter distances has been reported in French bean hypocotyls to anthracnose infection (Elliston et al., 1971) although subsequent work by Skipp and Deverall (1973) suggested that the induced resistance may only be localized. Kuc et al. (1976) summarized the work on this system and suggested that phytoalexins were involved. Protection over limited distances has been reported in carrot root slices to Botrytis cinerea (Heale and Sharman, 1977) and in sweet potato root tissue to Ceratocystis (Weber and Stahmann, 1966). Hubert and Helton (1967) described a translocated resistance phenomenon in Italian prune trees induced by prior infection with Cytospora cinta. Flax inoculated with rust showed induced resistance to subsequent infection for at least 30 days in experiments carried out by Hoes and Dorrell (1979). Significant

protective effects have also been described by Sutton (1979) in French bean plants where foliage became resistant to anthracnose after prior inoculation of lower leaves with the same fungus. More recently Gessler and Heller (1981) have reported the occurrence of the phenomenon in tomatoes infected with Colletotrichum phomoides and have shown that penetration of the fungus is affected as in the cucumber system. Tjamos (1979) protected cucumber against wilt by pre-treatment of lower leaves with Verticillium conidia and achieved some effects by pre-treatment with culture filtrates.

Apart from the work reported by Kuc (1981) involving active extracts from inducer leaves there has been little successful work on isolating agents responsible for the systemic induction of resistance. Anderbrhan et al. (1980) found novel proteins associated with the induction of resistance but these are non-specific and may only be part of a general response to stress (personal communication).

Cross-protection by avirulent and non-pathogens

There exist numerous reports of the induction of resistance, often called cross-protection, by non-pathogens and avirulent pathogens against pathogens; the topic was reviewed thoroughly by Matta in 1971. Many of these however are of a localized nature only and involve direct antagonism or the induction of phytoalexins. Such cases include many rusts such as Melampsora lini on flax (Littlefield, 1969), Puccinia coronata and Puccinia recondita on oats (Tani et al., 1980) and Uromyces phaseoli and Puccinia helianthi on beans and sunflower (Yarwood, 1956).

Avirulent bacterial cells either of different species or different races have also been used to induce localized resistance to pathogens. Non-pathogenic Corynebacterium insidiosum cells induce resistance to virulent races in alfalfa (Carrol and Lukezic, 1972) and Pseudomonas fluorescens has been shown to antogonise infection by Pseudomonas

phaseolicola (Teliz-Ortiz and Burkhelder, 1960). Goodman (1967) protected apple tissue against Erwinia amylovora using virulent strains and three other bacterial species.

Instances of virus infected plants being locally protected against subsequent fungal infection exist between TMV and rust in bean (Wilson, 1958) and TMV and Phytophthora parasitica var. nicotianae in tobacco, the latter having a systemic as well as a localized effect. There is also a report however of enhanced susceptibility to attack by Puccinia recondita of wheat previously infected with wheat streak mosaic virus (Raju et al., 1969). Russel (1966) reported both induced susceptibility and induced resistance with different strains of beet yellows and beet mild yellowing virus to the downy mildew fungus. Downy mildew of tobacco has been shown to induce complete resistance to subsequent attack by the powdery mildew fungus Erysiphe cichoracearum (Coheen et al., 1975) while cultivar non-pathogenic races of Phytophthora parasitica var. nicotianae protect tobacco against subsequent infection by the same fungus (McIntyre and Miller, 1978). Chamberlain (1972) protected soybeans against attack by Phytophthora megasperma var. sojae by prior treatment with any of four non-pathogenic fungi. Phytoalexin production has been linked with protection of soybean by the non-pathogen Phytophthora cactorum against Phytophthora megasperma var. sojae (Svoboda and Paxton, 1972) but Rahe et al. (1969) working with hypocotyls and French bean anthracnose postulated that phytoalexins were not involved because of the timing of the response. Deverall et al. (1979) also failed to implicate antifungal substances in cross-protection of wheat against take-all by prior colonisation of the roots with the Phialophora state of Gauemannomyces cylindrosporus. Wang (1975) cross-protected against wheat and oat take-all fungi by Gauemannomyces graminis var. graminis while Fusarium oxysporum f.sp. dianthi can be used to immunize tomatoes against Fusarium

oxysporum f.sp. lycopersici (Zazzerini, 1976). Sinha and Das (1972) and Sinha and Trivedi (1972) both reported localized cross-protection by avirulent or mildly virulent races against virulent strains of Helminthosporium oryzae in rice plants. Helminthosporium maydis infection of maize can also be reduced by prior treatment with an avirulent race (Nelson and Tung, 1973). Pre-inoculation with weak or avirulent races and species can protect mint against Verticillium nigrescens (Melonk and Homer, 1975), cotton against Verticillium albo-atrum (Schnathorst and Mathre, 1966) and tomato against Verticillium wilt (Matta and Garabaldi, 1977).

Elicitors

Protection can also be induced by so-called elicitors, substances often of bacterial or fungal origin that cause changes in the host which may lead a susceptible host to display resistant responses.

Albersheim and Anderson (1975) reviewed the effect that such substances, especially carbohydrates and proteins, have on cell surfaces and their role in pathogenesis while Albersheim (1980) summarized the work on elicitors and their role in recognition in plant-parasite interactions. Keen (1975) claimed to have found a race-specific elicitor of phytoalexin activity in Phytophthora megasperma var. sojae although most reports including those of Anderson (1978a, b) show elicitors to be non-specific in their action. Yoshikawa (1978) postulated that constitutive phytoalexins are due to a synthesis/degradation system and that abiotic elicitors suppress degradation while biotic elicitors stimulate synthesis. Thus elicitors might still play a role in specificity. Further hypotheses of the mode of action of elicitors have been discussed by Hadwiger and Schwochau (1969).

The majority of cases indicating elicitors as inducers of resistance report localized effects only and many have been linked to the phytoalexin

response. Ricci et al. (1980) reported that zoospore germination fluids and culture filtrates of Phytophthora parasitica elicited resistance because higher levels of phytoalexins are produced in response to the challenge inoculation. Phytoalexins were also induced by treatment of soybean hypocotyls with cell-free culture filtrates of Phytophthora megasperma var. sojae (Keen et al., 1972). Autoclaved conidia and sonicated conidia have been shown to cause the accumulation of terpenoids in potato tuber slices (Lisker and Kuc, 1977) while Dixon and Fuller (1977) characterized components from culture filtrates of Botrytis cinerea which stimulated phaseollin biosynthesis in Phaseolus vulgaris cell suspension cultures. Bell and Presley (1969) found that heat-inhibited or heat-killed conidia of Verticillium albo-atrum induced resistance in cotton and that the level of acquired resistance was directly related to the amount of phytoalexin induced. Sinha and Trivedi (1969) found that germination fluids and heated spore suspensions protected rice against Helminthosporium oryzae infection while Shepherd and Mandryk (1962) isolated inhibitory substances from tobacco leaves above stem infections of Peronospora tabacina which were quantitatively related to the degree of protection induced. Berard et al. (1972) found a cultivar-specific protection factor in incompatible interactions of green beans with Colletotrichum lindemuthianum although this was only a localized effect. Beretta et al. (1972) claimed to have induced systemic resistance to coffee rust by prior treatment with extracts from autoclaved urediniospores. Heat-killed bacteria were found to protect tobacco against wildfire disease (Lovrekovich and Farkas, 1965) while Sequeira et al. (1977) showed that the interaction of bacteria with tobacco cell walls was related to the induction of resistance. Further work (Graham et al., 1977) showed that bacterial lipopolysaccharides were the factors responsible, inducing ultrastructural changes in the host while increases in

peroxidases associated with the induced resistance were not directly involved (Nadolny and Sequeira, 1980). McIntyre et al. (1975) found that DNA extracts from virulent or avirulent Erwinia amylovora protected pear against fireblight both locally and systemically. Related DNA, but not unrelated DNA, from various sources has also been shown to induce systemic resistance to pear against fireblight (Kuc et al., 1976).

One notable case where elicitors induce systemic resistance is that reported by Heale and Sharman (1977) where cell-free germination fluid of Botrytis cinerea was applied to carrot root slices and resistance to subsequent infection with Botrytis cinerea resulted in cells some distance from those treated. Tjamos (1979) also reported systemic resistance induced by culture filtrates of Verticillium to subsequent wilt infection in tomato. Schönbeck et al. (1980) proposed that there is a resistance potential in every plant independent of its genetic constitution which can be activated by appropriate inducers. He found that 10-15% of bacterial and fungal isolates tested contained inducers in culture filtrates or cell extracts which induced systemic resistance to Uromyces phaseoli in beans.

Chemical and physical inducers of resistance

In an article on disease control Kuc (1968) pointed out the potential of substances that induce "natural" disease resistance in plants whilst Cartwright et al. (1977) described a substance that itself did not stimulate phytoalexin production but increased the capacity of rice to synthesise phytoalexins in response to infection. Davis and Dimond (1952) tested a large number of chemicals for fungicidal potential and concluded that in vitro effects could not be correlated to in vivo effects. The same conclusion was reached by Horsfall and Dimond (1951) working with Fusarium wilt of tomato. Langcake and Wickens (1978) found that the protective effect of the dichlorocyclopropane fungicides in

rice against blast disease was closely correlated with their ability to enhance peroxidase activity in the leaves and the production of a brown pigment which may restrict fungal growth. Further work by Langcake et al. (1977) concluded that the dichlorocyclopropanes sensitize the rice plant such that on subsequent challenge with Pyricularia oryzae a resistant type of response occurs. The response involves a rapid accumulation of melanin-like material around the infection zone and its accompanied by the production of phytoalexin-like compounds. The soybean phytoalexin, hydroxyphaseollin, has been found to be induced by various fungicides including maneb, butylamine, benomyl, ethylenediamine, cupric chloride and nabam (Reilly and Klarman, 1972). Bompeix et al. (1980) described a substance, TEPA, which had no in vitro effect but which led to a rapid accumulation of phytoalexins and disease restriction when treated tomato plants were inoculated with Phytophthora capsici or Phytophthora parasitica. Two hypotheses were suggested, either that the substance had a complementary action to the pathogens elicitors or that it raised the level of repression shown by suppressors of the pathogens in defence reactions.

Rathmell (1981) has suggested that synthetic compounds that can induce resistance to pathogens in plants are surprisingly common. He observed a highly significant correlation between the ability of a compound to cause some form of phytotoxicity when applied to soil in which rice or wheat seedlings were growing and its ability to induce resistance to a subsequent inoculation of the plant's foliage with Pyricularia oryzae or Puccinia recondita. Between two and five percent of compounds tested caused phytotoxicity and induced resistance in treated plants; the linkage between the two effects was statistically highly significant.

Gianinazzi and Kassanis (1974) induced resistance to various virus diseases in tobacco by pretreatment with polyacrylic acid, a substance

having no in vitro effects. Polyacrylic acid treatment has since been shown to elicit systemic effects against Colletotrichum lagenarium in cucumber (Mills and Wood, unpublished). Aspirin (acetylsalicylic acid) has also been shown to have a similar mode of action against virus disease (White, 1979) and this too has also been demonstrated in the cucumber/Colletotrichum system (Mills and Wood, unpublished).

Davis and Dimond (1953) investigated the chemotherapeutic activity of various plant growth regulators and several were found that had no in vitro fungitoxicity but induced effects in the host. Indole-3-acetic acid retarding compounds were shown by Andel (1968) to induce resistance while indole-3-acetic acid itself increased susceptibility. Stahmann et al. (1966) increased disease resistance of sweet potato tissue to black rot by exposure to ethylene whilst tomatoes gassed with ethylene showed induced resistance to Verticillium albo-atrum attack (Pegg, 1976). Catechol and ethephon treatment of tomato roots inhibited expression of wilt symptoms (Retig and Chet, 1974; Retig, 1974). The induced resistance in both cases was correlated to an increase in peroxidase and polyphenoloxidase activities.

Physical treatments such as exposure to heat and ultra-violet (UV) irradiation have been suggested as possible inducers of resistance in plants. Rahe (1973) exposed French bean hypocotyls to heat and induced levels of phytoalexins to the extent that a shift from susceptibility to resistance was achieved. Heat treatment of sugar cane leaves by Byther and Steiner(1975) led to induced resistance to Helminthosporium sacchari and to helminthosporoside. Exposure to UV irradiation was shown to lead to increased levels of phytoalexin accumulation in French bean hypocotyls by Anderbrhan (1979). Theodorou (1979) correlated phytoalexin increases due to irradiation of French bean leaves with alteration of disease response. All these physical treatments led only to localized induced resistance there being no reported systemic effects.

III. MATERIALS AND METHODS

1. Plant Material

a) Vines (*Vitis vinifera*)

i) Seedlings - Seedlings were grown from grape seed obtained from ICI Plant Protection. They were vernalized by soaking in ice cold distilled water for 3 days, the water being changed twice daily. They were then drained and mixed with an equal volume of vermiculite and stored at 2-3°c for 4 weeks. The seeds were germinated in trays and pricked out at the cotyledon stage into 1½" pots. Seedlings were transferred from Jealott's Hill Research Station to Imperial College at South Kensington at this stage and grown on in the greenhouse (25-35°c; 16 h. photoperiod). At the 2-3 leaf stage plants were repotted into 3" pots or larger in a peat/compost mixture (1:1).

ii) Cuttings - Hardwood cuttings were taken from mature vines (cvs. Black Hamburg, Cabernet Sauvignon, Sauvignon Blanc, Semillon, Alicante Bouschet and Rubired) in the late autumn/early winter by removing stem sections, approximately 2" long, including a bud. Cuttings were pressed into moist peat and placed in propagating cases in the greenhouse (c. 30°c; 16 h. photoperiod) to induce bud burst. Cuttings not required immediately were stored in plastic bags at 5°c. Softwood cuttings from an actively growing vine were rooted and grown in a similar manner. These were more susceptible to fungal attack, especially by *Botrytis cinerea*, and were therefore sprayed with a commercial preparation of benomyl (Benlate, DuPont) to exclude unwanted infection. Softwood cuttings were not stored.

iii) Exclusion of powdery mildew (*Uncinula necator*) - Contamination by the vine powdery mildew fungus was a recurrent problem. Spores were introduced both on plant material obtained from Jealott's Hill

Research Station and from outside the greenhouse in South Kensington. Daily drenching of the leaves with water or a detergent solution (0.01% Tween 80) kept the fungus in check but did not eliminate it, especially from the undersurfaces of the leaves which tended to escape treatment. A major problem was the escalation of small scale infections when experimental plants were placed in growth cabinets. Initially Karathane was applied as a wettable powder (Dinocap, Murphy Chemicals Ltd.) but "scorching" of the leaves often resulted. Delp (1954) suggested the use of high temperatures to control vine powdery mildew and it was confirmed that maintenance of a temperature of at least 32°c for approximately six hours successfully eliminated all infection. Growing plants were intermittently exposed to such temperatures to kill any infective material in the greenhouse.

iv) Use of leaf disks - Leaf disks were cut with a No. 5 or No. 10 cork borer (8 mm or 15 mm) and floated abaxial surface uppermost on distilled water in glass staining dishes. They remained green and in good condition for several weeks.

b) French beans (Phaseolus vulgaris)

i) Seedlings - French bean seeds cv. Canadian Wonder were pre-germinated at 25°c for 2 days and then planted into 3" pots in peat and grown on in the greenhouse (c. 25°c; 14 h. photoperiod). Primary leaves were fully expanded after seven to ten days' growth.

c) Broad beans (Vicia faba)

i) Seedlings - Seeds of broad bean cv. "The Sutton" were soaked overnight and surface treated for 10 minutes in a 20% Parazone solution (10% active chlorine). They were pre-germinated in high relative humidity at 25°c for 2 days then planted in compost in 3" pots and kept in the greenhouse (c. 25°c; 14 h. photoperiod). Plants were generally 3-4 weeks old when used and at this stage possessed 2-3 fully expanded pairs of leaflets.

Seedlings were also grown in Long Ashton Nutrient Solution, seeds being germinated in the usual way and supported on muslin at the top of wide necked 300 ml bottles. Roots grew into the solution which was supplemented as required. Bottles were covered with aluminium foil to exclude light and reduce algal growth.

Long Ashton Nutrient Solution -

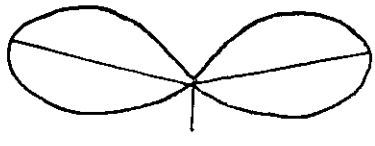
		g/200 ml	
1.	$Ca(NO_3)_2\ 4H_2O$	110.0	
2.	KNO_3	50.5	
3.	$NaH_2PO_4\ 2H_2O$	20.0	
4.	$MgSO_4\ 7H_2O$	36.0	
5.	FeNa EDTA	1.22	
	(adjusted to pH 5.6 with IN NaOH (4^oc))		
6.	Micronutrients - $MnSO_4\ 4H_2O$	0.223	in 200 ml of distilled water
	$CuSO_4\ 5H_2O$	0.024	
	$ZnSO_4\ 7H_2O$	0.029	
	$(NH_4)\ Mo_7\ 4H_2O$	0.0035	
	H_3Bo_3	0.186	

5 ml each of 1-6 was made up to 2.5 1 with distilled water.

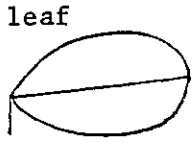
ii) Use of detached leaflet pairs (bifoliate leaves) - Fully expanded bifoliate leaves were detached from 3-4 week old plants. Wicks of absorbent paper were attached to their petioles and they were suspended on plastic grids over water in plastic boxes. Leaves remained in good condition for some time; sufficient for the duration of experiments.

iii) Definitions -

a) Bifoliate leaf (leaflet pair)



b) Half of a bifoliate leaf



c) One side of half a bifoliate leaf



2. Pathogens

a) Plasmopara viticola - grape vine downy mildew

i) Source - P. viticola was obtained from Jealott's Hill Research Station.

ii) Maintenance - Cultures were maintained on live plant material, either whole plants or leaf disks floated on distilled water in glass staining dishes. Stock inoculum was transferred onto fresh leaf material every 7 days.

iii) Spore suspensions - Leaves or leaf disks showing actively sporulating fungus on the undersurface were agitated in ice cold distilled water to produce a sporangial suspension. This was filtered through muslin and the concentration adjusted (1×10^5 sporangia ml^{-1}) using a haemocytometer.

iv) Inoculation - Leaf tissue was inoculated by spraying spore suspension on to the undersurface using a 'Jet-pak' aerosol cylinder (Shandon Southern Products Ltd., Cheshire). Where discrete areas of tissue were inoculated or a standard amount of inoculum was used droplets of spore suspension (5 µl) were placed on the tissue using an 'Agla' syringe (Wellcome Reagents Ltd.). Leaf material was incubated at 100% relative humidity ($24^{\circ}c$; 14 h photoperiod) for penetration to occur. Incubation was continued at a lower humidity for 6-8 days when material was again placed in 100% relative humidity for 24 hours to induce sporulation.

v) Disease assessment - On whole leaves disease was assessed on a visual scale as devised at Jealott's Hill Research Station. Grades 0-4 were assigned to a range of percentages of leaf area showing sporulation.

Grade	% leaf area showing sporulation
0	none
1	1-25
2	26-50
3	51-75
4	76-100

Where disks were used assessment could be carried out more precisely. A known quantity of inoculum was placed on a uniform leaf area using an Agla syringe. After incubation and sporulation induction disks were placed in 20% ethanol to preserve the sporangia and agitated using a "Whirly-mix" to produce a spore suspension. The number of sporangia per disk was calculated using a haemocytometer. However, with experience, visual assessment was shown to produce very similar results to direct counting and as the latter method was considerably time consuming it was rarely used.

Where several replicate disks from one leaf were assessed the modal disease grade was recorded i.e. that occurring most frequently. This was considered preferable to a normal mean as the situation when an inoculum droplet accidentally rolled off a leaf disk did not affect the final result.

vi) Viability of sporangia - Sporangial germination tests were carried out as follows:-

a) glass slides were cleaned by:

i) washing thoroughly in hot water and a non-ionic detergent;

ii) rinsing in hot tap water and then distilled water to remove detergent;

iii) standing in glass distilled water for 48 hours;

iv) draining and soaking in Analar acetone for a few hours;

v) draining and drying in a hot oven;

b) 3 x 20 µl drops of sterile ice cold distilled water were placed on 3 cleaned slides;

c) 2 µl of sporangial suspension in ice cold water was added to each drop at a concentration of 4 x 10^5 sporangia ml^{-1};

d) slides were placed in plastic boxes lined with moist absorbent paper and incubated in the dark at 17°c for 24 hours;

e) 100 sporangia were counted for each of 6 drops. The number of empty sporangia indicated the percentage germination, an allowance being made for those already germinated before the start of the test.

b) Colletotrichum lindemuthianum - French bean anthracnose

i) Source - C. lindemuthianum (race δ - virulent on French bean cv. Canadian Wonder) was obtained from the Imperial College culture collection.

ii) Maintenance - Cultures were kept on V8 juice agar in 250 ml conical flasks at 25°c in a "black light" incubator.

V8 juice agar -

V8 juice	200 ml	Autoclaved at 15 psi for 15 minutes
distilled water	800 ml	
agar	20 g	

The fungus was subcultured every 14 days by washing the surface of the V8 juice agar with approximately 1 ml of a 1 x 10^6 ml^{-1} spore suspension.

iii) Spore suspensions - The surfaces of 14 day old cultures were washed with 5-10 ml of sterile distilled water, filtered through sterile muslin and washed twice by centrifugation. Spores were resuspended in

sterile distilled water and the concentration adjusted using a haemocytometer. The spore concentration used was 1 x 10^6 spores ml^{-1} unless otherwise stated.

iv) Inoculation - a) Injection - Spore suspensions were injected directly into the laminae of leaves using syringes fitted with fine needles (1ere, Paris; No. 30G) (Theodorou, 1979).

b) Spraying - Spore suspensions were sprayed on to leaves using an air brush (Aerograph 63, DeVilbiss Co. Ltd., London). When it was required to inoculate only discrete areas the rest of the plant was protected with a thin plastic film. Inoculated plants were incubated in high relative humidity in growth cabinets (20^{o}c; 14 h photoperiod). In spray inoculated plants anthracnose symptoms developed after 5-6 days incubation. Where leaves had been injected with spore suspensions inoculated areas of the laminae collapsed after 3 days.

v) Disease assessment - Injected plants were assessed from the extent to which the lamina had collapsed as described by Theodorou and Smith (1978). Spray inoculated plants were assessed visually from the extent of necrosis which was visible mainly on the undersurfaces of the leaves. Attempts were made to develop a more objective assay whereby standard inoculum droplets were placed on the larger veins on the undersurfaces of leaves. However the method did not allow a better disease assay than visual assessment which was more reliable and considerably less time consuming.

c) Botrytis fabae - chocolate spot of broad bean

i) Source - B. fabae was obtained from the Imperial College culture collection. Initially the virulence was not high but was considerably improved after reisolation from lesions on the plant.

ii) Maintenance - B. fabae was grown on solid Medium X in petri dishes.

Medium X -

glucose	10 g)	
mycological peptone	2 g)	
casein hydrolysate (acid)	3 g)	
KH_2PO_4	1.5 g)	
$NaNO_3$	6 g)	(1)
KCl	0.5 g)	
yeast nucleic acids	0.5 g)	
distilled water	200 ml)	
$MgSO_4$ $7H_2O$	0.5 g)	(2)
distilled water	100 ml)	
Oxoid agar	20 g)	(3)
distilled water	700 ml)	

(3) was heated in a water bath (100^{o}c) to dissolve the agar and was then added to (1) and (2) prior to autoclaving for 20 minutes at 15 psi.

The fungus was incubated at 25^{o}c in the dark and sub-cultured on to new media every 6-8 days.

iii) Spore suspensions - Culture surfaces were flooded with 5-10 ml of sterile distilled water and agitated with a glass rod to dislodge the spores. The suspension was filtered through muslin and washed twice by centrifugation. The spores were resuspended in sterile distilled water and the concentration adjusted using a haemocytometer. This was generally 1 x 10^{6} spores ml^{-1} unless otherwise stated.

iv) Inoculation - Standard droplets (5 µl x 20/leaf) of spore suspension were placed directly on the upper surfaces of leaves, either on whole plants or detached leaves, using an Agla syringe. Inoculated leaves were incubated in high relative humidity to maintain droplets

(20°c; 14 h photoperiod). Symptoms appeared after 36-48 h. Leaves could also be inoculated by direct injection of spore suspension from a syringe fitted with a fine needle (1ere, Paris; No. 30G). They were incubated under the same conditions as droplet inoculated leaves; injected areas became necrotic after 24-36 h.

v) Disease assessment - The necrotic lesions resulting from the droplet inoculation of leaves were assessed using a grading scale modified from that used by Mansfield (1972):-

Grade	Lesion type
1 (s)	Spreading; necrosis beyond area of original droplet
2 (100)	100% necrosis under area of droplet
3 (51-99)	51-99% of area under droplet necrotic
4 (1-50)	1-50% of area under droplet necrotic (flecking)
5 (0)	no necrosis

Results were generally assessed after 3 days incubation when in the controls at least 85% of lesions would be of grade 1. If assessed after only 2 days the majority of the lesions would be of grade 2 and would not have started to spread.

d) Botrytis cinerea - grey mould

i) Source - B. cinerea was obtained from the Imperial College culture collection.

ii) Maintenance - B. cinerea was maintained on solid Medium X in petri dishes (as for B. fabae - see above) and sub-cultured every 4-5 days. Cultures were incubated at 25°c in the dark.

iii) Spore suspensions - These were prepared as for B. fabae.

iv) Inoculation - Leaves were inoculated by placing droplets of spore suspension on the upper surface or by direct injection as for

B. fabae. Symptoms appeared after 2 days incubation but in the controls were much less pronounced than were those caused by B. fabae even when inoculum was directly injected into leaves.

v) Disease assessment - Disease was assessed as for B. fabae but inoculation rarely resulted in symptom grades higher than 4. A proportion of inoculum droplets did not cause symptoms particularly when leaves were on whole plants. Detached leaves were slightly more susceptible; in controls the majority of droplets caused grade 4 lesions.

e) Uromyces fabae - broad bean rust

i) Source - U. fabae spores were obtained from infected material supplied by the Imperial College Field Station at Silwood Park.

ii) Maintenance - U. fabae was maintained on broad bean plants (cv. "The Sutton") in the greenhouse (c. 25°c; 14 h photoperiod). The fungal infection was sub-cultured on to fresh plant material every 10-14 days by spraying whole plants with a spore suspension and incubating them in high humidity for 2-3 days. Rust pustules appeared on the leaves after 6-7 days incubation.

iii) Spore suspensions - Plant material bearing rust pustules was agitated in a 0.01% Tween 80 solution. The resulting spore suspension was filtered through muslin and washed twice by centrifugation. The spores were taken up in 0.01% Tween 80 solution and their concentration adjusted to 5×10^5 spores ml^{-1} using a haemocytometer.

iv) Inoculation - Plants were inoculated by spraying with a solution of rust spores using a "Jet-pak" aerosol. Where discrete areas of plants were to be inoculated the remaining area was protected by covering it with a thin plastic film. Inoculated plants were incubated in high humidity (25°c; 14 h photoperiod) for 2 days. After a further 4-5 days incubation rust pustules appeared on the leaves.

v) Disease assessment - Disease was assessed by counting the number of sites at which a primary pustule developed.

f) Uromyces phaseoli - French bean rust

i) Source - U. phaseoli spores were obtained from infected material supplied by the Imperial College Field Station at Silwood Park.

ii) Maintenance - U. phaseoli was maintained on French bean plants (cv. Canadian Wonder) in the greenhouse (25°c; 14 h photoperiod). The fungus was sub-cultured on to fresh plant material every 10-14 days by spraying whole plants with a spore suspension and incubating them in high humidity for 2-3 days. Rust pustules appeared on the leaves after 6-7 days incubation.

iii) Spore suspensions - These were prepared as for Uromyces fabae (see above).

iv) Inoculation - Leaves were inoculated as for Uromyces fabae (see above).

v) Disease assessment - as for U. fabae (see above).

g) Pseudomonas phaseolicola - Haloblight of French bean

i) Source - P. phaseolicola was obtained from Dr. A. Slusarenko at Imperial College.

ii) Maintenance - Wild type and mutant strains of the bacterium were grown on Kings B agar slopes in McCartney bottles at 25°c.

Kings B medium -

Proteose peptone No. 3	2.0%
Bacto agar	1.5%
Glycerol B.P.	1.0%
K_2HPO_4 (anhydrous)	0.15%
$MgSO_4$ $7H_2O$	0.15%

Autoclaved at 121°c for 15 minutes then if necessary pH adjusted to 7.2.

24 hour old cultures were stored at 5°c and sub-cultured every few weeks.

iii) Cell suspensions - Fresh 24 hour cultures were flooded with 5-10 ml of sterile distilled water. The bacterial concentration (normally 1 x 10^7 cells ml^{-1}) was adjusted using a nephelometer, the standard curve being kindly supplied by Dr. A. Slusarenko.

iv) Inoculation - French beans were inoculated by directly injecting bacterial suspensions into the leaves from a syringe fitted with a fine needle (Aere, Paris; No. 30G). An area approximately 3 cm x 3 cm at the base of the petiole was infiltrated. Plants were also inoculated by spraying the leaves with bacterial suspension using a "Jet-pak" aerosol. Inoculated plants were incubated in high humidity at 20°c with a 14 hour photoperiod. Symptoms were apparent after 5-6 days incubation.

h) Tobacco necrosis virus (TNV) - on French bean; a local lesion host.

i) Source - Purified TNV was supplied by Mr. S. Oldfield at Imperial College.

ii) Maintenance - Purified TNV was stored at -20°c until required.

iii) Inoculation - French bean leaves were dusted with carborundum (Silicon carbide and aloxite - F.500) and then rubbed with purified virus. Plants were washed and incubated at 25°c with a 14 hour photoperiod for 3 days.

iv) Assessment - The number of local lesions appearing on the leaves was counted.

3. Preparation of biotic inducers

a) Cell-free germination fluid

Suspensions of spores of Botrytis fabae, Botrytis cinerea and Colletotrichum lindemuthianum were prepared at concentrations of 1 x 10^6 spores ml^{-1}. Uromyces fabae and Plasmopara viticola spore suspensions were prepared at 5 x 10^6 spores ml^{-1}. c. 70 ml of each was transferred

to a sterile medical flat bottle and incubated horizontally at 25°c in the dark for 48 h. Suspensions were then passed first through a size 0.80 µm Millipore filter and then through a 0.22 µm filter. This treatment excluded all spores whether germinated or not and P. viticola zoospores. Bacteria present would also have been excluded; cell-free germination fluids were thus obtained. They were used immediately after preparation and were not stored.

b) Heat-killed spores/bacterial cells

Suspensions of Botrytis fabae, Botrytis cinerea, and Colletotrichum lindemuthianum spores were prepared at concentrations of 1 x 10^6 spores ml^{-1} and of Uromyces fabae and Plasmopara viticola at 5 x 10^5 spores ml^{-1}. Spores were heat-killed in three ways:-

i) 10 ml of each suspension was placed in a test-tube in a water bath at 50°c. When the temperature of the suspensions reached 50°c they were left for a further 10 minutes then removed and allowed to cool to room temperature. This method was adapted from that described by Bell and Presley (1969).

ii) Following the method described by Beretta et al. (1977) spore suspensions were autoclaved at 121°c for 30 minutes. They were stirred at room temperature for one hour and then passed through a Millipore filter (0.80 µm pore size).

iii) Using the method of Heale and Sharman (1977) spore suspensions were passed through a Millipore filter (0.45 µm pore size). The deposit of spores on the surface of the filter was washed by passing 2 x 20 ml aliquots of sterile distilled water through it. The spores were removed by shaking the filter in 20 ml of sterile distilled water in a test tube. The concentration was adjusted to 1 x 10^6 spores ml^{-1} and the test tube placed in a water bath at 50°c. When the temperature of the suspension (10 ml) reached 50°c it was heated for a further 5 minutes

then allowed to cool to room temperature.

Bacterial suspensions were heat-killed by placing 10 ml volumes in a test tube in a water bath at 50°c. When the suspension had reached 50°c it was heated for a further 10 minutes then allowed to cool to room temperature.

c) Crude cell wall extracts

Botrytis fabae and Botrytis cinerea were grown in Czapek Dox liquid medium in 250 ml conical flasks (20 per fungus) on a shaking incubator (150 rpm; 18°c) for 14 days.

Czapek Dox liquid medium -

sucrose	60 g
$NaNO_3$	6 g
KH_2PO_4	2 g
$MgSO_4\ 7H_2O$	1 g
KCl	1 g
yeast extract	2 g
$FeSO_4$	0.1 g
distilled water	2 l

Autoclaved at 15 psi for 15 minutes.

The mycelium was then filtered through muslin and 5 ml distilled water per g wet weight of fungus added. This was homogenised for 5 minutes in a blender and again filtered through muslin. This process of homogenising and filtering was repeated first using chloroform: methanol (1:1; v/v) and then acetone. The mycelium was finally dried in air and stored overnight at -20°c. Before use 1 g of dried extract was added to 100 ml of distilled water and autoclaved at 121°c for 30 minutes. The product was filtered through muslin and allowed to cool to room temperature. For longer storage the crude wall extract was kept at -20°c at the air-dried stage.

d) Crude culture filtrates

i) Following the method of Osman (1977) extracts were prepared from 3 week old cultures of B. fabae and B. cinerea growing on solid Medium X (see above) in petri dishes. The surfaces of the cultures (5 per fungus) were each rinsed for a few seconds with 10 ml of sterile distilled water and the aliquots pooled. The suspensions were centrifuged for 5 minutes to deposit the majority of the spores and the supernatant passed through a Millipore filter (0.45 µm pore size). The spore-free samples were then condensed on a rotavapor at 37°c to 1/10th of the original volume. Filtrates prepared in this manner were not stored but used immediately after preparation.

ii) Filtrates of cultures of B. fabae and B. cinerea were also prepared by a method modified from that of Theodorou (1979). Fungi were grown in Czapek Dox liquid medium (see above) for 14 days on a shaking incubator (150 rpm; 18°c). The mycelium was removed by filtering through muslin and the filtrate then passed through a Büchner funnel (Whatman No. 41 paper; x 2 thicknesses). It was further cleaned by passing through a Millipore filter (0.45 µm pore size) and dialysed against a large volume of distilled water for 48 h at 5°c. The water was changed a total of 5 times throughout the 48 hour period. The filtrates were stored at -20°c until required.

4. Abiotic inducers

a) Growth regulating substances

i) Indole-3-acetic acid (IAA) (MW = 175.18)

ii) Maleic hydrazide (MH) (1,2-Dihydro-3,6-pyridazinedione) (MW = 112.09)

iii) Gibberellic acid (GA) (MW = 346.37)

iv) Cycocel (2-chloroethyl trimethyl ammonium chloride) (MW = 450.27)

v) Ethephon (2-chloroethyl phosphonic acid) (MW = 144.5)

vi) Kinetin (6-furfurylaminopurine) (MW = 215.21)

Indole-3-acetic acid, maleic hydrazide, gibberellic acid, cycocel and kinetin were all supplied by the Sigma Chemical Company. Ethephon was obtained from A.H. Marks and Co. Ltd. (Wyke, Bedford).

All growth regulating susbstances were made up as aqueous solutions, some were initially dissolved in a small volume of ethanol which was then evaporated off.

b) Polyacrylic acid (Dispex N40)

Supplied by Allied Colloids Ltd. Polyacrylic acid was dissolved in 0.1 M phosphate buffer (pH 6.5-7.0) to a concentration of 0.4% (w/v).

c) Gentisic acid (2,5-Dihydroxybenzoic acid)

Supplied by the Sigma Chemical Company. Gentisic acid was dissolved in ·1 M phosphate buffer (pH 6.5-7.0) at a concentration of 0.02% (w/v).

d) Aspirin = Acetylsalicyclic acid (2-Acetoxy benzoic acid)

Supplied by the Sigma Chemical Company. Aspirin was dissolved in 0.1 M phosphate buffer (pH 6.5-7.0) at a concentration of 0.02% (v/w).

e) ICI test fungicide compounds (Nos. 1-7)

These were made up as aqueous solutions. Those not presented as salts were initially dissolved in a small volume of ethanol which was then evaporated off. All solutions were freshly prepared before use.

f) Ultra-violet irradiation

Leaf material was exposed to UV irradiation supplied by a Phillips TUV 30 watt germicidal single tube with an output of 8 watts at 2537Å waveband at a distance of 140 cm.

5. Extraction and assessment of phytoalexins from vines

Leaf material (c. 2 g fresh weight) was weighed and homogenised with 50 ml of 80% methanol. Samples were dried using a rotary evaporator at 40°c then redissolved in 25 ml ethyl acetate and 25 ml distilled

water. They were partitioned three times and the organic phases pooled. These were dried by the addition of anhydrous sodium sulphate then evaporated on a rotary evaporator at 40°c. Samples were stored overnight at -20°c then spotted onto t.l.c. plates (Scheicher and Schüll, F1500 LS. 254; silica gel) in 500 µl of ethyl acetate. Plates were developed in dichloromethane:methanol (4:1) for approximately 90 minutes. They were examined under a UV light source (254 nm and 366 nm) to determine the position of extracted substances. Each plate was then cut into 3 sections lengthways. The sections were used for i) a Cladosporium cucumerinum bioassay (Bailey and Burden, 1973), ii) a reagent test for phenolics using diazotised nitroaniline and iii) spectrophotometric analysis.

Diazotised nitroaniline -

5 ml of 0.5% 4-nitroaniline solution in 2 N HCl was mixed under cooling with 0.5 ml of 5% aqueous $NaNO_2$ solution and 15 ml of 20% aqueous sodium acetate solution was added. Phenolic compounds form products of an orange/brown colour with this reagent.

ii) The reagent was sprayed directly onto tlc plates and reacted immediately with any phenolics present.

iii) Spectrophotometric analysis - samples were removed from the surface of the tlc plates and eluted from the silica gel with ethanol. They were scanned on a Beckman spectrophotometer (series 30; Model 35) for UV absorbance between 200 and 360 nm. Peaks in absorbance spectra were compared with published values (Langcake and Pryce, 1977).

6. Statistical analysis of data

Statistical tests were carried out on data; controls and treatments were considered significantly different with a greater than 95% probability if:-

$$\pm \bar{x}_c - \bar{x}_T > 2 \quad (SE_T^2 + SE_c^2)$$

where:- $\bar{x}_c$ = mean of controls

$\bar{x}_T$ = mean of treatments

SE_T = standard error of treatments

SE_c = standard error of controls

given that:-

$$\text{standard error} = \frac{\text{standard deviation}}{\sqrt{(\text{no. of replicates})}}$$

$$\text{standard deviation} = \sqrt{\left(\frac{\Sigma x^2 - (\Sigma x)^2/\text{no. of replicates}}{\text{no. of replicates} - 1}\right)}$$

IV. EXPERIMENTAL

A. Induction of systemic resistance by pre-inoculation with live pathogens

(1) Vitis vinifera: Plasmopara viticola

a) Preinoculation with P. viticola

Small vine seedlings (10 replicates) with 3-4 leaves were inoculated on the lowest leaf with P. viticola sporangial suspension at a concentration of 1×10^5 sporangia ml^{-1} and infection developed as expected. Controls were similarly treated with distilled water. After 3, 7 and 10 days incubation the upper leaves were inoculated (the challenge inoculation) at a concentration of 1×10^5 sprangial ml^{-1} either by directly inoculating whole leaves on the plant or by inoculating disks taken from the upper leaves. Those plants incubated for 7 and 10 days were either challenged after the initial infection had been induced to sporulate or before. Plants incubated for 3 days were all challenged before the induction of sporulation. The experiment was repeated with different sporangial concentrations, pre-inoculating 2 lower leaves rather than one, not inducing the challenge inoculation to sporulate and challenging disks from upper leaves. Inoculum levels at 1×10^5 and 5×10^5 sporangia ml^{-1} were used for pre-inoculation and 1×10^4 and 5×10^4 sporangia ml^{-1} for challenges. There was no evidence of systemic induced resistance in any of the challenged leaf disks (Tables 1-12).

The experiment was repeated using plants grown from cuttings (5 replicates) (cv. Black Hamburg) pre-inoculating both one and two lower leaves at 3 spore concentrations and challenging leaf disks taken from upper leaves at 3 spore concentrations after 7 days (Tables 13-18).

More limited experiments were carried out on cuttings (10 replicates) of the varieties Sauvignon Blanc, Semillon, Rubired, Alicante Bouschet

and Pinot Noir. The two lower leaves were pre-inoculated at a concentration of 1 x 10^5 sporangia ml^{-1} and disks cut from the upper leaves were challenged at concentrations at 1 x 10^5 and 5 x 10^4 sporangia ml^{-1} after 7 days incubation without inducing sporulation on the lower leaves.

No evidence of systemic induced resistance was seen in any of the varieties tested (Tables 19-23).

The degree of necrosis caused by any infection varied considerably between the different types of plants used. Seedling plants, Black Hamburg and Alicante Bouschet cuttings generally showed more necrosis than any of the other varieties. However the greatest variation in the occurrence and the extent of necrosis was seen between individual plants of the same cultivar and type. This was particularly so in the seedling plants which were inherently variable in many respects. At spore concentrations just sufficient to produce dense sporulation the concentration had no apparent effect on the extent of necrosis. At higher spore concentrations (5 x 10^5 sporangia ml^{-1}) overall necrosis was greater although there was still considerable variation between individual plants.

On seedling plants attempts were made to induce resistance in the leaves by pre-inoculating the cotyledons. Cotyledons could be infected by inoculating in the usual manner but for some reason inoculation was not always successful. Plants (20 replicates) with inoculated cotyledons (1 x 10^5 sporangia ml^{-1}) were thus incubated for 6 days when sporulation was induced. Controls were treated with distilled water. Those plants with cotyledons showing sporulation (c. 60% of those inoculated) were challenged after 7 days by inoculating disks taken from the leaves (sporangial concentration 1 x 10^5 ml). No evidence of any systemic induced resistance was seen (Table 24). Vine cotyledons, unlike the leaves, can be injected so inoculum (1 x 10^5 sporangia ml^{-1}) was introduced directly via a syringe fitted with a fine neede. Sporulation

did not follow such inoculations. Challenging of disks taken from leaves after 6 days showed no evidence for systemic induced resistance (Table 24).

To see if protection could be demonstrated over shorter distances within a single leaf large leaf disks 20 mm in diameter were cut from large vine leaves (cv. Black Hamburg). Five disks were cut from each of 4 leaves from the same vine. 5 µl inoculum droplets (concentration 1 x 10^5 sporangia ml^{-1}) were placed on one side of each disk, control disks being similarly treated with distilled water. After 3 and 5 days incubation the opposite side of each disk was challenged with another inoculum droplet (concentration 1 x 10^5 sporangia ml^{-1}). Disease developed to a similar degree in all disks with no evidence of induced resistance (Tables 25 and 26).

2. Phaseolus vulgaris: Colletotrichum lindemuthianum.

a) Pre-inoculation with Colletotrichum lindemuthianum

Following the methods reported by Sutton (1979) French bean plants with fully expanded primary leaves and 1 small trifoliate leaf (6 replicates) were inoculated with C. lindemuthianum race δ. Spore suspension (1 x 10^6 spores ml^{-1}) was sprayed on to the primary leaves using an airbrush and disease developed as expected; controls were treated with distilled water. Plants were also treated by injecting the primary leaves with spore suspension (concentration 1 x 10^6 spores ml^{-1}) (Materials and Methods). Control plants were injected with distilled water. After 5 and 10 days incubation in high humidity the plants were sprayed entirely with spore suspension at concentrations of 1 x 10^5 and 1 x 10^6 spores ml^{-1}. After a further 5 days incubation disease was assessed. Plants were also challenged by injecting the trifoliate leaves with spore suspension (1 x 10^6 spores ml^{-1}). After 4 days incubation these were assessed by the degree of tissue collapse.

On plants challenged by spraying necrotic lesions developed after 5 days, leaves becoming totally necrotic after 10 days. Neither the tissue collapse reaction (Materials and Methods) nor necrosis on the lower leaves had any apparent effect on the development of disease on the trifoliates. Injected leaves collapsed (the susceptible reaction) after 3-4 days incubation. This reaction on the trifoliates was unaffected by collapse or necrosis of the lower leaves.

Pre-inoculation of an area of tissue (c. 2.5 cm diameter) on one side of a primary leaf or at the leaf tip had no effect on subsequent challenging of non-infected portions on the opposite side or end of the same leaf (areas were separated by c. 6 cm). The spore concentration for pre-inoculation and challenge was 1×10^6 spores ml^{-1}; plants were incubated for 3 and 5 days before challenging. Controls were pretreated with distilled water.

b) Pre-inoculation with TNV

Primary leaves (6 replicates) were inoculated with TNV and 3 and 6 days later the trifoliate leaves challenged by spraying with a spore suspension of C. lindemuthianum (1×10^6 spores ml^{-1}). Controls were pretreated with distilled water. TNV local lesions (several hundred per leaf) developed after 48 h incubation. Their presence on the primary leaves had no apparent effect on the development of C. lindemuthianum on the trifoliate leaves.

Primary leaves were also pre-inoculated on one side of the leaf or at the leaf tip on an area approximately 2.5 cm in diameter. Challenging of the opposite side of or the petiole end of the leaf (c. 6 cm distant) with C. lindemuthianum (1×10^6 spores ml^{-1}) after 3 and 5 days incubation showed no evidence of any systemic induced resistance between parts of the same leaf.

c) Pre-inoculation with Pseudomonas phaseolicola

Primary leaves were injected at the base of the petiole with P. phaseolicola cell suspensions (1 x 10^7 cells ml^{-1}) of a wild type strain and a toxin-less mutant. Controls were injected with distilled water. Severe chlorosis of the inoculated area occurred after 3 days incubation in bacteria-injected but not control plants. Challenge inoculation of the trifoliate leaves by spraying with C. lindemuthianum was carried out after 3 and 6 days. The development of necrosis in treated plants compared to controls was unaffected by either of the bacterial pre-treatments.

Bacterial suspensions were also injected into small areas (approximately 2.5 cm diameter) on one side of or at the leaf tip of primary leaves. Challenge inoculations were made on the opposite side and at the petiole ends of leaves (c. 6cm distant) but there was no evidence of systemic induced resistance within leaves.

d) Pre-inoculation with Uromyces phaseoli

Primary leaves (10 replicates) were inoculated with the French bean rust fungus U. phaseoli by spraying with and injecting with a spore suspension (5 x 10^5 spores ml^{-1}). Controls were similarly treated with a 0.01% Tween 80 solution. Plants were incubated for 7 and 12 days, large numbers of rust pustules (up to 100 per leaf) appearing on inoculated plants after 7 days. Fewer pustules were produced from injected than from sprayed plants; the former tended to cause the development of chlorotic areas. Challenge inoculations were carried out by spraying the trifoliates with a spore suspension of C. lindemuthianum (1 x 10^6 spores ml^{-1}) and disease developed as expected in control plants. There was no significant difference between plants pre-inoculated with the rust and control plants.

3. Vicia faba: Botrytis fabae/Botrytis cinerea. Uromyces fabae

a) Pre-inoculation with Botrytis fabae and B. cinerea

3-4 week old broad bean plants (3 replicates) were selected and the lower 2 bifoliate leaves were inoculated with a spore suspension (1×10^6 spores ml^{-1}) either by direct injection or by placing droplets on the leaf surface (20 x 5 µl per bifoliate leaf). Controls were similarly treated with distilled water. After high humidity incubation for 3 and 5 days the upper leaves were detached and challenged by droplet inoculation (5 µl x 20 per bifoliate) at a concentration of 1×10^6 spores ml^{-1}. Lower leaves injected with B. fabae were completely necrotic after 2 days and those inoculated with droplets of inoculum had 100% lesions (i.e. the area under the inoculum droplet completely necrotic but no spreading necrosis). Leaves injected with B. cinerea had limited necrosis only and were somewhat desiccated despite incubation at high humidity.

In a series of experiments various combinations of "inducing" and "challenging" pathogens were used. B. fabae was used as the inducer and challenged with B. fabae or B. cinerea. B. cinerea was used as the inducer and challenged with B. cinerea or B. fabae. Where B. cinerea was used as an inducer it was applied by injection only as droplet inoculations on whole plants produced little if any reaction. In all cases challenges were assessed after 3 days.

Plants challenged with B. cinerea were unaffected by any of the pre-inoculation treatments. However plants injected with B. fabae and challenged with B. fabae after 3 days developed no spreading lesions compared to 95% spreading lesions in water injected controls. Plants where B. fabae had been applied as droplets also produced no spreading lesions when challenged compared to 92% in controls. Plants injected with B. cinerea and challenged with B. fabae after 3 days produced 17%

spreading lesions compared to 95% in the controls. Results were essentially similar in the 5 day incubated plants. Leaves from plants injected with B. fabae where the spreading of lesions had been totally suppressed appeared somewhat desiccated despite incubation at high humidity (Tables 27-34).

The experiment was repeated five times with essentially similar results on three occasions. In the other two experiments there was no evidence of any induced resistance.

Detached bifoliate leaves (3 replicates) were inoculated on one side with droplets of B. fabae or injected with B. cinerea (spore concentration 1 x 10^6 ml^{-1}). Controls were similarly treated with distilled water. After 3 days incubation the opposite halves were challenged with droplets of B. fabae and B. cinerea spore suspension (concentration 1 x 10^6 spores ml^{-1}); 10 per leaflet). No evidence of any resistance induced systemically across the leaf was seen (Table 35).

b) Pre-inoculation with Uromyces fabae

3-4 week old plants (10 replicates) were inoculated on the 3 lowest bifoliate leaves with a suspension of spores of U. fabae (5 x 10^5 spores ml^{-1}). Controls were treated with 0.01% Tween 80 solution. Rust pustules (c. 30-50 per leaf) appeared on inoculated leaves after 6-7 days incubation. All plants were challenged 6 days later with U. fabae (spore concentration 5 x 10^5 ml^{-1}). The development of rust pustules on the upper leaves (c. 30-50 per leaf) was the same in all plants, there being no evidence of any systemic induced resistance (Table 36).

Plants (6 replicates) pre-inoculated on the lower leaves with U. fabae were also challenged with B. fabae and B. cinerea (spore concentration 1 x 10^6 ml^{-1}) by droplet inoculation of detached upper leaves. Again necrosis developed to the usual extent in all plants with no indication of any systemic induced resistance (Table 37).

The converse experiment where the 2 lower bifoliates were pre-inoculated with B. fabae or B. cinerea and the upper leaves were challenged with U. fabae also failed to show any evidence of systemic induced resistance (Table 38).

B. Induction of systemic resistance by pre-treatment with the products and/or constituents of pathogens

(i) Cell-free germination fluid

a) P. viticola: Vitis vinifera

It was soon found that vine leaves cannot be infiltrated with liquid by injection or spraying under pressure. This meant that treatments could only be applied to the surfaces of leaves.

Droplets (5 µl) of cell-free germination fluid were placed on leaf disks taken from leaves on a large Black Hamburg vine (10 replicates) and incubated for 24 and 72 hours under the usual conditions. Controls were treated with distilled water. All the droplets were then removed from the disks and replaced by inoculum droplets (5 µl; concentration 5×10^5 sporangia ml^{-1}).

Droplets of germination fluid were also applied to one side of larger leaf discs (Black Hamburg; 15 mm diameter) and the opposite side challenged with inoculum after 24 and 72 h.

Results were assessed according to the amount of sporulation. Pre-treatment with cell-free germination fluid had no effect on disease development either locally or systemically (Tables 39 and 40).

b) U. fabae)
B. fabae) Vicia faba
B. cinerea)

The lower 2 bifoliate leaves of broad bean plants (3 replicates) were injected with cell-free germination fluid from U. fabae. B. fabae, and B. cinerea. Control plants were injected with 0.01% Tween 80 in the

case of U. fabae and distilled water in the case of the two Botrytis species. After 3 and 7 days incubation under the usual conditions for each disease the upper leaves were detached and challenged with U. fabae, B. fabae or B. cinerea. Plants pre-treated with B. fabae germination fluid was also challenged with B. cinerea and vice-versa.

None of the germination fluids had any effect on the development of disease on the upper leaves (Tables 41 and 42).

Germination fluids were also injected into one half of detached bifoliate leaves (Materials and Methods) (3 replicates). Controls were injected with 0.01% Tween 80 or distilled water as previously. The opposite halves of leaves were challenged after 3 and 5 days incubation. Pre-injection of germination fluid had no effect on the development of disease on the opposite half-leaf. Again B. fabae germination fluid was challenged against B. cinerea inoculum and vice-versa (Tables 43 and 44).

An experiment was also carried out to see if any effect was transmitted between opposite sides of one half of a bifoliate leaf (Materials and Methods). One half (3 replicates) was infiltrated with germination fluid, (or water or 0.01% Tween 80 in the case of controls) and the opposite half challenged after 2 and 4 days. In all cases disease developed normally, the pre-treatments having no apparent effects (Table 45).

c) C. lindemuthianum: Phaseolus vulgaris

Cell-free germination fluid was injected into both primary leaves (6 replicates) at the base of the petiole infiltrating an area approximately 2.5 cm in diameter. Controls were similarly injected with distilled water. Plants were incubated under the usual conditions for 2 and 5 days and then challenged by spraying with a spore suspension (1×10^6 spores ml^{-1}) of C. lindemuthianum. The development of necrosis was

unaffected by pre-treatment with germination fluid.

Germination fluid was also injected into one primary leaf and the opposite primary leaf was challenged by spraying after 2 and 5 days incubation. There was no evidence for any induction of resistance between the primary leaves. To test for any effects within individual leaves an area of tissue on one side of or at the leaf tip of a primary leaf was infiltrated with germination fluid or distilled water (controls). After 2 and 5 days the opposite side or the opposite end of the leaf was challenged with C. lindemuthianum inoculum. Pre-treatment had no effect on disease development.

(2) Heat-killed spores/bacterial cells

a) P. viticola: Vitis vinifera

Sporangial suspensions were heat-killed according to the method of Bell and Presley (1969) (Materials and Methods).

The cotyledons of seedling vines (10 replicates) with 2-3 leaves were injected with a heat-killed sporangial suspension (5×10^6 sporangia ml^{-1}) or distilled water (controls). After 4 and 7 days incubation under the usual conditions disks (5 replicates) were removed from the upper leaves and challenged with P. viticola (5×10^5 sporangia ml^{-1}). There was no significant difference in sporulation on control and treatment disks (Table 46).

Droplets (5 µl x 1) of heat-killed sporangial suspension were placed centrally on 8 cm leaf disks taken from leaves of a Black Hamburg vine (10 replicates). Controls were treated similarly with distilled water. After 24 and 72 hours droplets were removed and replaced by droplets of P. viticola inoculum (5×10^5 sporangia ml^{-1}).

Droplets of heat-killed sporangial suspension (5 µl x 1) were also placed to one side of larger leaf disks (15 mm diameter; 10 replicates) from a Black Hamburg vine. Controls were again treated with distilled

water. Challenging was carried out by placing inoculum droplets (x 1 per disk) on the opposite sides of leaf disks.

The results show no evidence of induced resistance by heat-killed sporangial preparations either locally or systemically (Tables 47 and 48).

Heat-killed sporangial suspensions were also applied to the roots of Black Hamburg vines (3-4 leaf stage; 6 replicates). Preparations were watered onto the compost in which the vines were growing at the rate of 50 ml per day for 4 days. Controls were similarly treated with distilled water. Disks (5 replicates) cut from the leaves were challenged with P. viticola (5×10^5 sporangia ml^{-1}) after 7 and 9 days (from the start of the experiment). Pre-treatment with heat-killed sporangia in this manner showed no effect on the development of disease in the leaves as assessed by the extent of sporulation (Table 49).

b) U. fabae: Vicia faba

Spore suspensions (5×10^5 spores ml^{-1}) were heat-killed according to the methods of Beretta et al. (1977) and of Heale and Sharman(1977) (Materials and Methods).

Both preparations were each injected into the lower 2 bifoliate leaves of 3-4 week old plants (6 replicates). Controls were injected with 0.01% Tween 80 solution. Upper leaves were challenged by spraying with inoculum after 3 and 7 days incubation. There was no evidence of any systemic induced resistance, the number of rust pustules developing on treatment and control plants being similar (Table 50).

Heat-killed preparations were also injected into one half of detached bifoliate leaves (Materials and Methods) (6 replicates). Controls were injected with 0.01% Tween 80 solution. The opposite half of the leaf was challenged with droplets of U. fabae spore suspension (5×10^5 spores ml^{-1}) after 2 and 4 days incubation under the usual conditions.

Results showed no evidence of any systemic induced resistance (Table 51).

Both preparations were each applied to the roots of plants (6 replicates) by watering onto the soil at a rate of 50 ml per day for 4 days. Controls were treated similarly with 0.01% Tween 80 solution. All plants were challenged by spraying with U. fabae inoculum (5×10^5 spores ml^{-1}) after 7 and 9 days incubation (from the start of the experiment). There was no significant difference in the number of rust pustules produced on the leaves of treated and non-treated plants (Table 52).

Beretta et al. (1977) achieved significant effects by painting lower leaves with heat-killed preparations. Spores heat-killed by this method were thus painted onto both surfaces of 2 lower bifoliate leaves (6 replicates). Controls were similarly treated with 0.01% Tween 80 solution. Upper leaves were detached and challenged after 3 and 7 days incubation but results showed no alteration in disease response (Table 53).

Heat-killed spore preparations were also painted on to the lower surfaces of detached leaves (6 replicates) and the upper surfaces challenged after 24 and 72 hours. Results showed the number of pustules produced on treated leaves to be similar to that produced on control leaves (Table 54).

c) U. fabae)
B. fabae) Vicia faba
B. cinerea)

Samples were prepared according to the methods of Bell and Presley (1969) and of Heale and Sharman (1977) (Materials and Methods).

Preparations were each injected into the two lower bifoliate leaves of 3-4 week old plants (3 replicates). Controls were injected with distilled water. Upper leaves were detached and challenged

(1×10^6 spores ml^{-1}) after 3 and 7 days incubation under the usual conditions. In a series of experiments B. fabae heat-killed preparations were challenged with B. fabae and B. cinerea inoculum and B. cinerea heat-killed preparations challenged with B. cinerea and B. fabae inoculum. Assessment of lesions showed no alteration of response induced by pre-treatment with heat-killed conidia (Tables 55-58).

Both preparations were also injected into one half of detached bifoliate leaves (Materials and Methods) (3 replicates; distilled water controls) and the opposite halves challenged after 2 and 4 days incubation. As previously preparations from both species were challenged with inoculum from both species but there was no evidence of alteration of disease response in any of the combinations (Tables 59-62).

Both heat-killed preparations from B. fabae and B. cinerea were each applied to the roots of plants (3 replicates) by watering onto the soil at a rate of 50 ml per day for 4 days. Controls were similarly treated with distilled water. After 7 and 9 days (from the start of the experiment) leaves were detached and challenged with B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions developed to a similar extent in treated and control plants (Tables 63-66).

d) C. lindemuthianum: Phaseolus vulgaris

Spores were heat-killed according to the method described by Heale and Sharman(1977) (Materials and Methods).

Preparations or distilled water (controls) were injected into both primary leaves (6 replicates) at the base of the petiole watersoaking an area approximately 2.5 cm in diameter. After 2 and 5 days incubation under the usual conditions plants were inoculated by spraying with C. lindemuthianum spore suspension (1×10^6 spores ml^{-1}). Visual assessment of the degree of necrosis showed no significant difference between treated plants and controls.

Heat-killed preparations were also injected into one primary leaf (6 replicates) and the other leaf challenged after 3 and 7 days. There was no evidence of any systemic induced resistance in the leaves.

A small area of tissue approximately 2 cm in diameter on one side of a primary leaf or at the leaf tip was infiltrated with heat-killed preparation or distilled water (controls). Challenging of the opposite side or the opposite end of the leaf with C. lindemuthianum inoculum showed no evidence of systemic induced resistance within a single leaf.

Heat-killed spore preparations were also applied to the roots by watering onto the soil at a rate of 50 ml per day for 4 days. After 7 and 9 days (from the start of the experiment) plants (6 replicates) were challenged by spraying with C. lindemuthianum inoculum. There was no significant difference in the extent of necrosis on treated and control plants.

e) Pseudomonas phaseolicola/C. lindemuthianum: Phaseolus vulgaris

Bacterial suspensions (1×10^8 cells ml^{-1}) were heat-killed as described previously (Materials and Methods). Heat-killed preparations were directly injected into primary leaves (6 replicates) infiltrating an area approximately 2.5 cm in diameter at the base of the petiole. Controls were similarly treated with distilled water. After 3 and 6 days incubation under the usual conditions plants were challenged by spraying with C. lindemuthianum inoculum (1×10^6 spores ml^{-1}). The development of necrosis was similar on treated and control plants.

Heat-killed bacterial suspensions were also applied to the roots by watering onto the soil at a rate of 50 ml per day for 4 days. Controls were similarly treated with distilled water. After 7 and 9 days incubation (from the start of the experiment) plants were challenged by spraying with C. lindemuthianum inoculum (1×10^6 spores ml^{-1}). Necrosis developed to a similar extent on treated and control plants.

(3) Crude cell wall extracts B. fabae/B. cinerea: V. faba

Crude cell wall extracts were prepared from B. fabae and B. cinerea cultures.

The two lower bifoliate leaves of 3-4 week old plants (3 replicates) were injected with crude cell wall extracts of B. cinerea and B. fabae or distilled water (controls). Plants were incubated under the usual conditions for 2 and 5 days when the upper leaves were detached and challenged with B. fabae or B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but none of the pre-treatments had any effect on infection of the upper leaves (Table 67).

Both cell wall extracts were also injected into one half of detached bifoliate leaves (Materials and Methods) (3 replicates). Controls were injected with distilled water. After 2 and 4 days incubation the opposite halves were inoculated with droplets of B. fabae and B. cinerea spore suspensions (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but there was no evidence of any effect being transmitted between the two halves of bifoliate leaves (Table 68).

Broad beans (3 replicates) were grown from seed in Long Ashton nutrient solution containing cell wall extracts. Plants were grown in 200 ml of Long Ashton nutrient solution plus 50 ml of crude cell wall extract; controls were grown in Long Ashton nutrient solution only. Jars were supplemented with Long Ashton nutrient solution as required. After approximately ten days in the usual incubation conditions in a growth cabinet seedlings had grown to produce two bifoliate leaves; treated plants tended to be c. 4-5 cm shorter and their roots stubbier than controls. Leaves were detached after 12 days and challenged with B. fabae and B. cinerea (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but there was no evidence of any difference in disease response between treated plants and controls (Table 69).

(4) Crude filtrates from cultures of B. fabae/B. cinerea: V. faba

i) Crude culture filtrates were prepared from B. fabae and B. cinerea liquid cultures according to the methods of Osman (1977) as described in Materials and Methods.

Filtrates were painted onto the upper and lower surfaces of detached bifoliate leaves (3 replicates; 0.01% Tween 80 controls). After 24 hours incubation under the usual conditions treated and untreated surfaces were challenged with droplets of B. cinerea and B. fabae spore suspension (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but results showed no alteration in response either systemically, i.e. through the thickness of the leaf, or locally (Table 70).

Filtrates were also tested for any effect after uptake by the petiole of detached leaves. Leaves (3 replicates; distilled water controls) were treated for 2 and 5 days then inoculated with B. fabae or B. cinerea inoculum (1×10^6 spores ml^{-1}). After 3 days lesions were assessed but if constituents of the filtrates were taken up by the leaves they induced no changes in host response to disease (Table 71).

The filtrates were also injected into the 2 lower bifoliate leaves of 3-4 week old plants (3 replicates). Controls were injected with distilled water. After 2 and 5 days incubation under the normal conditions both treated and upper leaves were detached and challenged with B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but there were no significant differences between treated plants or controls either on injected or upper leaves (Table 72).

ii) Crude culture filtrates were also prepared using a method modified from that described by Theodorou (1979) (Materials and Methods).

Filtrates or distilled water (controls) were directly injected into the 2 lower bifoliate leaves on 3-4 week old plants (3 replicates) or one half of detached bifoliate leaves (3 replicates). After 5 and 3

days incubation respectively detached upper leaves and opposite half leaves were challenged with B. fabae and B. cinerea inoculum (1 x 10^6 spores ml^{-1}). Lesions were assessed after 3 days but there was no significant difference between treated plants or leaves and controls (Table 73).

Seedlings (3 replicates) were also grown in culture filtrate (50 ml) plus Long Ashton nutrient solution (200 ml). Controls were grown in pure Long Ashton nutrient solution, all jars being supplemented with nutrient solution only as necessary. After 12 days incubation under the usual conditions plants possessed 2 bifoliate leaves which were detached and challenged with B. fabae and B. cinerea inoculum (1 x 10^6 spores ml^{-1}). Treatment with culture filtrates in this manner had no effect on the development of lesions on the leaves (Table 74).

C. Induction of systemic resistance by pre-treatment with abiotic inducers

(1) Growth regulating substances

a) B. fabae/B. cinerea: Vicia faba

Growth regulating substances tested were indole-3-acetic acid, maleic hydrazide, gibberellic acid, cycocel, ethephon and kinetin. All were made up as aqueous solutions at a concentration of 50 mgl^{-1} except for indole-3-acetic acid which was made up at 100 mgl^{-1}. The results obtained by Anderbrhan (1978) in similar experiments were used as a guide for the concentrations used.

The lower 2 bifoliate leaves of 3-4 week old broad bean plants (3 replicates) were injected with growth regulating substances or distilled water (controls). After 3 and 7 days incubation under the usual conditions upper leaves were detached and challenged with B. fabae and B. cinerea inoculum (1 x 10^6 spores ml^{-1}). Assessment of lesions after 3 days showed no evidence for any alteration of host response systemically

induced by growth regulating substances.

Halves of detached bifoliate leaves (3 replicates) were also injected with growth regulating substances and the opposite halves challenged after 2 and 4 days incubation. There was no significant difference between treated leaves injected with growth regulating substances and distilled water injected controls.

Detached leaves (3 replicates) were challenged with B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}) after 4 days incubation with their petioles dipping into solutions of growth regulating substances or distilled water (controls). Lesions were assessed after 3 days but none of the growth regulating substances induced any apparent effects on their development.

Broad bean plants (3 replicates) were grown for 3-4 weeks in vermiculite supplemented with Long Ashton nutrient solution. The roots were then washed free and the plants placed in jars containing solutions of growth regulating substances or distilled water (controls). After 3 and 7 days incubation leaves were detached and challenged with droplets of B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days incubation but growth regulating substances applied to plants in this manner apparently induced no effect on the response of leaves to disease (Tables 75-86).

(2) Polyacrylic acid; gentisic acid; aspirin

Aspirin and gentisic acid were dissolved in 0.1 M phosphate buffer pH 6.5-7 at a concentration of 0.02%. Polyacrylic acid was also dissolved in the phosphate buffer but at a concentration of 0.4%. Previous work by Mills and Wood (unpublished) was used as a guide for the concentrations used. Phosphate buffer was used as a control in the aspirin and gentisic acid treatments and buffer plus 0.05% Tween 80 in the polyacrylic acid treatments.

a) B. fabae/B. cinerea: Vicia faba

The two lower bifoliate leaves of 3-4 week old plants (3 replicates) and one half of detached bifoliate leaves (Materials and Methods) (3 replicates) were injected with aspirin, gentisic acid and polyacrylic acid. Detached upper leaves and opposite half leaves were challenged after 3 and 7 days with B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days incubation under the usual conditions. Plants treated by direct injection of the lower leaves showed no change in response to B. fabae or B. cinerea. However, where one side of a leaf was injected with polyacrylic acid, gentisic acid or aspirin the number of 100% lesions (i.e. where 100% of area beneath the inoculum droplet is necrotic) produced in response to B. fabae infection was reduced by 47%, 35% and 24% compared to the controls. The response to B. cinerea infection in treated leaves was not significantly different from the controls.

Plants were also treated with polyacrylic acid, gentisic acid and aspirin by applying solutions to the roots. Plants (3 replicates) were grown for 3-4 weeks in vermiculite supplemented with Long Ashton nutrient solution. The roots were then washed free and the plants placed in jars containing test solutions or distilled water (controls). After 3 and 7 days incubation leaves were detached and challenged with B. fabae and B. cinerea inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days but there was no significant difference between treated and control plants (Tables 87 and 88).

b) C. lindemuthianum: Phaseolus vulgaris

Primary leaves of 2-3 week old plants (6 replicates) were injected at the base of the petiole with each of the three solutions by water-soaking an area approximately 2.5 cm in diameter. After 3 and 7 days incubation under the usual conditions plants were challenged by spraying

with C. lindemuthianum inoculum (1 x 10^6 spores ml^{-1}). After 5 days incubation the degree of necrosis was assessed visually. Resistance was not induced by pre-treatment with any of the 3 substances.

Plants with fully expanded primary leaves were uprooted, washed and the roots steeped in solutions of aspirin, gentisic acid and polyacrylic acid for 3 and 7 days. All plants were then sprayed with C. lindemuthianum inoculum (1 x 10^6 spores ml^{-1}) and the extent of necrosis assessed after 5 days. There was no indication of any induced resistance.

(3) ICI test fungicide compounds

Aqueous solutions of 7 test fungicide compounds were prepared at concentrations of 50, 100, 500, 1000 and 5000 ppm. Those presented as salts were directly dissolved in distilled water. The others were initially dissolved in a small volume of ethanol which was then evaporated off by gentle heating. Solutions were freshly prepared for each experiment.

a) B. fabae: Vicia faba

Phytotoxicity tests

Whole plants - All 7 substances were each injected into leaves on whole plants at concentrations of 500 and 1000 ppm. After 2 days incubation under the usual conditions leaves were assessed for phytotoxicity. Necrosis was recorded on a scale from - through + and ++ to +++ which represented no necrosis, some necrosis, extensive necrosis and very extensive necrosis respectively. All substances except no. 3 caused necrosis at both concentrations (Table 89).

Detached leaves - Leaves were injected with all 7 compounds at concentrations of 100, 500, 1000 and 5000 ppm. After 2 days incubation phytotoxicity was assessed as for whole plants. All compounds except no. 3 caused necrosis at the two higher concentrations but only one had

this effect at the two lower concentrations (Table 89).

All 7 compounds were injected into the lower 2 bifoliate leaves of broad bean plants (3 replicates) at concentrations of 500 and 100 ppm. Controls were similarly injected with distilled water. Plants were incubated under the usual conditions for 3 days. All substances except no. 3 caused necrosis in injected leaves. Plants injected with compound no. 3 showed no adverse effects even after 1 week's incubation. Upper leaves were detached and challenged with droplets of B. fabae inoculum (1×10^6 spores ml^{-1}). Lesions were assessed after 3 days on the basis of whether necrosis had spread beyond the area of the inoculum droplet or not. Compounds 1, 2 and 4 apparently induced no changes in response to B. fabae infection. Compounds 3, 5, 6 and 7 induced some restriction of lesion spread particularly at the higher concentration. Results between replicates were not always consistent and a larger number would have been preferable. Compound no. 3 restricted lesion spread totally in the 1000 ppm treatment despite causing no phytotoxicity on the injected leaves or elsewhere (Table 90).

b) C. lindemuthianum: Phaseolus vulgaris

Compounds were directly injected into the primary leaves at concentrations of 50 and 100 ppm by watersoaking an area approximately 2.5 cm in diameter at the base of each petiole (6 replicates). Controls were similarly injected with distilled water. Plants were incubated for 3 days under the usual conditions; compounds 2, 5 and 7 caused severe chlorosis in injected areas after 2-3 days (compounds 1, 3, 4 and 6 had no apparent effects). Plants were challenged by spraying with C. lindemuthianum inoculum (1×10^6 spores ml^{-1}) and necrosis was assessed visually after 5 days incubation. There was no significant difference in the extent of necrosis on treated and control plants.

Compounds were also applied to plants (6 replicates) via the roots.

2-3 week old plants were uprooted, washed and steeped in test solutions or distilled water (controls). They were incubated under the usual conditions for 3 and 7 days. Severe chlorosis appeared in all plants treated with compounds no. 2, 5 and 7 after 3 days incubation; those treated with 1, 3, 4 and 6 had no apparent effects. Challenging with C. lindemuthianum inoculum (1 x 10^6 spores ml^{-1}) however showed no induction of resistance in any of the plants whether phytotoxicity had occurred or not.

c) TNV: Phaseolus vulgaris

Compounds were injected into both primary leaves of 2-3 week old plants (3 replicates) at concentrations of 500 and 1000 ppm water-soaking an area approximately 2.5 cm in diameter at the base of each petiole. Controls were injected with distilled water. All plants were incubated under the usual conditions for 4 days, all areas injected with test compounds becoming severely necrotic after 2-3 days. Trifoliate leaves were challenged with purified TNV and the lesions counted after 48 hours. Compounds 1, 3, 4 and 5 had no effect on the number of lesions produced on the upper leaves. Plants treated with 500 ppm of nos. 2, 6 and 7 and 1000 ppm of nos. 2 and 6 showed fewer lesions than controls (Table 91).

(4) Ultra-violet irradiation

a) P. viticola: Vitis vinifera

Leaf disks (15 mm diameter; 5 replicates) were cut from young Black Hamburg leaves and the undersurfaces exposed to UV light for 0, 1, 2, 3, 5 and 10 minutes. After 0, 24 and 72 hours incubation under the usual conditions disks were inoculated with droplets of P. viticola sporangial suspension (5 x 10^4 sporangia ml^{-1}). Sporulation was assessed after 6 days. Similar results were obtained whether leaf disks were kept for 72, 24 or 0 hours prior to inoculation. Sporulation was totally

inhibited by 5 and 10 minute exposures; UV damage was considerable after 10 minutes exposure. Sporulation occurred in all disks exposed for 3, 2, 1 and 0 minutes although the density of sporulation was decreased with increasing exposure (Table 92).

Leaf disks (15 mm diameter; 5 replicates) were cut from young Black Hamburg leaves and ½ of the undersurface covered by aluminium foil. Disks were then exposed to UV light for 0, 2 and 10 minutes 48 and 24 hours prior to inoculation. Immediately prior to inoculation the disks were bisected to separate the UV treated and non-UV treated halves. All ½-disks were inoculated with droplets of P. viticola sporangial suspension (5×10^4 sporangia ml^{-1}) and incubated for 6 days after which sporulation was assessed. There was no significant difference between disks inoculated at different times after exposure nor any reduction in sporulation on unexposed half-disks; exposed disks reacted as previously. There was thus no evidence for any systemic induced resistance (Table 93).

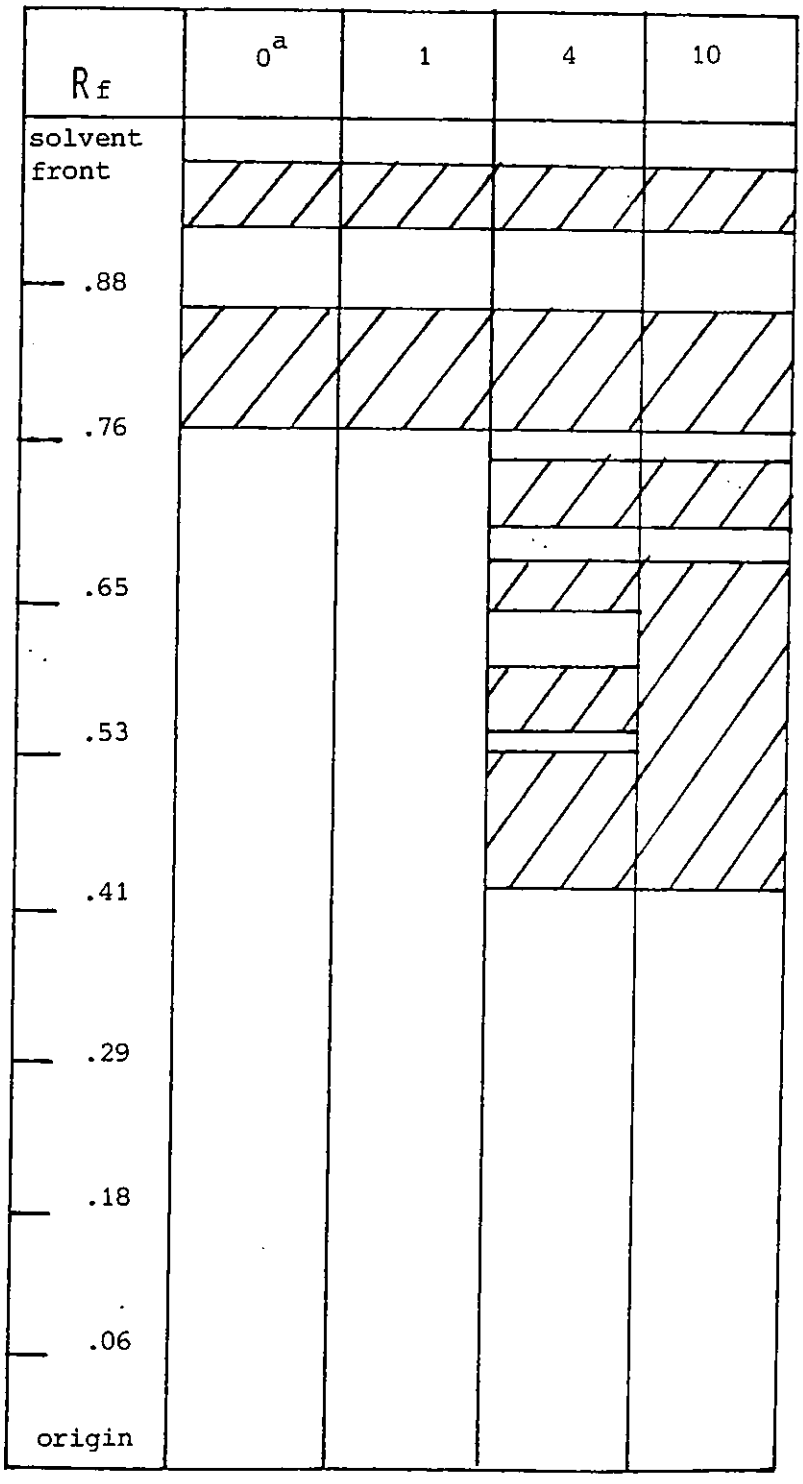
Uniform vine plants (cv. Black Hamburg) grown from hardwood cuttings with 4-5 fully expanded leaves were selected. The growing point and all the leaves except the two lowest were covered with aluminium foil and the plants then exposed to UV light for 0, 2 and 10 minutes (10 replicates per treatment). After 72 hours the whole of each plant was spray inoculated with P. viticola (5×10^4 sporangia ml^{-1}); sporulation was assessed after 6 days. Disease on the upper, non-exposed tissue in both control and treated plants was similar indicating that systemic resistance had not been induced by pre-treating the lower leaves with UV light. On exposed leaves disease developed in the 0 and 2 minute treatments but not in the 10 minute treatment where visible UV damage occurred (Table 94).

Leaf disks exposed to UV irradiation which subsequently developed

local resistance to P. viticola infection were tested for the presence of phytoalexins. Extracts from treated leaves were subjected to a Cladosporium bioassay, a reagent test for phenolics and spectrophotometric analysis. The Cladosporium bioassay revealed the presence of several antifungal substances which increased in number and quantity with increasing exposure time (Figure 1). Treatment with diazotised nitroaniline showed many of these substances to be phenolic in nature (Table 95). Spectrophotometric analysis confirmed that several of these substances were phytoalexins previously isolated from vine leaves as described by Langcake and Pryce (1977).

Figure 1

Cladosporium cucumerinum bioassay of extracts from UV irradiated vine leaves after tlc on silica gel.



a - UV exposure time (minutes).

V DATA SECTION.

Table 1

Pre-inoculation of 1 lower leaf of vine seedlings with P.viticola ($1x10^5$ sporangia per ml): disks from upper leaves challenged with P.viticola ($1x10^5$ sporangia per ml) after 3,7 and 10 days (sporulation not induced).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	2	4	4	3
2	0	4	4	4	4	4
3	2	4	4	4	2	4
4	4	3	4	0	2	3
5	4	1	0	3	0	3
6	4	4	4	3	4	0
7	4	4	4	4	4	4
8	4	4	1	4	4	4
9	4	4	4	4	4	4
10	3	4	4	4	4	4
Mean	2.9	3.6	3.1	3.4	3.2	3.3
S.E.	.53	.31	.48	.40	.44	.40

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 2

Pre-inoculation of 1 lower leaf of vine seedlings with P.viticola ($1x10^5$ sporangia per ml): disks from upper leaves challenged with P.viticola ($1x10^5$ sporangia per ml) after 3,7 and 10 days (sporulation induced in 7 and 10 day treatments).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	0	4	4	3	1
2	4	4	4	4	4	1
3	2	4	4	1	0	3
4	4	4	0	3	4	4
5	4	3	0	3	4	4
6	4	3	0	3	4	0
7	3	3	4	4	3	4
8	4	4	3	0	4	4
9	1	4	4	4	0	4
10	4	4	4	3	4	3
Mean	3.0	3.3	2.7	2.9	3.0	2.8
S.E.	.47	.40	.60	.43	.52	.49

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 3

Pre-inoculation of 1 lower leaf of vine seedlings with P.viticola ($1x10^5$ sporangia per ml): disks from upper leaves challenged wtih P.viticola ($1x10^5$ sporangia per ml) after 3,7 and 10 days (sporulation not induced).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	4	0	4	0
2	0	4	4	4	4	4
3	0	3	4	3	4	4
4	4	4	4	4	0	4
5	3	0	0	4	4	3
6	4	4	0	2	3	1
7	4	4	3	1	1	4
8	3	4	1	4	4	4
9	4	3	0	4	3	0
10	4	3	4	0	4	4
Mean	2.6	3.3	2.4	2.6	3.1	2.8
S.E.	.58	.40	.60	.54	.46	.55

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 4

Pre-inoculation of 1 lower leaf of vine seedlings with P.viticola ($1x10^5$ sporangia per ml):disks from upper leaves challenged with P.viticola($1x10^5$ sporangia per ml) after 3,7 and 10 days (sporulation induced in 7 and 10 day treatments).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	4[a]	0	4	0	1	0
2	4	0	4	0	3	0
3	0	4	4	4	3	2
4	3	4	4	4	3	3
5	4	1	3	4	4	4
6	4	4	4	4	4	4
7	1	4	2	4	4	4
8	4	3	0	4	4	4
9	4	4	4	4	4	4
10	4	4	4	4	4	4
Mean	3.2	2.8	3.3	3.2	3.4	2.9
S.E.	.47	.55	.42	.53	.31	.53

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 5

Pre-inoculation of 2 lower leaves of vine seedlings with *P.viticola* ($1x10^5$ sporangia per ml): disks from upper leaves challenged with *P.viticola* ($1x10^5$ sporangia per ml) after 3,7 and 10 days (sporulation not induced).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	2	4	3	2
2	4	0	2	4	4	1
3	4	0	4	4	4	4
4	4	0	4	4	4	4
5	1	4	4	4	4	4
6	2	4	4	4	3	4
7	4	4	4	3	0	0
8	4	4	0	3	4	4
9	4	3	0	1	2	4
10	4	4	0	0	4	3
Mean	3.1	2.7	2.4	3.1	3.2	3.0
S.E.	.48	.60	.58	.46	.42	.47

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 6

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola (1×10^5 sporangia per ml): disks from upper leaves challenged with P.viticola (1×10^5 sporangia per ml) after 3,7 and 10 days (sporulation induced in 7 and 10 day treatments).

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	4	4	0	4
2	3	4	4	4	1	4
3	4	3	4	4	4	4
4	4	4	0	0	3	4
5	4	2	1	4	1	4
6	1	4	4	4	4	4
7	0	4	3	0	4	4
8	4	0	4	3	4	1
9	4	0	4	4	4	0
10	4	4	4	4	4	2
Mean	2.8	2.9	3.2	3.1	2.9	3.1
S.E.	.55	.53	.47	.53	.50	.48

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 7

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola ($1x10^5$ sporangia per ml):disks from upper leaves challenged with P.viticola ($1x10^5$ sporangia per ml) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	4[a]	0	4	4	4	4
2	4	4	0	4	0	4
3	4	4	4	4	4	4
4	4	3	4	4	3	4
5	0	4	3	0	3	4
6	3	4	4	4	4	4
7	4	4	4	4	4	0
8	3	3	3	4	4	0
9	4	1	4	4	4	3
10	4	4	3	4	4	4
Mean	3.4	3.1	3.3	3.6	3.4	3.1
S.E.	.40	.46	.40	.40	.40	.53

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 8

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola ($1x10^5$ sporangia per ml): disks from upper leaves challenged with P.viticola ($1x10^5$ sporangia per ml) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	4[a]	4	4	4	4	1
2	4	4	4	4	4	4
3	4	4	4	4	4	4
4	2	4	4	4	4	4
5	3	0	4	4	4	4
6	4	4	4	4	4	4
7	4	3	3	0	0	3
8	4	3	3	4	3	4
9	0	4	3	4	3	4
10	4	4	4	4	1	4
Mean	3.3	3.4	3.7	3.5	3.1	3.6
S.E.	.42	.40	.15	.40	.46	.31

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 9

Pre-inoculation of 2 lower leaves of vine seedlings with *P.viticola* ($1x10^5$ sporangia per ml): disks from upper leaves challenged with *P.viticola* ($1x10^4$ sporangia per ml) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	4[a]	3	4	4	4	4
2	4	3	4	0	4	4
3	4	0	3	4	4	3
4	4	2	4	4	4	3
5	4	4	4	4	4	4
6	0	3	4	4	4	4
7	3	2	4	4	3	2
8	4	4	4	4	3	4
9	3	4	4	4	4	4
10	3	4	3	3	0	4
Mean	3.3	3.3	3.8	3.5	3.4	3.6
S.E.	.40	.40	.13	.40	.40	.22

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 10

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola ($1x10^5$ sporangia per ml): disks from upper leaves challenged with P.viticola ($5x10^4$ sporangia per ml) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	4	4	4	4
2	4	4	3	4	3	4
3	4	4	1	4	4	4
4	4	4	4	0	4	4
5	3	4	4	4	0	2
6	4	4	4	4	4	4
7	4	4	0	2	4	4
8	4	4	4	4	4	0
9	4	4	4	3	4	3
10	4	3	4	4	4	3
Mean	3.5	3.9	3.2	3.3	3.5	3.3
S.E.	.40	.10	.47	.42	.40	.42

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 11

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola ($5x10^5$ sporangia per ml) : disks from upper leaves challenged with P.viticola ($1x10^4$ sporangia per ml) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	3[a]	3	4	4	1	4
2	4	0	4	4	3	3
3	4	4	4	4	3	4
4	0	4	4	0	4	4
5	4	3	0	4	4	4
6	3	1	1	1	4	3
7	0	4	4	4	4	4
8	4	4	3	3	3	4
9	4	3	4	4	0	0
10	4	4	4	4	1	4
Mean	3.0	3.0	3.2	3.2	2.7	3.4
S.E.	.52	.45	.47	.47	.47	.40

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 12

Pre-inoculation of 2 lower leaves of vine seedlings with P.viticola ($5x10^5$ sporangia per ml^{-1}) ; disks from upper leaves challenged with P.viticola ($5x10^4$ sporangia ml^{-1}) after 3,7 and 10 days.

Incubation time	3 days		7 days		10 days	
Replicates	C	T	C	T	C	T
1	0[a]	4	4	4	4	4
2	4	1	3	4	4	3
3	3	4	4	4	3	4
4	4	4	4	0	4	4
5	4	3	4	3	3	0
6	4	4	4	3	0	4
7	3	4	3	2	2	4
8	4	3	3	4	3	3
9	3	4	4	3	4	3
10	4	4	3	3	4	4
Mean	3.3	3.5	3.6	3.0	3.1	3.3
S.E.	.40	.31	.16	.39	.41	.40

a - modal disease of 5 disks per plant

C - controls

T - treatments

Table 13

Pre-inoculation of one lower leaf of Black Hamburg vines with P.viticola ($5x10^4$ sporangia per ml) ; upper leaves challenged with P.viticola ($1x10^4$,$5x10^4$,$1x10^5$ sporangia per ml) after 7days.

challenge $conc^n$	Replicates	C	T
$1x10^4$	1	3[a]	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	3.8	4
	S.E.	.2	0
$5x10^4$	1	4	4
	2	4	4
	3	3	4
	4	4	4
	5	4	4
	Mean	3.8	4
$1x10^5$	1	0	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	3.2	4
	S.E.	.8	0

a - modal disease grade of 5 disks per plant

Table 14

Pre-inoculation of 1 lower leaf of Black Hamburg vines with P.viticola ($1x10^5$ sporangia per ml); upper leaves challenged with P.viticola ($5x10^4$, 1x104, $1x10^5$ sporangia per ml) after 7 days.

challenge conc^n	Replicates	C	T
$1x10^4$	1	4[a]	3
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	4	3.8
	S.E.	0	.2
$5x10^4$	1	4	4
	2	3	4
	3	4	4
	4	4	4
	5	4	4
	Mean	3.8	4
	S.E.	.2	0
$1x10^5$	1	4	4
	2	4	4
	3	3	4
	4	4	4
	5	3	4
	Mean	3.6	4
	S.E.	.2	0

a- modal disease grade of 5 disks per plant

Table 15

Pre-inoculation of 1 lower leaf of Black Hamburg vines with P.viticola ($5x10^5$ sporangia per ml);upper leaves challenged with P.viticola ($1x10^4$,$5x10^4$,$1x10^5$ sporangia per ml) after 7 days.

challenge conc^n	Replicates	C	T
$1x10^4$	1	4[a]	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	4	4
	S.E.	0	0
$5x10^4$	1	4	3
	2	3	4
	3	4	4
	4	4	4
	5	4	4
	Mean	3.8	3.8
	S.E.	.2	.2
$1x10^5$	1	4	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	4	4
	S.E.	0	0

a - modal disease grade of 5 disks per plant

Table 16

Pre-inoculation of 2 lower leaves of Black Hamburg vines with P.viticola ($5x10^4$ sporangia ml^{-1}) ; upper leaves challenged with P.viticola ($1x10^4$, $5x10^4$ and $1x10^5$ sporangia ml^{-1}) after 7 days.

challenge conc^n	Replicates	C	T
$1x10^4$	1	3[a]	4
	2	4	4
	3	4	3
	4	4	4
	5	3	4
	Mean	3.6	3.8
	S.E.	.2	.2
$5x10^4$	1	4	3
	2	4	3
	3	3	3
	4	4	4
	5	3	4
	Mean	3.6	3.4
	S.E.	.2	.2
$1x10^5$	1	4	4
	2	4	4
	3	4	4
	4	3	4
	5	4	4
	Mean	3.8	4
	S.E.	.2	0

a - modal disease grade of 5 disks per plant

C - controls

T - treatments

Table 17

Pre-inoculation of 2 lower leaves of Black Hamburg vines with P.viticola ($1x10^5$ sporangia ml^{-1}) ; upper leaves challenged with P.viticola ($1x10^4$, $5x10^4$ and $1x10^5$ sporangia ml^{-1}) after 7 days.

challenge conc^n	Replicates	C	T
$1x10^4$	1	4[a]	4
	2	3	4
	3	4	4
	4	4	4
	5	4	4
	Mean	3.8	4
	S.E.	.2	0
$5x10^4$	1	4	4
	2	4	4
	3	3	3
	4	4	3
	5	4	4
	Mean	3.8	3.6
	S.E.	.2	.2
$1x10^5$	1	4	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	4	4
	S.E.	0	0

a - modal disease grade of 5 disks per plant

C - controls

T - treatments

Table 18

Pre-inoculation of 2 leaves of Black Hamburg vines with *P.viticola* ($5x10^5$ sporangia ml^{-1}) ; upper leaves challenged with *P.viticola* ($1x10^4$, $5x10^4$ and $1x10^5$ sporangia ml^{-1}) after 7 days.

challenge conc^n	Replicates	C	T
$1x10^4$	1	4[a]	4
	2	4	4
	3	4	4
	4	3	4
	5	4	4
	Mean	3.8	4
	S.E.	.2	0
$5x10^4$	1	4	4
	2	0	4
	3	4	4
	4	4	3
	5	4	4
	Mean	3.2	3.8
	S.E.	.8	.2
$1x10^5$	1	4	4
	2	4	4
	3	4	4
	4	4	4
	5	4	4
	Mean	4	4
	S.E.	0	0

a - modal disease grade of 5 disks per plant

C - controls

T - treatments

Table 19

Pre-inoculation of lower leaves of Pinot Noir vines with P.viticola ($1x10^5$ sporangia per ml^{-1}); upper leaves challenged with P.viticola ($1x10^5$ and $5x10^4$ sporangia per ml^{-1}) after 7 days.

	$1x10^5$		$5x10^4$	
Replicates	C	T	C	T
1	[a] 3	0	1	4
2	4	3	3	4
3	4	4	4	4
4	3	4	4	3
5	2	2	4	4
6	4	4	3	4
7	3	3	3	4
8	4	4	4	3
9	4	4	0	4
10	3	4	4	4
Mean	3.4	3.2	3.0	3.8
S.E.	.70	1.32	1.41	.42

a - modal disease grade of 5 leaf disks per plant

C - controls

T - treatments

Table 20

Pre-inoculation of lower leaves of Semillon vines with P.viticola ($1x10^5$ sporangia ml^{-1}); upper leaves challenged with P.viticola ($1x10^5$ and $5x10^4$ sporangia ml^{-1}) after 7 days.

	$1x10^5$		$5x10^4$	
Replicates	C	T	C	T
1	0[a]	4	4	3
2	2	4	4	4
3	4	3	2	4
4	4	0	4	4
5	3	3	3	3
6	4	3	4	3
7	4	4	4	4
8	3	2	0	4
9	4	4	0	4
10	4	4	4	0
Mean	3.2	3.1	2.9	3.3
S.E.	1.32	1.29	1.66	1.25

a - modal disease grade of 5 leaf disks per plant

C - controls

T - treatments

Table 21

Pre-inoculation of lower leaves of Rubired vines with P.viticola ($1x10^5$ sporangia ml^{-1}); upper leaves challenged with P.viticola ($1x10^5$ and $5x10^4$ sporangia ml^{-1}) after 7 days.

	$1x10^5$		$5x10^4$	
Replicates	C	T	C	T
1	3[a]	4	3	4
2	4	0	3	3
3	3	2	4	3
4	4	4	4	4
5	2	3	3	3
6	4	3	3	3
7	0	4	4	4
8	4	3	4	0
9	4	4	3	4
10	0	4	0	4
Mean	2.8	3.1	3.1	3.2
S.E.	1.62	1.29	1.29	1.23

a - modal disease grade of 5 leaf disks per plant

C - controls

T - treatments

Table 22

Pre-inoculation of lower leaves of Alicante Bouschet vines with P.viticola ($1x10^5$ sporangia ml^{-1}) ; upper leaves challenged with P.viticola ($1x10^5$ and $5x10^4$ sporangia ml^{-1}) after 7 days.

challenge conc^n	$1x10^5$		$5x10^4$	
Replicates	C	T	C	T
1	2 [a]	0	1	3
2	4	2	3	3
3	0	1	4	2
4	4	3	4	4
5	2	3	3	2
6	4	2	3	4
7	3	4	3	3
8	2	2	4	3
9	1	4	3	2
10	4	4	3	0
Mean	2.7	2.5	3.1	2.6
S.E.	1.49	1.43	.88	1.17

a - modal disease grade of 5 leaf disks per plant

C - controls

T - treatments

Table 23

Pre-inoculation of lower leaves of Sauvignon Blanc vines with P.viticola ($1x10^5$ sporangia ml^{-1}) ; upper leaves challenged with P.viticola ($1x10^5$ and $5x10^4$ sporangia ml^{-1}) after 7 days.

challenge conc^n	$1x10^5$		$5x10^4$	
Replicates	C	T	C	T
1	3 [a]	4	4	4
2	4	0	4	4
3	4	4	4	4
4	4	4	0	4
5	3	4	4	4
6	4	4	4	4
7	3	3	4	4
8	4	3	4	4
9	4	2	4	4
10	4	1	4	4
Mean	3.7	2.9	3.6	4
S.E.	.48	1.45	1.26	0

a - modal disease grade of 5 leaf disks per plant

C - controls

T - treatments

Table 24

Pre-inoculation of cotyledons of seedling vines with P.viticola ($1x10^5$ sporangia per ml) by injection and droplets; leaves challenged later with P.viticola ($1x10^5$ sporangia per ml).

	Injection		Droplets	
Replicates	C	T	C	T
1	4[a]	3	4	0
2	0	3	3	3
3	3	4	4	4
4	2	4	4	1
5	4	4	3	4
6	4	0	0	3
7	3	1	2	4
8	4	1	4	0
9	4	4	1	4
10	4	3	3	3
11	0	4	4	4
12	4	4	3	4
Mean	3.0	2.9	2.9	2.8
S.E.	.44	.42	.38	.46

a - modal sporulation grade of 5 leaf disks per plant

C - controls

T - treatments

Table 25

Pre-inoculation of one side of leaf disks from Black Hamburg vines with P.viticola ($1x10^5$ sporangia ml^{-1}) ; opposite sides of disks challenged with P.viticola ($1x10^5$ sporangia ml^{-1}) after 3 days.

Replicates		C	T
Leaf 1	1	0[a]	4
	2	3	4
	3	3	4
	4	4	4
	5	4	1
Leaf 2	6	4	4
	7	4	3
	8	3	3
	9	3	3
	10	4	4
Leaf 3	11	4	4
	12	4	4
4	13	4	
	14	4	4
	15	3	0
Leaf 4	16	4	3
	17	3	4
	18	0	0
	19	4	4
	20	4	4
Mean		3.30	3.25
S.E.		.27	.30

a - disease grade

C - controls

T - treatments

Table 26

Pre-inoculation of one side of leaf disks from Black Hamburg vines with P.viticola ($1x10^5$ sporangia ml^{-1}) ; opposite sides of disks challenged with P.viticola ($1x10^5$ sporangia ml^{-1}) after 5 days.

Replicates		C	T
Leaf 1	1	4[a]	0
	2	0	4
	3	3	4
	4	3	3
	5	4	4
Leaf 2	6	4	3
	7	4	4
	8	2	4
	9	4	3
	10	4	3
Leaf 3	11	4	4
	12	3	4
	13	4	3
	14	4	4
	15	4	4
Leaf 4	16	3	4
	17	4	4
	18	3	2
	19	4	4
	20	4	4
Mean		3.45	3.50
S.E.		.22	.22

a - disease grade

C - controls

T - treatments

Table 27

Broad beans pre-inoculated on the lower leaves with B.fabae by direct injection and surface droplets; upper leaves challenged with B.fabae after 3 days incubation.

	Replicates	C	T
Direct injection	1	95[a]	0
	2	90	0
	3	100	0
	Mean	95	0
	S.E.	2.9	0
Surface droplets	1	90	0
	2	90	0
	3	95	0
	Mean	91.7	0
	S.E.	1.7	0

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 28

Broad beans pre-inoculated on the lower leaves with B.fabae by direct injection and surface droplets ; upper leaves challenged with B.fabae after 5 days incubation.

	Replicates	C	T
Direct injection	1	85[a]	5
	2	95	5
	3	95	0
	Mean	91.7	3.3
	S.E.	3.4	1.7
Surface droplets	1	95	0
	2	95	0
	3	90	5
	Mean	93.3	1.7
	S.E.	1.7	1.7

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 29

Broad beans pre-inoculated on the lower leaves with B.cinerea by direct injection; upper leaves challenged with B.fabae after 3 days incubation.

Replicates	C	T
1	90[a]	20
2	100	15
3	95	15
Mean	95	16.7
S.E.	2.9	1.7

a - %of lesions of greater than 100%

C - controls

T - treatments

Table 30

Broad beans pre-inoculated on the lower leaves with B.cinerea (direct injection) ; upper leaves challenged with B.fabae after 5 days incubation.

Replicates	C	T
1	85[a]	10
2	90	25
3	95	20
Mean	90	18.3
S.E.	2.9	4.5

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 31

Broad beans pre-inoculated on the lower leaves with B.fabae by direct injection and surface droplets ; upper leaves challenged with B.cinerea after 3 days incubation.

	Replicates	C	T
Direct injection	1	80[a]	70
	2	80	75
	3	80	85
	Mean	80	77
	S.E.	0	7.6
Surface droplets	1	75	75
	2	75	75
	3	75	80
	Mean	75	77
	S.E.	0	2.9

a - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 32

Broad beans pre-inoculated on the lower leaves with B.fabae by direct injection and surface droplets ; upper leaves challenged with B.cinerea after 5 days incubation.

	Replicates	C	T
Direct injection	1	75[a]	80
	2	75	70
	3	75	70
	Mean	75	73
	S.E.	0	5.7
Surface droplets	1	70	75
	2	70	70
	3	75	75
	Mean	72	73
	S.E.	2.9	2.9

a - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 33

Broad beans pre-inoculated on the lower leaves with B.cinerea by direct injection ; upper leaves challenged with B.cinerea after 3 days incubation.

Replicates	C	T
1	75[a]	80
2	75	75
3	80	80
Mean	77	78
S.E.	2.9	2.9

a - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 34

Broad beans pre-inoculated on the lower leaves with B.cinerea by direct injection; upper leaves challengedwith B.cinerea after 5 days incubation.

Replicates	C	T
1	80[a]	90
2	80	80
3	80	80
Mean	80	83
S.E.	0	3.4

a - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 35

Detached bifoliate leaves inoculated on one side with B.fabae (droplets) or B.cinerea (injection); challenged on the opposite sides with B.fabae and B.cinerea droplets after 3 days.

	Pre-treatment fungus	B.fabae		B.cinerea	
Challenge fungus	Replicates	C	T	C	T
B.fabae	1	90[a]	100	100	90
	2	100	100	100	100
	3	100	100	100	100
	Mean	97	100	100	97
	S.E.	3.4	0	0	3.4
B.cinerea	1	80[b]	80	90	80
	2	80	90	80	80
	3	80	80	80	80
	Mean	80	83	83	80
	S.E.	0	3.4	3.4	0

a - %of lesions of greater than 100% necrosis

b - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 36

Lower leaves of broad bean plants inoculated with U.fabae ; upper leaves challenged with U.fabae after 6 days.

Replicates	C	T
1	55[a]	33
2	49	48
3	38	25
4	44	45
5	47	46
6	40	54
7	28	51
8	35	29
9	51	32
10	48	32
Mean	43.5	39.5
S.E.	2.6	3.3

a - no. of pustules per leaf

C - controls

T - treatments

Table 37

Lower leaves of broad bean plants inoculated with U.fabae; Upper leaves challenged after 6 days with B.fabae and B.cinerea.

	B.fabae		B.cinerea	
Replicates	C	T	C	T
1	80[a]	95	90	80[b]
2	95	100	90	80
3	100	100	80	80
4	85	80	75	75
5	85	80	80	90
6	90	85	80	80
Mean	89	90	83	81
S.E.	3.0	3.9	2.5	2.0

a - % of lesions of greater than 100% necrosis

b - % of lesions of 1-50% necrosis

C - controls

T - treatments

Table 38

Lower leaves of broad bean plants inoculated with B.fabae or B.cinerea; upper leaves challenged after 3 days with U.fabae.

Pre-treatment fungus		C	T
B.fabae	1	53[a]	48
	2	28	54
	3	41	44
	4	46	33
	5	39	39
	6	45	40
	Mean	42	43
	S.E.	3.4	3.0
B.cinerea	1	40	50
	2	52	47
	3	38	40
	4	45	50
	5	46	30
	6	28	36
	Mean	42	42
	S.E.	3.7	3.4

a - no. of pustules per leaf

C - controls

T - treatments

Table 39

Pre-treatment of vine leaf disks with cell-free germination fluid of P.viticola; disks challenged with P.viticola after 24 and 72 hours.

	24 hours		72 hours	
Replicates	C	T	C	T
1	[a] 4	4	4	4
2	4	3	4	4
3	4	4	3	4
4	4	4	3	4
5	4	4	3	4
6	4	2	4	4
7	4	4	4	4
8	4	4	4	4
9	4	4	4	4
10	4	4	4	4
Mean	4	3.7	3.7	4
S.E.	0	.21	.21	0

a - disease grade (sporulation) on inoculated disks

C - controls

T - treatments

Table 40

Pre-treatment of one side of vine leaf disks with cell-free germination fluid of P.viticola; opposite sides of disks challenged with P.viticola after 24 and 72 hours.

	24 hours		72 hours	
Replicates	C	T	C	T
1	[a] 3	4	3	4
2	4	4	3	4
3	4	2	3	4
4	3	2	4	4
5	2	4	4	4
6	4	4	3	4
7	4	4	4	2
8	4	3	4	3
9	4	4	4	3
10	3	4	4	3
Mean	3.5	3.5	3.6	3.5
S.E.	.22	.27	.16	.22

a - disease grade (sporulation) on inoculated half of leaf disk

C - controls

T - treatments

Table 41

Pre-treatment of lower leaves of broad beans with germination fluids of B.fabae, B.cinerea and U.fabae; challenged after 3 and 5 days with inoculum of the same fungi.

	Replicates	C[3]	T	C[5]	T
U.fabae	1	35[a]	42	29	42
	2	44	41	41	39
	3	51	39	36	38
	Mean	43	40	35	40
	S.E.	4.7	0.9	3.6	1.2
B.fabae	1	90[b]	85	75	80
	2	80	75	80	80
	3	80	75	80	80
	Mean	83	78	78	80
	S.E.	3.4	3.4	1.7	0
B.cinerea	1	85[c]	80	90	80
	2	85	80	80	80
	3	75	75	80	85
	Mean	82	78	83	82
	S.E.	3.4	1.7	3.4	1.7

a - no. of pustules per leaf

b - % no. of lesions of greater than 100% necrosis

c - % no. of lesions of 1-50 % necrosis

C - controls

T - treatments

Table 42

Pre-treatment of lower leaves of broad bean with germination fluids of B.fabae and B.cinerea; challenged after 3 and 5 days with B.cinerea and B.fabae respectively.

		3 days		5 days	
	Replicates	C	T	C	T
B.fabae germination fluid challenged with B.cinerea	1	75[a]	80	80	80
	2	80	85	75	85
	3	80	75	70	75
	Mean	78	80	75	80
	S.E.	1.7	2.9	2.9	2.9
B.cinerea germination fluid challenged with B.fabae	1	85[b]	85	100	100
	2	80	80	85	80
	3	90	85	90	85
	Mean	85	83	92	88
	S.E.	2.9	1.7	4.5	6.2

a - % of lesions of 1-50% necrosis

b - % of lesions of greater than 100% necrosis

C - controls

T - treatments

Table 43

One half of detached bifoliate leaves of broad bean injected with germination fluids of U.fabae, B.fabae and B.cinerea; opposite half leaves challenged with inoculum of the same fungi after 3&5 days.

	Replicates	3 C	3 T	5 C	5 T
U.fabae	1	21[a]	23	19	20
	2	18	17	23	17
	3	15	16	20	21
	Mean	18	18	20	19
	S.E.	3.0	3.2	1.5	2.1
B.fabae	1	80[b]	80	90	80
	2	80	80	80	80
	3	70	90	70	80
	Mean	77	83	80	80
	S.E.	3.4	3.4	5.9	0
B.cinerea	1	90[c]	100	90	80
	2	100	100	90	100
	3	100	80	90	90
	Mean	97	93	90	90
	S.E.	3.4	6.8	0	5.9

a - no. of pustules per leaf

b - % no. of lesions of greater than 100% necrosis

c - % no. of lesions of 1-50 % necrosis

C - controls

T - treatments

Table 44

One half of detached bifoliate leaves of broad bean injected with germination fluids of B.cinerea and B.fabae ; opposite half leaves challenged with B.fabae and B.cinerea respectively after 3 and 5 days.

		3 days		5 days	
	Replicates	C	T	C	T
B.cinerea germination fluids challenged with B.fabae	1	100[a]	80	90	100
	2	90	90	70	90
	3	80	100	80	80
	4	100	100	80	80
	5	90	90	90	80
	6	100	80	90	80
	Mean	93	90	83	85
	S.E.	3.3	3.7	3.3	3.4
B.fabae germination fluid challenged with B.cinerea	1	70[b]	80	80	90
	2	80	90	70	80
	3	60	70	90	70
	4	90	70	80	60
	5	80	70	90	70
	6	80	70	80	80
	Mean	77	75	82	75
	S.E.	4.2	3.4	3.1	4.3

a - % of lesions of greater than 100% necrosis

b - % of lesios of 1-50% necrosis

C - controls

T - tretments

Table 45

One side of one half of a bifoliate leaf (broad bean) injected with germination fluids of U.fabae, B.fabae or B.cinerea; opposite sides challenged with the same fungi after 2 and 4 days.

	Replicates	2 C	2 T	4 C	4 T
U.fabae	1	10[a]	8	11	13
	2	9	4	6	7
	3	5	12	9	8
	Mean	8.0	8.0	8.7	9.3
	S.E.	1.6	2.4	1.5	1.9
B.fabae	1	80[b]	90	100	70
	2	70	80	80	70
	3	90	80	80	100
	Mean	80	83	87	80
	S.E.	5.9	3.4	6.8	10.2
B.cinerea	1	60[c]	80	80	80
	2	70	70	90	80
	3	80	70	80	80
	Mean	70	73	83	80
	S.E.	5.9	3.4	3.4	0

a - no. of pustules per leaf

b - % no. of lesions of greater than 100% necrosis

c - % no. of lesions of 1-50 % necrosis

C - controls

T - treatments

Table 46

Cotyledons of seedling vines injected with heat-killed sporangial suspensions (P.viticola); leaves challenged after 4 and 7 days with P.viticola.

	4 days		7 days	
Replicates	C	T	C	T
1	3[a]	3	3	4
2	4	3	4	3
3	4	4	4	3
4	3	4	4	4
5	2	4	4	4
6	4	4	3	2
7	4	3	2	4
8	3	3	3	4
9	4	4	3	3
10	4	4	4	4
Mean	3.5	3.6	3.4	3.5
S.E.	.22	.16	.22	.22

a - modal disease grade of 5 disks from each plant replicate

C - controls

T - treatments

Table 47

Droplets of heat-killed sporangial suspension (P.viticola) placed on leaf disks for 24 and 72 hours; disks challenged after 24 and 72 hours with P.viticola.

	24 hours		72 hours	
Replicates	C	T	C	T
1	4[a]	4	4	4
2	3	4	4	4
3	4	4	4	4
4	4	4	4	3
5	4	4	3	4
6	3	4	4	4
7	4	4	4	4
8	4	4	4	4
9	4	4	4	4
10	4	4	3	4
Mean	3.8	4	3.8	3.9
S.E.	.13	0	.13	.10

a - disease grade (sporulation)

C - controls

T - treatments

Table 48

Droplets of heat-killed sporangial suspension placed to one side of leaf disks; opposite sides challenged with P.viticola inoculum after 24 and 72 hours.

	24 hours		72 hours	
Replicates	C	T	C	T
1	a 4	3	4	4
2	4	3	4	4
3	4	4	4	4
4	4	4	3	3
5	4	4	4	3
6	3	4	4	4
7	4	4	3	4
8	4	4	4	4
9	4	4	3	4
10	4	4	4	4
Mean	3.9	3.8	3.7	3.8
S.E.	.10	.13	.15	.13

a - disease grade (sporulation) on inoculated half of leaf disk

C - controls

T - treatments

Table 49

Heat-killed sporangial suspensions of P.viticola applied to vine roots ; leaves challenged after 7 and 9 days.

	7 days		9 days	
Replicates	C	T	C	T
1	4[a]	3	4	4
2	4	3	4	4
3	3	4	4	4
4	4	4	3	4
5	4	4	4	4
6	3	4	3	4
Mean	3.7	3.7	3.7	4.0
S.E.	.2	.2	.2	0

a - modal disease grade of 5 disks from each plant replicate

C - controls

T - treatments

Table 50

Heat-killed preparations of U.fabae spores injected into lower leaves ofbroad beans; upper leaves challenged with U.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	40[a]	30	22	42
	2	42	44	50	45
	3	38	22	38	29
	4	34	51	44	34
	5	44	45	48	54
	6	41	44	41	42
	Mean	39.8	39.3	40.5	41.0
	S.E.	1.4	4.5	4.1	3.6
Preparation (2)	1	30	35	40	42
	2	44	40	45	42
	3	41	48	32	36
	4	48	58	51	48
	5	39	50	49	50
	6	43	41	43	41
	Mean	40.8	45.3	43.3	43.2
	S.E.	2.5	3.4	2.8	2.1

a - no. of pustules per leaf

C - controls

T - treatments

Table 51

Heat-killed preparations of U.fabae spores injected into one half of detached bifoliate leaves ; opposite halves challenged with U.fabae after 2 and 4 days.

		2 days		4 days	
	Replicates	C	T	C	T
Preparation (1)	1	21[a]	22	22	21
	2	19	22	20	25
	3	18	25	25	22
	4	24	15	19	18
	5	15	13	21	23
	6	18	19	26	24
	Mean	19.2	19.3	22.2	22.2
	S.E.	1.3	1.9	1.1	1.0
Preparation (2)	1	20	21	21	22
	2	20	23	22	20
	3	21	26	25	19
	4	17	18	19	25
	5	24	24	21	20
	6	25	24	24	22
	Mean	21.2	22.7	22.0	21.3
	S.E.	1.2	1.1	0.9	0.9

a - no. of pustules per leaf

C - controls

T - treatments

Table 52

Heat-killed preparations of U.fabae spores applied to the roots of broad bean plants; leaves challenged after 7 and 9 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	50 [a]	51	40	34
	2	48	42	43	38
	3	35	39	45	42
	4	42	50	41	40
	5	40	41	38	50
	6	40	44	49	50
	Mean	42.5	44.5	42.7	42.3
	S.E.	2.3	2.0	1.6	2.7
Preparation (2)	1	42	40	43	39
	2	50	45	46	47
	3	44	40	52	49
	4	37	41	37	38
	5	45	51	48	47
	6	48	40	48	48
	Mean	44.3	42.8	45.7	44.7
	S.E.	1.9	1.8	2.1	2.0

a - no. of pustules per leaf

C - controls

T - treatments

Table 53

Heat-killed preparations of U.fabae spores applied to the surfaces of the lower leaves of broad bean plants; upper leaves challenged with U.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	38[a]	50	48	39
	2	40	50	38	40
	3	50	42	40	51
	4	44	44	43	42
	5	44	41	43	42
	6	41	40	45	40
	Mean	42.8	44.5	42.8	42.3
	S.E.	1.7	1.8	1.5	1.8
Preparation (2)	1	39	49	48	44
	2	34	38	42	40
	3	48	42	38	38
	4	50	43	49	50
	5	46	45	43	40
	6	48	45	43	44
	Mean	44.2	43.7	43.8	42.7
	S.E.	2.6	1.5	1.7	1.8

a - no. of pustules per leaf

C - controls

T - treatments

Table 54

Heat-killed preparations of U.fabae spores painted onto lower surfaces of detached leaves; upper surfaces challenged after 24 and 72 hours with U.fabae.

		24 hours		72 hours	
	Replicates	C	T	C	T
Preparation (1)	1	42[a]	39	38	27
	2	50	48	42	45
	3	38	49	47	39
	4	40	44	42	50
	5	46	44	50	51
	6	45	45	48	47
	Mean	43.5	44.8	44.5	39.8
	S.E.	1.8	1.4	1.9	6.8
Preparation (2)	1	39	41	28	38
	2	42	45	50	44
	3	44	38	44	41
	4	44	50	42	43
	5	46	39	48	48
	6	40	46	41	50
	Mean	42.5	43.2	42.2	44.0
	S.E.	1.6	1.9	3.2	1.8

a - no. of pustules per leaf

C - controls

T - treatments

Table 55

Heat-killed preparations (2) of B.fabae spores injected into lower leaves of broad bean plants; upper leaves challenged with B.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	85[a]	95	95	90
	2	90	90	85	85
	3	90	90	85	95
	Mean	88	92	88	90
	S.E.	1.7	1.7	3.4	2.9
Preparation (2)	1	80	100	95	95
	2	95	90	90	90
	3	95	85	90	100
	Mean	90	92	92	95
	S.E.	5.1	4.5	1.7	2.9

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 56

Heat-killed preparations (2) of B.fabae spores injected into lower leaves of broad bean plants; upper leaves challenged with B.cinerea after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	85[a]	80	85	75
	2	75	80	80	75
	3	80	80	75	80
	Mean	80	80	80	77
	S.E.	2.9	0	2.9	1.7
Preparation (2)	1	80	75	75	85
	2	75	75	85	80
	3	70	85	75	80
	Mean	75	78	78	82
	S.E.	2.9	3.4	3.4	1.7

a - % of lesions of 1-50%

C - controls

T - treatments

Table 57

Heat-killed preparations (2) of B.cinerea spores injected into lower leaves of broad bean plants; upper leaves challenged with B.cinerea after 3 adn 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	75[a]	86	85	70
	2	80	80	75	70
	3	80	75	80	90
	Mean	78	80	80	77
	S.E.	1.7	2.9	2.9	6.8
Preparation (2)	1	85	85	80	75
	2	75	80	85	85
	3	75	80	80	75
	Mean	78	82	82	78
	S.E.	3.4	1.7	1.7	3.4

a - % of lesions of 1-50%

C - controls

T - treatments

Table 58

Heat-killed preparations (2) of B.cinerea spores injected into lower leaves of broad bean plants; upper leaves challenged with B.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	100[a]	90	95	95
	2	90	95	90	90
	3	85	100	85	85
	Mean	92	95	90	90
	S.E.	4.5	2.9	2.9	2.9
Preparation (2)	1	95	85	90	90
	2	90	90	95	90
	3	90	95	80	85
	Mean	92	90	88	88
	S.E.	1.7	2.9	4.5	1.7

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 59

Heat-killed preparations (2) of B.fabae spores injected into one half of detached bifoliate leaves; opposite half leaves challenged with B.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	80[a]	80	90	80
	2	80	90	90	90
	3	85	95	90	85
	Mean	82	88	90	85
	S.E.	1.7	4.5	0	2.9
Preparation (2)	1	95	85	80	95
	2	85	80	85	90
	3	85	95	85	95
	Mean	88	87	83	93
	S.E.	3.4	4.5	1.7	1.7

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 60

Heat-killed preparations (2) of B.fabae spores injected into one half of detached bifoliate leaves; opposite half leaves challenged with B.cinerea after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	80[a]	85	85	75
	2	85	75	80	75
	3	85	80	75	75
	Mean	83	80	80	75
	S.E.	1.7	2.9	2.9	0
Preparation (2)	1	70	70	75	75
	2	70	75	75	85
	3	90	85	80	75
	Mean	77	77	77	78
	S.E.	6.8	4.5	1.7	3.4

a - % of lesions of 1-50%

C - controls

T - treatments

Table 61

Heat-killed preparations (2) of B.cinerea spores injected into one half of detached bifoliate leaves; opposite half leaves challenged with B.cinerea after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	80[a]	85	90	80
	2	80	85	80	75
	3	75	80	80	70
	Mean	78	83	83	75
	S.E.	1.7	1.7	3.4	2.9
Preparation (2)	1	80	75	85	85
	2	80	85	75	80
	3	80	75	75	80
	Mean	80	78	78	82
	S.E.	0	3.4	3.4	1.7

a - % of lesions of 1-50%

C - controls

T - treatments

Table 62

Heat-killed preparations (2) of B.cinerea spores injected into one half of detached bifoliate leaves; opposite half leaves challenged with B.fabae after 3 and 7 days.

		3 days		7 days	
	Replicates	C	T	C	T
Preparation (1)	1	80[a]	100	95	85
	2	95	95	80	85
	3	90	95	80	90
	Mean	88	97	85	88
	S.E.	4.5	1.7	5.1	3.4
Preparation (2)	1	85	95	90	90
	2	90	85	85	80
	3	80	85	90	80
	Mean	85	88	88	83
	S.E.	2.9	3.4	1.7	3.4

a - % of lesions of greater than100%

C - controls

T - treatments

Table 63

Heat-killed preparations (2) of B.cinerea applied to the roots of broed bean plants; leaves challenged with B.fabae after 7 and 9 days.

		7 days		9 days	
	Replicates	C	T	C	T
Preparation (1)	1	85[a]	85	95	90
	2	90	80	80	90
	3	90	80	80	75
	Mean	88	82	85	85
	S.E.	1.7	1.7	5.1	5.1
Preparation (2)	1	95	85	85	85
	2	85	90	80	90
	3	85	80	85	80
	Mean	88	85	83	85
	S.E.	3.4	2.9	1.7	2.9

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 64

Heat-killed preparations (2) of B.cinerea applied to the roots of broad bean plants; leaves challenged with B.cinerea after 7 and 9 days.

		7 days		9 days	
	Replicates	C	T	C	T
Preparation (1)	1	75[a]	90	85	80
	2	80	85	80	75
	3	75	90	75	85
	Mean	77	88	80	80
	S.E.	1.7	1.7	2.9	2.9
Preparation (2)	1	75	80	85	75
	2	85	80	75	80
	3	75	90	80	80
	Mean	78	83	80	78
	S.E.	3.4	3.4	2.9	1.7

a - % of lesions of 1-50%

C - controls

T - treatments

Table 65

Heat-killed preparations (2) of B.fabae applied to the roots of broad bean plants; leaves challenged with B.cinerea after 7 and 9 days.

		7 days		9 days	
	Replicates	C	T	C	T
Preparation (1)	1	90[a]	90	85	85
	2	80	85	85	80
	3	80	90	80	75
	Mean	83	88	83	80
	S.E.	3.4	1.7	1.7	2.9
Preparation (2)	1	75	90	90	80
	2	80	80	80	75
	3	75	75	80	80
	Mean	77	82	83	78
	S.E.	1.7	4.5	3.4	1.7

a - % of lesions of 1-50%

C - controls

T - treatments

Table 66

Heat-killed preparations (2) of B.fabae applied to the roots of broad bean plants; leaves challenged with B.fabae after 7 and 9 days.

		7 days		9 days	
	Replicates	C	T	C	T
Preparation (1)	1	85[a]	90	85	90
	2	90	90	90	90
	3	90	90	80	75
	Mean	88	90	85	85
	S.E.	1.7	0	2.9	5.1
Preparation (2)	1	90	85	85	85
	2	85	95	80	90
	3	90	85	80	80
	Mean	88	88	82	85
	S.E.	1.7	3.4	1.7	2.9

a - % of lesions of greater than 100%

C - controls

T - treatments

Table 67

Lower leaves of broad beans injected with crude cell wall extracts of B.fabae and B.cinerea; challenged after 2 and 5 days with B.fabae and B.cinerea.

cell wall extract	challenge organism	Replicates	2 days		5 days	
			T	C	T	C
B.fabae	B.fabae	1	85[a]	90	90	95
		2	80	95	90	95
		3	80	80	75	100
		Mean	82	88	85	97
		S.E.	1.7	4.5	5.1	1.7
	B.cinerea	1	80	75	90	85
		2	85	80	75	80
		3	85	85	80	80
		Mean	83	80	82	82
		S.E.	1.7	2.9	4.5	1.7
B.cinerea	B.cinerea	1	75	75	80	85
		2	80	80	80	85
		3	75	85	90	80
		Mean	77	80	83	83
		S.E.	1.7	2.9	3.4	1.7
	B.fabae	1	85	85	85	90
		2	90	80	90	85
		3	90	80	80	90
		Mean	88	82	85	88
		S.E.	1.7	1.7	2.9	1.7

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

Table 68

Crude cell wall extracts of B.fabae and B.cinerea injected into one half of detached leaves; opposite halves challenged after 2 and 4 days with B.fabae and B.cinerea.

cell wall extract	challenge organism	Replicates	2 days		5 days	
			T	C	T	C
B.fabae	B.fabae	1	85[a]	95	95	100
		2	90	85	95	90
		3	90	90	80	85
		Mean	88	90	90	92
		S.E.	1.7	2.9	5.1	4.5
	B.cinerea	1	85	80	80	85
		2	90	75	80	80
		3	90	80	85	80
		Mean	88	78	82	82
		S.E.	1.7	1.7	1.7	1.7
B.cinerea	B.cinerea	1	75	80	75	80
		2	80	80	85	85
		3	75	85	90	85
		Mean	77	82	83	83
		S.E.	1.7	1.7	4.5	1.7
	B.fabae	1	85	85	90	85
		2	95	90	90	90
		3	85	80	75	80
		Mean	88	85	85	85
		S.E.	3.5	2.9	5.1	2.9

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

Table 69

Cell wall extracts of B.fabae and B.cinerea applied to broad bean plants via the roots; leaves challenged with B.fabae and B.cinerea after 12 days.

cell wall extract	challenge fungus	replicates	C	T
B.fabae	B.fabae	1	85[a]	95
		2	80	80
		3	85	80
		Mean	83	85
		S.E.	1.7	5.1
	B.cinerea	1	80	70
		2	80	85
		3	85	75
		Mean	82	77
		S.E.	1.7	4.5
B.cinerea	B.cinerea	1	80	75
		2	80	85
		3	90	75
		Mean	83	78
		S.E.	3.4	3.4
	B.fabae	1	95	90
		2	90	95
		3	100	85
		Mean	95	90
		S.E.	2.9	2.9

a - % of lesions in usual category ie. 1-50% for B.cinerea and greater than 100% for B.fabae.

Table 70

Filtrates of cultures of B.fabae and B.cinerea prepared according to the method of Osman (1977) were painted onto upper and lower surfaces of detached broad bean leaves; upper surfaces were challenged with B.fabae and B.cinerea after 24 hours.

culture filtrate	challenge organism	Replicates	lower surface		upper surface	
			T	C	T	C
B.fabae	B.fabae	1	80[a]	85	80	90
		2	95	90	95	90
		3	90	80	90	85
		Mean	88	85	88	88
		S.E.	4.5	2.9	4.5	1.7
	B.cinerea	1	85	85	85	70
		2	80	75	75	85
		3	75	70	75	75
		Mean	80	77	78	77
		S.E.	2.9	4.5	3.4	4.5
B.cinerea	B.cinerea	1	85	90	80	75
		2	80	85	80	75
		3	80	90	90	75
		Mean	82	88	83	75
		S.E.	1.7	1.7	3.4	0
	B.fabae	1	95	85	85	85
		2	85	80	90	90
		3	85	80	80	80
		Mean	88	82	85	85
		S.E.	3.4	1.7	2.9	2.9

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

Table 71

Filtrates of cultures of B.fabae and B.cinerea prepared according to the method of Osman (1977) applied to detached leaves via the petiole; leaves challenged with B.fabae and B.cinerea after 2 and 5 days.

culture filtrate	challenge organism	Replicates	2 days		5 days	
			T	C	T	C
B.fabae	B.fabae	1	95[a]	85	90	95
		2	85	90	90	85
		3	85	80	85	85
		Mean	88	85	88	88
		S.E.	3.4	2.9	1.7	3.4
	B.cinerea	1	80	85	70	80
		2	80	80	85	85
		3	85	75	75	85
		Mean	82	80	77	83
		S.E.	1.7	2.9	4.5	1.7
B.cinerea	B.cinerea	1	80	85	80	85
		2	80	80	80	75
		3	85	75	75	70
		Mean	82	80	78	77
		S.E.	1.7	2.9	1.7	4.5
	B.fabae	1	90	85	90	85
		2	95	80	90	90
		3	80	90	90	80
		Mean	88	85	90	85
		S.E.	4.5	2.9	0	2.9

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

Table 72

Filtrates of cultures of B.fabae and B.cinerea prepared according to the method of Osman (1977) injected into lower leaves of broad beans; injected leaves and upper leaves challenged with B.fabae and B.cinerea after 2 and 5 days.

culture filtrate	challenge organism	Replicates	upper		injected	
			T	C	T	C
B.fabae	B.fabae	1	90[a]	85	95	90
		2	95	80	95	90
		3	80	80	100	95
		Mean	88	82	97	92
		S.E.	4.5	1.7	1.7	1.7
	B.cinerea	1	85	80	90	75
		2	80	80	85	80
		3	75	75	75	75
		Mean	80	78	83	77
		S.E.	2.9	1.7	4.5	1.7
B.cinerea	B.cinerea	1	80	90	80	90
		2	80	80	85	85
		3	85	80	85	90
		Mean	82	83	83	88
		S.E.	1.7	3.4	1.7	1.7
	B.fabae	1	85	85	85	95
		2	90	80	80	80
		3	80	85	85	80
		Mean	85	83	83	85
		S.E.	2.9	1.7	1.7	5.1

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

Table 73

Filtrates of cultures of B.fabae and B.cinerea prepared according to the method of Theodorou (1979) injected into lower leaves or half of detached leaves ;upper leaves or opposite half leaves challenged with B.fabae and B.cinerea after 5 and 3 days respectively.

culture filtrate	challenge organism	Replicates	½1		1	
			T	C	T	C
B.fabae	B.fabae	1	90[a]	90	100	85
		2	85	95	90	90
		3	95	100	85	95
		Mean	90	95	92	90
		S.E.	2.9	2.9	4.5	2.9
	B.cinerea	1	85	85	80	75
		2	80	80	80	85
		3	80	75	80	70
		Mean	82	80	80	77
		S.E.	1.7	2.9	0	4.5
B.cinerea	B.cinerea	1	75	90	85	80
		2	80	75	90	80
		3	80	80	80	85
		Mean	78	82	85	82
		S.E.	1.7	4.5	2.9	1.7
	B.fabae	1	90	95	85	85
		2	95	95	80	80
		3	100	85	95	85
		Mean	95	92	87	83
		S.E.	2.9	3.4	4.5	1.7

a - % of lesions , 1-50% for B.cinerea and greater than 100% for B.fabae

½1- ½ leaf injection 1 - whole leaf injection

Table 74

Seedlings grown in filtrate of cultures of B.fabae and B.cinerea prepared according to the method of Theodorou (1979); leaves challenged with B.fabae and B.cinerea after 12 days.

culture filtrate	challenge organism	replicates	T	C
B.fabae	B.fabae	1	80[a]	90
		2	80	95
		3	85	80
		Mean	82	88
		S.E.	1.7	4.5
	B.cinerea	1	75	80
		2	80	75
		3	80	80
		Mean	78	78
		S.E.	1.7	1.7
B.cinerea	B.cinerea	1	80	85
		2	80	80
		3	80	75
		Mean	80	80
		S.E.	0	2.9
	B.fabae	1	80	95
		2	90	85
		3	85	85
		Mean	85	88
		S.E.	2.9	3.4

a - % of lesions in usual category ie.1-50% for B.cinerea and greater than 100% for B.fabae.

Table 75

Broad bean plants treated with indole-3-acetic acid by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B. fabae after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	85 [a]	100	90	80
	2	95	95	90	95
	3	85	95	90	90
	Mean	88	97	90	88
	S.E.	3.4	1.7	0	4.5
3 day controls	1	95	80	95	85
	2	80	95	85	90
	3	80	90	85	80
	Mean	85	88	88	85
	S.E.	5.1	4.5	3.4	2.9
7 day treatments	1	90	85	*	*
	2	90	90	*	*
	3	85	80	*	*
	Mean	88	85	-	-
	S.E.	1.7	2.9	-	-
7 day controls	1	85	95	95	85
	2	85	90	85	90
	3	90	90	85	90
	Mean	88	92	88	88
	S.E.	3.4	1.7	3.4	1.7

a - %no. of lesions of > 100% necrosis

1 - whole leaf injection

½1 - ½ leaf injection

p - petiole uptake

r - root uptake

* - phytotoxicity: not challenged

Table 76

Broad bean plants treated with indole-3-acetic acid by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.cinerea after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80[a]	75	85	90
	2	80	80	80	85
	3	75	75	80	90
	Mean	78	77	82	88
	S.E.	1.7	1.7	1.7	1.7
3 day controls	1	70	85	80	85
	2	85	80	80	75
	3	75	75	85	70
	Mean	77	80	82	77
	S.E.	4.5	2.9	1.7	4.5
7 day treatments	1	75	85	*	*
	2	75	85	*	*
	3	75	75	*	*
	Mean	75	78	-	-
	S.E.	0	3.4		
7 day controls	1	75	80	80	80
	2	85	85	75	90
	3	75	85	85	90
	Mean	78	83	80	83
	S.E.	3.4	1.7	2.9	3.4

a - %no. of lesions of 1-50% necrosis
1 - whole leaf injection
½1 - ½ leaf injection
p - petiole uptake
r - root uptake
* - phytotoxicity: not challenged

Table 77

Broad bean plants treated with maleic hydrazide by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B. fabae after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	95[a]	85	85	90
	2	95	90	95	90
	3	100	90	85	90
	Mean	97	88	88	90
	S.E.	1.7	1.7	3.4	0
3 day controls	1	85	95	85	85
	2	90	85	80	80
	3	80	85	85	80
	Mean	85	88	83	82
	S.E.	2.9	3.4	1.7	1.7
7 day treatments	1	90	85	90	85
	2	90	80	95	80
	3	75	90	80	85
	Mean	85	85	88	83
	S.E.	5.1	2.9	4.5	1.7
7 day controls	1	90	95	90	85
	2	85	80	90	90
	3	90	80	90	80
	Mean	88	85	90	85
	S.E.	1.7	5.1	0	2.9

a - %no. of lesions of > 100% necrosis

1 - whole leaf injection

½1- ½ leaf injection

p - petiole uptake

r - root uptake

Table 78

Broad bean plants treated with maleic hydrazide by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with *B cinerea* after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80[a]	90	80	90
	2	85	80	85	85
	3	85	80	85	90
	Mean	83	83	83	88
	S.E.	1.7	3.4	1.7	1.7
3 day controls	1	80	90	85	80
	2	80	90	85	75
	3	85	85	80	80
	Mean	82	88	83	78
	S.E.	1.7	1.7	1.7	1.7
7 day treatments	1	90	85	80	80
	2	80	80	80	80
	3	75	75	75	75
	Mean	82	80	78	78
	S.E.	4.5	2.9	1.7	1.7
7 day controls	1	90	85	85	75
	2	85	80	85	80
	3	75	80	75	75
	Mean	83	82	82	77
	S.E.	4.5	1.7	3.4	1.7

a - %no. of lesions of 1-50% necrosis

1 - whole leaf injection

½1 - ½ leaf injection

p - petiole uptake

r - root uptake

Table 79

Broad bean plants treated with gibberellic acid by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.fabae after 3 and 7 days.

Method of treatment		l	½l	p	r
3 day treatments	1	100[a]	85	95	100
	2	90	90	90	90
	3	85	90	95	85
	Mean	92	88	93	92
	S.E.	4.5	1.7	1.7	4.5
3 day controls	1	85	95	85	95
	2	90	95	85	90
	3	90	80	95	95
	Mean	88	90	88	93
	S.E.	1.7	5.1	3.4	1.7
7 day treatments	1	90	95	90	95
	2	95	90	85	90
	3	85	100	90	90
	Mean	90	95	88	92
	S.E.	2.9	2.9	1.7	1.7
7 day controls	1	90	95	80	95
	2	90	85	95	85
	3	90	85	95	90
	Mean	90	88	90	90
	S.E.	0	3.4	5.1	2.9

a - %no. of lesions of > 100% necrosis
l - whole leaf injection
½l- ½ leaf injection
p - petiole uptake
r - root uptake

Table 80

Broad bean plants treated with gibberellic acid by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.cinereaafter 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80[a]	80	85	80
	2	85	80	75	75
	3	85	80	80	70
	Mean	83	80	80	75
	S.E.	1.7	0	2.9	2.9
3 day controls	1	75	85	70	70
	2	75	75	75	70
	3	80	80	85	90
	Mean	77	80	77	77
	S.E.	1.7	2.9	4.5	6.8
7 day treatments	1	85	85	80	75
	2	80	75	80	75
	3	80	75	80	85
	Mean	82	78	80	78
	S.E.	1.7	3.4	0	3.4
7 day controls	1	75	85	80	75
	2	75	80	85	85
	3	75	75	80	75
	Mean	75	80	82	78
	S.E.	0	2.9	1.7	3.4

a - %no. of lesions of 1-50% necrosis
1 - whole leaf injection
½1- ½ leaf injection
p - petiole uptake
r - root uptake

Table 81

Broad bean plants treated with cycocel by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B. fabae after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	85[a]	100	95	85
	2	90	80	90	90
	3	95	85	100	90
	Mean	90	88	95	88
	S.E.	2.9	6.2	2.9	1.7
3 day controls	1	90	85	90	85
	2	90	80	95	80
	3	90	95	100	85
	Mean	90	87	95	83
	S.E.	0	4.5	2.9	1.7
7 day treatments	1	85	85	*	*
	2	80	95	*	*
	3	80	90	*	*
	Mean	82	90	-	-
	S.E.	1.7	2.9	*	*
7 day controls	1	80	85	95	100
	2	95	80	95	85
	3	90	90	85	90
	Mean	88	85	92	92
	S.E.	4.5	2.9	3.4	4.5

a - %no. of lesions of > 100% necrosis
1 - whole leaf injection
½1 - ½ leaf injection
p - petiole uptake
r - root uptake
* - phytotoxicity: not challenged

Table 82

Broad bean plants treated with cycocel by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.cinereaafter 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	75 [a]	85	80	85
	2	80	90	80	90
	3	80	80	70	80
	Mean	78	85	77	85
	S.E.	1.7	2.9	3.4	2.9
3 day controls	1	85	90	85	80
	2	80	75	80	80
	3	75	80	80	85
	Mean	80	82	82	82
	S.E.	2.9	4.5	1.7	1.7
7 day treatments	1	75	85	*	*
	2	80	75	*	*
	3	80	75	*	*
	Mean	78	78	-	-
	S.E.	1.7	3.4	-	-
7 day controls	1	80	75	80	80
	2	80	85	85	80
	3	75	70	70	80
	Mean	78	77	78	80
	S.E.	1.7	4.5	4.5	0

a - %no. of lesions of 1-50% necrosis
1 - whole leaf injection
½1- ½ leaf injection
p - petiole uptake
r - root uptake
* - phytotoxicity: not challenged

Table 83

Broad bean plants treated with ethephon by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.fabae after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	90 [a]	90	90	90
	2	95	95	85	90
	3	100	85	95	95
	Mean	95	90	90	92
	S.E.	2.9	2.9	2.9	1.7
3 day controls	1	100	85	95	100
	2	90	90	95	90
	3	90	95	90	85
	Mean	93	90	93	92
	S.E.	3.4	2.9	1.7	4.5
7 day treatments	1	95	90	95	*
	2	90	90	90	*
	3	100	90	85	*
	Mean	95	90	90	*
	S.E.	2.9	0	2.9	*
7 day controls	1	95	95	90	95
	2	80	90	90	90
	3	90	90	90	85
	Mean	88	92	90	90
	S.E.	4.5	1.7	0	2.9

a - %no. of lesions of 100% necrosis

1 - whole leaf injection

½1 - ½ leaf injection

p - petiole uptake

r - root uptake

* - phytotoxicity: not challenged

Table 84

Broad bean plants treated with ethephon

by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B. cinerea after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80 [a]	85	75	80
	2	75	80	80	80
	3	80	75	80	80
	Mean	78	80	78	80
	S.E.	1.7	2.9	1.7	0
3 day controls	1	75	70	80	80
	2	75	80	85	75
	3	80	80	80	70
	Mean	77	77	82	75
	S.E.	1.7	3.4	1.7	2.9
7 day treatments	1	80	85	80	*
	2	75	85	80	*
	3	80	80	75	*
	Mean	78	83	78	-
	S.E.	1.7	1.7	1.7	-
7 day controls	1	90	75	75	80
	2	80	80	75	80
	3	75	80	80	80
	Mean	82	78	77	80
	S.E.	4.5	1.7	1.7	0

a - %no. of lesions of 1-50% necrosis

1 - whole leaf injection

½1 - ½ leaf injection

p - petiole uptake

r - root uptake

* - phytotoxicity: not challenged

Table 85

Broad bean plants treated with kinetin by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.fabae after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80 [a]	85	90	80
	2	90	90	90	80
	3	95	90	90	85
	Mean	88	88	90	82
	S.E.	4.5	1.7	0	1.7
3 day controls	1	85	80	90	90
	2	90	85	95	80
	3	90	85	80	85
	Mean	88	83	88	85
	S.E.	1.7	1.7	4.5	2.9
7 day treatments	1	80	90	85	90
	2	90	90	90	85
	3	85	90	85	85
	Mean	85	90	87	87
	S.E.	2.9	0	1.7	1.7
7 day controls	1	90	95	90	85
	2	85	85	90	80
	3	85	85	85	95
	Mean	87	88	88	87
	S.E.	1.7	3.4	1.7	4.5

a - %no. of lesions of > 100% necrosis

1 - whole leaf injection

½1- ½ leaf injection

p - petiole uptake

r - root uptake

Table 86

Broad bean plants treated with kinetin by injection of lower leaves, injection of half detached leaves, and via the roots and petiole, challenged on upper leaves, opposite half leaves and leaves with B.cinerea after 3 and 7 days.

Method of treatment		1	½1	p	r
3 day treatments	1	80 [a]	80	80	85
	2	85	75	75	80
	3	80	80	75	80
	Mean	82	78	77	82
	S.E.	1.7	1.7	1.7	1.7
3 day controls	1	85	80	75	85
	2	75	80	75	90
	3	75	80	75	70
	Mean	78	80	75	82
	S.E.	3.4	0	0	6.1
7 day treatments	1	85	80	80	80
	2	80	85	75	80
	3	80	90	75	80
	Mean	82	85	77	80
	S.E.	1.7	2.9	1.7	0
7 day controls	1	90	85	80	75
	2	75	90	80	75
	3	80	90	75	85
	Mean	82	88	78	82
	S.E.	4.5	1.7	1.7	3.4

a - %no. of lesions of 1-50 % necrosis

1 - whole leaf injection

½1- ½ leaf injection

p - petiole uptake

r - root uptake

Table 87

Broad bean plants treated with polyacrylic acid, gentisic acid and aspirin by injecting into lower leaves and half detached leaves, and via the roots; challenged after 3 and 7 days with B.fabae.

Incubation time		3 days			7 days		
Method of treatment		½l	l	r	½l	l	r
Polyacrylic acid	C	94[a]	96	98	90	98	94
	T	50	92	96	48	96	93
Gentisic acid	C	94	93	88	93	92	94
	T	63	92	90	59	90	94
Aspirin	C	94	93	95	92	93	95
	T	72	93	91	70	91	92

a - % of lesions of greater than 100% necrosis

½l- ½ leaf injection

l - whole leaf injection

r - root uptake

C - controls

T - treatments.

Table 88

Broad bean plants treated with polyacrylic acid, gentisic acid and aspirin by injecting into lower leaves and half detached leaves, and via the roots; challenged after 3 and 7 days with B.cinerea.

Incubation time		3 days			7 days		
Method of treatment		½l	l	r	½l	l	r
Polyacrylic acid	C	82[a]	81	84	81	80	82
	T	86	76	84	86	78	80
Gentisic acid	C	82	88	85	81	80	87
	T	84	86	83	82	84	82
Aspirin	C	81	83	80	81	83	83
	T	80	82	80	86	84	80

a - % no. of lesions of 1-50% necrosis ie. grade 4

½l- ½ leaf injection

l - whole leaf injection

r - root uptake

C - controls

T - treatments.

Table 89

Assessment of phytotoxicity of test fungicide compounds when injected into broad bean leaves both on whole plants or detached.

	Whole plants		Detached leaves			
	500ppm	1000ppm	100ppm	500ppm	1000ppm	5000ppm
1	++[a]	++	-	-	++	++
	++	+++	-	-	++	+++
2	+	++	-	++	++	++
	+	++	-	+	++	++
3	-	-	-	-	-	-
	-	-	-	-	-	-
4	+++	+++	++	+++	+++	+++
	+++	+++	++	+++	++	+++
5	++	+++	+	+	+++	++
	++	+++	+	++	++	++
6	++	++	+	+	+	++
	++	++	++	+	++	+++
7	++	++	+	+	++	++
	++	+++	-	+	++	++

a - degree of necrosis

- none
+ some necrosis
++ extensive necrosis
+++ very extensive necrosis.

Table 90

Injection of lower leaves of broad bean plants with test fungicide compounds at 500 and 1000 ppm.; upper leaves challenged with B.fabae after 3 days.

	500ppm					1000ppm				
	Replicates (3)			Mean	S.E.	Replicates (3)			Mean	S.E.
1	90[a]	100	92	94	3	100	100	92	97	3
2	83	100	92	92	5	100	83	83	89	6
3	17	67	67	50	17	0	0	0	0	0
4	100	100	100	100	0	92	100	100	97	3
5	25	67	67	53	14	25	50	50	42	8
6	50	83	67	67	10	67	75	50	64	7
7	33	83	50	55	15	50	33	33	39	6

H_2O controls (6replicates)						Mean	S.E.
100	83	92	100	100	50	88	8

a - % no. of spreading lesions.

Table 91

Injection of the primary leaves of French beans with test fungicide compounds at concentrations of 500 and 1000 ppm.; upper leaves challenged with purified TNV after 4 days.

	500ppm					1000ppm				
	Replicates (3)			Mean	S.E.	Replicates (3)			Mean	S.E.
1	286[a]	194	262	247	28	381	105	223	236	80
2	150	147	62	120	29	94	153	63	103	27
3	222	308	252	261	25	289	303	197	263	33
4	300	211	183	231	35	265	101	304	223	62
5	407	134	160	234	87	402	147	197	263	33
6	37	120	50	69	26	23	45	110	59	26
7	70	150	70	97	27	300	147	262	237	46

H_2O controls (6replicates)							Mean	S.E.
	187	211	291	120	257	220	214	24

a - no. of TNV lesions.

Table 92

Leaf disks from Black Hamburg vines exposed to UV irradiation for 0,1,2,3,5 and 10 minutes; challenged after 0,24 and 72 hours with P.viticola inoculum.

Incubation time (h)	Exposure time(mins)	Sporulation (no.disks)	Sporulation density	Visible damage(no.disks)
72	10	0	0	10
	5	0	0	1
	3	10	+	0
	2	10	++	0
	1	10	+++	0
	0	10	+++	0
24	10	0	0	8
	5	0	0	0
	3	9	+	0
	2	9	++	0
	1	10	+++	0
	0	10	+++	0
0	10	0	0	8
	5	0	0	0
	3	10	+	0
	2	10	++	0
	1	10	+++	0
	0	10	+++	0

+,++,+++ - increasing density of sporulation.

Table 93

Leaf disks from Black Hamburg vines half exposed to UV irradiation for 0,2 and 10 minutes; exposed and non-exposed halves separated and challenged with P.viticola after 24 and 48 hours.

		24[b]	48
Unexposed			
½-disks	0[a]	4[c]	5
	2	4	4
	10	3	4
Exposed			
½-disks	0	4	4
	2	4	4
	10	0	0

a - time (minutes) disks exposed to UV

b - incubation time before inoculation (hours)

c - no.of ½-disks showing dense sporulation.

Table 94

Lower leaves of Black Hamburg vines exposed to UV irradiation for 0,2 and 10 minutes ; plants challenged by spray inoculating with P.viticola after 72 hours.

	0[a]	2	10
1[b]	3[c]	4	4
2	2	3	3
3	4	1	2
4	4	2	3
5	4	3	4
6	2	4	2
7	3	3	2
8	4	3	3
9	4	4	3
10	2	4	4
Mean	3.2	3.1	3.0
S.E.	.29	.31	.26

a- time (minutes) of UV exposure

b- 10 replicates

c- assessment of disease on upper leaves.

Table 95

Reaction of extracts from UV irradiated vine leaves to diazotised nitroaniline reagent.

	0 [a]	1	4	10
Rf values[b]			.40	.40
			.50	.50
			.60	.60
			.66	.66
			.72	.72
		.76	.76	.76
	.80	.80	.80	.80
	.88	.88	.88	.88
	.96	.96	.96	-

a - time (minutes) vine leaves exposed to UV irradiation.

b - Rf values of bands reacting to reagent, ie. phenolics.

VI. DISCUSSION

A. Systemic induced resistance by pre-inoculation with live pathogens

The immunization of cucumber by Colletotrichum lagenarium to Colletotrichum lagenarium has been well established by Kuc (1981) both in laboratory and field experiments. Similar phenomena have been described with Peronospora parasitica in tobacco, Colletotrichum lindemuthianum in French bean and more recently with Colletotrichum phomoides in tomato. Induced resistance in virus systems is also well documented and fairly widespread. Caruso and Kuc (1977) suggested that the phenomenon may be common in plant-parasite interactions. However this work has shown that, under the conditions described, immunization of grape vine against Plasmopara viticola by Plasmopara viticola is not possible nor could the system described in French bean with Colletotrichum lindemuthianum be repeated under the conditions described by Sutton (1979) or otherwise. Pre-inoculation of French beans with either TNV, Pseudomonas phaseolicola, or Uromyces phaseoli also failed to induce systemic resistance to subsequent infection by Colletotrichum lindemuthianum. Broad beans could not be immunized against rust by prior infection with the broad bean rust Uromyces fabae and Botrytis cinerea infection of the lower leaves had no effect on the development of the same fungus on the upper leaves. However systemic induced resistance was shown to occur in broad beans using Botrytis fabae. A degree of systemic protection also resulted when lower leaves were infected with Botrytis cinerea and upper leaves subsequently challenged with Botrytis fabae. The degree of protection achieved was thus greater where the more virulent pathogen was used as the inducer. Pre-inoculation with Botrytis fabae had no effect on the rather poor infection of the upper leaves with Botrytis cinerea, i.e. there was no evidence for any induced

susceptibility.

The importance of necrosis in the inducing infection has been shown in all cases of viral induced resistance and has been discussed by Kuc (1981) in relation to the cucumber system. He states that "in all cases reported to date with cucurbits, macroscopic or microscopic necrosis caused by an infectious agent is associated with immunization". Varying degrees of necrosis resulted in experiments using different varieties of grape vine and a consistently high degree in the variety most thoroughly investigated, Black Hamburg. Necrosis was also always associated with inducing inoculations of French bean with Colletotrichum lindemuthianum yet in neither of these cases could systemic induced resistance by demonstrated. Necrosis is however a feature of Botrytis fabae and Botrytis cinerea infection where induced systemic protection was achieved. Its demonstration is however, as with the tomato/Colletotrichum phomoides system, much less reliable and its effect less dramatic than that described by Kuc in cucumbers. Moreover no work has yet been carried out to determine whether the immunization has features similar to those shown in cucumbers.

The desiccated appearance of broad bean plants in some cases where systemic induced resistance was observed may or may not be significant. An alteration in the water balance in the protected leaves of tobacco (against viruses) has previously been shown by Casells et al. (1978) and as Wagih (1981) points out for viruses if water stress is involved in the phenomenon then all challenge experiments may be inherently irreproducible. This may account for the varied results achieved in this work and those reported by Gessler and Heller (1981) working on tomato with Colletotrichum phomoides.

B. Induction of systemic resistance by pre-treatment with the products and/or constituents of pathogens

Biotic elicitors such as germination fluids, extracts from heat-killed cells, cell wall extracts and culture filtrates have been shown in many cases to induce localized resistance through direct antagonistic effects or the induction of accumulation of phytoalexins. There are few cases however of such substances eliciting systemic effects and several of these are active only over relatively short distances. Heale and Sharman (1977) induced systemic resistance in carrot root slices to Botrytis cinerea by treating cut surfaces with cell-free germination fluid and culture filtrates of Verticillium were shown to induce systemic protection to wilt in tomatoes (Tjamos, 1979). In this work cell-free germination fluids and heat-killed cells prepared by various methods were used to treat vines, French beans and broad beans which were subsequently challenged with Plasmopara viticola, Colletotrichum lindemuthianum, and with Uromyces fabae, Botrytis fabae and Botrytis cinerea respectively. Broad beans were treated with crude cell wall extracts and crude culture filtrates from Botrytis fabae and Botrytis cinerea and subsequently challenged with both fungi. None of these substances elicited any detectable systemic effects in the host-parasite combinations described. This is in common with the Colletotrichum/cucumber system where biotic elicitors such as these induced no systemic protection (Kuc, 1981). Anderbrhan (1978) obtained only localized effects when treating cucumbers with elicitors from Colletotrichum spp. but did achieve significant protection in cucumber plants grown from seeds which had been soaked in elicitors. Kumamato (1948) reported reduced susceptibility to blast in plants growm from rice seed which had been treated with extracts of cultures of Pyricularia oryzae. Protection of rice seedlings and mature plants against Helminthosporium oryzae infection was also obtained when

seeds were soaked in mycelial extracts or culture filtrates of the pathogen (Trivedi and Sinha, 1976). In this work no effects were achieved when broad beans were grown in crude cell wall extracts and crude culture filtrates of Botrytis fabae and Botrytis cinerea.

Schönbeck et al. (1980) however proposed that there is a resistance potential in every plant which can be activated by appropriate inducers. He found that 10-15% of bacterial and fungal isolates tested contained such inducers in their culture filtrates or in cell extracts and that many of these induced systemic resistance at least to a certain degree e.g. across portions of a leaf. Testing of a greater number of isolates against a particular pathogen may thus have yielded positive results.

Although techniques used in this work have all been developed from reports where elicitors have induced resistance at the concentrations used in most cases the amount of substance used was very small. Concentrations at which a fungicide might be expected to be active in a plant are generally much higher. Concentration of substances may have yielded positive results.

C. Induction of systemic resistance by pre-treatment with abiotic inducers

1. Growth regulating substances

Growth regulating substances such as those used in the present work can undoubtably be used to alter the "normal" response of a plant to disease. Many of them are not fungitoxic in vitro and may act by induction of host mechanisms. This alteration of response, towards resistance or susceptibility, may be consistent for one growth regulating substance or may vary according to the concentration, or method of application, or other factors. Excepting cases where growth regulating substances or their breakdown products are directly translocated through the host plant induced resistance has so far been reported only as a localized effect (Anderbrhan, 1978). The present work was designed to see if

any of a range of growth regulating substances could be used to induce systemic resistance perhaps by triggering natural defence mechanisms within the host. None of the substances tested under the conditions described induced any systemic effects in broad beans when challenged with either Botrytis fabae or Botrytis cinerea. This was somewhat surprising where substances were directly taken up by plants particularly via the roots. In the light of results such as those of Anderbrhan (1978), Davis and Dimond (1953), Pegg (1976), Stahmann et al. (1966) and Andel (1968) root uptake of at least some of the substances tested was expected to result in an alteration of disease response in the leaves. Particularly in the case of ethephon some indication of the effect of the ethylene released by this compound was expected and this may cast some doubt on the activity of the compounds used. Several of the compounds are unstable and should not be stored for long periods.

2. Polyacrylic acid, aspirin and gentisic acid

Previous work (Gianinazzi and Kassanis, 1974; White, 1979; Mills and Wood, unpublished) has shown that substances such as polyacrylic acid, gentisic acid and aspirin while not toxic in vitro can induce systemic resistance in host plants. All three substances induced systemic resistance to Botrytis fabae, polyacrylic acid being the most effective. Resistance was induced only between the two parts of the bifoliate leaf of broad bean and not between different leaves on the same plant; this is in common with other systems. The same substances were also tested on French beans against Colletotrichum lindemuthianum but no protection was achieved. Wagih (1981) reports that polyacrylic acid induced resistance has many similar features to that induced by the pathogens and thus similar mechanisms may be involved. Why other substances such as acetylsalicylic acid, which are chemically unrelated, should also induce resistance is unknown.

3. Potential fungicide compounds as inducers of systemic resistance

Rathmell (1981) suggested that compounds that can induce resistance to pathogens in plants are surprisingly common. He found that between two and five per cent of compounds tested in fungicide screens induced resistance and caused phytotoxicity in treated plants and that the linkage between the two effects was statistically highly significant. In this work 7 compounds were chosen at random which fulfilled the following criteria:-

i) When incorporated into potato dextrose agar at 50 ppm showed no activity against growth of Botrytis cinerea;

ii) When sprayed on the foliage of young (2 true leaf) tomato plants gave rise to visible phytotoxicity (at 50 ppm spray);

iii) Under the conditions of ii) showed activity against Botrytis cinerea in vivo

Compounds were tested against Botrytis fabae in broad beans, and Colletotrichum lindemuthianum and tobacco necrosis virus in French beans. Phytotoxicity tests showed that much higher concentrations were needed to achieve effects than was found in experiments at Jealott's Hill Research Station on tomatoes. Four out of the seven compounds (No. 3, 5, 6 and 7) induced some restriction of lesion spread in broad beans, particularly at the higher concentration, but no effects were induced against Colletotrichum lindemuthianum in French beans by any of the compounds. Three compounds (No. 2, 6 and 7) reduced the number of tobacco necrosis virus lesions in French beans but only two of these (No. 6 and 7) were compounds active against Botrytis fabae in broad bean.

4. Physical inducers - ultra-violet irradiation

Ultra-violet irradiation has been shown to induce localized resistance to pathogens often through stimulation of phytoalexins (e.g. Anderbrhan, 1978). Vine leaves exposed to ultra-violet irradiation

produced phytoalexins with the effect that treated tissue became partially or totally resistant to Plasmopora viticola infection. The degree of resistance was directly dependent on the amount of phytoalexins present which was in turn dependent on the time that the tissue was exposed. There was however no evidence for any induction of systemic effects.

Conclusions

The present work has shown that while systemic induced resistance by pre-inoculation is undoubtably very effective in cucumber it is much less common, reliable and striking in some other systems than was previously suggested. It does not appear to be a widespread phenomenon or at least not one that is as easily demonstrated in most plants as it is in members of the Cucurbitaceae.

The induction of systemic resistance by chemicals has been demonstrated in several host-parasite combinations although there is as yet no evidence as to whether or not this protection acts in a similar manner to immunization with pathogens.

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