GENETIC INTERACTIONS BETWEEN *ERYSIPHE FISCHERI* (BLUMER) AND MEMBERS OF THE GENUS *SENECIO*

by

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A thesis submitted for the degree of
Doctor of Philosophy
in the Faculty of Science.

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The University,
Glasgow.

February 1990.

DECLARATION

I hereby declare that this thesis embodies the results of my own special work, that it has been composed by myself and that it does not include work forming part of a thesis presented for a degree at this or any other university. Due acknowledgement is made within the text for contributions from other sources.

FRASER S. CAMPBELL
FEBRUARY 1990

ACKNOWLEDGEMENTS

I would like firstly to thank Dr.D.D.Clarke for his supervision of my work, his tireless patience and helpfulness.

From all the people in the Botany Department, who helped and advised me during my stay, I particularly thank the following:-

Miss Aileen Adams, for invaluable technical help in looking after my plants and cultures.

Mr Robert Cuthbertson, for his help in setting up greenhouse and growth-room experiments.

Janice Smith, Jackie Smith and Andrew Madden, for a great many helpful and encouraging conversations.

Finally, Dr.A.M.Berrie and Professor R.J.Cogdell, who as heads of the Department, made available the facilities for this project.

The project was funded by the S.E.R.C., and I received personal sponsorship from the C.K.Marr Educational Trust. I convey my thanks to the governors of the Trust.

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SUMMARY

Twelve single spore isolates of *E.fischeri* were made and each was shown to possess a different virulence phenotype when inoculated onto 47 lines of *S. vulgaris*. Three crosses were made between resistant and susceptible lines of *S.vulgaris*, and the resistance phenotypes of F1 and F2 plants recorded, using available samples from the 12 isolates of the pathogen. Resistance was shown to be under the control of one or two major genes, with resistance either dominant or recessive. In some of the cases of recessive resistance there was evidence of a separate locus containing a gene that inhibited the expression of resistance genes. In one cross, seven resistance genes were shown to be linked.

Polyacrylamide gel electrophoresis was conducted on protein extracts from a number of lines of *S.vulgaris*, but no protein differences were revealed that could be correlated with resistance phenotype.

Radiate lines of *S.vulgaris* were shown to be more resistant to *E.fischeri* than were non-radiate lines collected from the same location. It is considered that the radiate lines resulted from introgressive hybridisation with *S.squalidus*, and that the increased resistance is due to the transfer of non-host resistance from *S.squalidus* to *S.vulgaris*. This new

resistance was investigated in a crossing experiment, and did not segregate to give clear Mendelian ratios in an F2 progeny.

The resistance phenotypes of a number of triazine resistant lines of S.vulgaris were examined, and the mutation to herbicide resistance found in a large number of different resistance backgrounds, giving evidence that mutation to triazine resistance was a relatively frequently occurring event.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Historical note and some definitions.

The importance of the effects of plant disease have been recognised since the first civilisations of the world raised crops in the delta of the river Nile. The Old Testament of the Bible refers to the injurious effects of mildew and blasting. The first person to study the effects of disease in cultivated plants was probably Theophrastus, a Greek philosopher of the fourth century BC. He observed the effects of rust on different crop species and in different environments. Following on from the Greeks, the Romans also noticed the devastating effects of diseases, such as rusts, on cereal crops, but added little to their scientific understanding (Agrios 1989).

Since these early and unscientific days the effect of plant disease on world crop production has been well documented. For example, late blight of potatoes, caused by the fungus *Phytophthora infestans*, resulted in severe famine and the death of nearly a quarter of a million Irish people between 1845 and 1846. A permanent displacement of many more people followed as a direct result (Day 1974).

Even today the loss of production due to plant disease causes serious problems worldwide. In 1982 the Food and Agricultural Organisation of the United Nations estimated the percentage of cereal crop production lost due to plant disease (including the effects of weeds), to be 17.8% in developed countries and 46.1% in developing countries (Agrios 1989).

Many different definitions of disease in plants have been advanced, but the essential elements can be summarised as follows: it is an abnormal and injurious condition, physiological in nature and resulting from the interaction between some agent and the plant (Wheeler 1980). The exact nature of what is normal and what is abnormal in a plant species - especially a wild species - is rather difficult to define (Dickinson and Lucas 1982). Therefore even this definition must be used with caution.

The living organisms causing disease in plants are collectively termed pathogens, and include fungi, bacteria, viruses and viroids, mycoplasmas, nematodes, insects and several species of higher plant. An organism living on a plant and deriving its nutriment from that plant, but not causing disease, may be termed a parasite (Day 1974). Micro-organisms in the first three groups listed above cause by far the greatest proportion of plant disease, and this project considers the effects of one species from the first category.

Disease can also be caused by non-living agents, such as physical and chemical factors of the environment. These factors include light and temperature extremes, mineral deficiences, and excesses of pollutants (Dickinson and Lucas 1982).

Plant pathology is directed towards preventing crop losses caused by disease, by increasing our knowledge of the pathogens, and their hosts and the environments they interact within.

1.2 Plant defences to disease.

The fact that most wild plant species complete their life cycles relatively unaffected by disease suggests that they have evolved successful strategies to avoid the harmful effects of pathogenic organisms. These can be divided into three strategies; tolerance, disease escape and disease resistance. Each of these will now be considered.

1.2.1 Tolerance.

Tolerance occurs when plants are able to grow and produce a good crop relatively unaffected by a pathogen. Tolerance may be to the pathogen itself, or to the injurious effects it causes. Simmons (1966), measured tolerance in a variety of oats to Puccinia coronata f.sp. avenae, by comparing the yield in rusted and non-rusted plots. He found that certain varieties were consistantly more tolerant than others, and that these differences were heritable. More recently, Ben-Kalio and Clarke (1979), investigated the effects of infection with Erysiphe fischeri on Senecio vulgaris. They found that the physiology of the plant was little affected and concluded that the small effects seen were due to unavoidable damage caused by the pathogen, Infection rates causing the colonisation of 75-100% of the leaf surface did not affect chlorophyll levels or the rate of dry matter production per unit area of leaf tissue.

Clarke (1984), suggests that natural selection will favour host pathogen interactions where damage to the host is minimised, thus ensuring the survival of susceptible host tissue on which the pathogen can reproduce. As a result, in wild plant pathosystems, high levels of infection often result in relatively little damage to the host. This is in contrast to the situation in crop species, where low levels of infection can result in serious losses (Clarke et al. 1987). It may be that modern crop species have lost their tolerance of pathogens during attempts at crop improvement.

Tolerance therefore provides a high degree of protection to the host, but still allows the pathogen to reproduce. When considering the use of tolerance as a character in a breeding programme, this is important. The reproductive capacity of the pathogen is not reduced, but tolerant plants will not impose strong selection pressures on the pathogen, therefore any evolution would be expected to progress slowly.

1.2.2 Disease escape.

This occurs when susceptible plants do not become infected, generally because a susceptible host, virulent pathogen and suitable environmental conditions do not coincide (Agrios 1989).

For example, plants are susceptible to some pathogens such as powdery mildews when young, and others such as Botrytis cinerea when older (Agrios 1989). If the suitable pathogen is absent at the susceptible growth stage, the plant will escape becoming infected, even if it is genetically susceptible.

Environmental factors such as temperature and humidity greatly affect the growth and reproduction of fungi, unfavourble conditions for them will again allow susceptible plants to escape disease.

These are just a few examples of how disease escape functions. It depends on both the heritable characters of the host and on environmental factors. It can therefore be actively managed by farmers and used to increase the resistance of field crops to disease (Agrios 1989).

1.2.3 Disease resistance

Resistance to disease is defined by Day (1974), as "the result of genetic modifications of the host, which renders it resistant to pathogens that would otherwise grow on it." The key phrase is "genetic modifications", and these are fully discussed in chapter four. Here, resistance will be discussed in broader terms, with an examination of the different types possible.

Resistance can be described in either functional or genetic terms, although the two are not necessarily correlated (Day 1974).

There are two main types of resistance described by genetic terms, oligenic and polygenic.

1. Oligogenic resistance is resistance determined by one or a few major genes. Resistance is usually dominant to susceptibility, although this is not always the case.

2. Polygenic resistance is determined by a large number of genes, each of which produces relatively small effects.

Oligogenic resistance is often specific in nature, operating against certain isolates only of a pathogen, whilst polygenic resistance may be general, effective against all isolates of a pathogen.

Van der Plank (1963), introduced the functional terms, vertical and horizontal resistance. Vertical resistance is effective against only certain races of a pathogen, whilst horizontal resistance is effective against all races of that pathogen. He envisaged that all plants would possess fairly equal, but low, levels of horizontal resistance to particular pathogens. Additionally they would possess a greater or smaller number of vertical resistances, effective against the races of that pathogen.

Thus resistance can be considered as being either race specific, effective against only certain races of a pathogen, or race non-specific, effective against all races of the pathogen (Day 1974).

1.3 The gene-for-gene concept.

To ensure the continuing survival of both host and pathogen species it would be predicted that changes in pathogen virulence would be matched by changes in host resistance, and vice-versa. A dynamic equalibrium would be produced, providing the best chance of survival for both organisms (Agrios 1989). It was Flor, working with flax and flax rust, *Melampsora lini*, who gave these ideas a conceptual framework, the gene-for-gene concept (Flor 1942, 1955). He determined that for each host gene for resistance there was a corresponding gene for virulence in the pathogen. The system is most likely to occur where resistance and virulence are determined by single diallelic genes; to test this, a simple quadratic check is used (Dickinson and Lucas 1982). Resistance occurs in only one out of the four classes, where the dominant allele for resistance is matched with the dominant allele for avirulence in the pathogen.

If resistance to a pathogen isolate is determined by more than one gene, the pathogen may possess a corresponding number of avirulence genes (Crute 1985). The gene combinations and resulting disease reaction types can then be tabulated in larger tables (Agrios 1989).

The gene-for-gene system has only been demonstrated in plants possessing oligogenic resistance to a specific pathogen, but it may in future also be demonstrated to occur where host resistance and pathogen virulence are polygenically determined (Agrios 1989).

1.4 Resistance to Infection in wild species compared to crop species.

Wild plant pathosystems have not been as extensively studied as crop pathosystems - probably because of economic considerations. Plant breeders have mainly concentrated on improving the resistance of food crops (Wheeler 1980).
Breeding for resistance to pathogens has often entailed incorporating new major genes for resistance into the crop genome. However this has led to problems; many resistance genes have been quickly overcome by mutations to virulence in the pathogen, resulting in the continual need to search for new resistance genes (Day 1974). However, there are examples where single gene resistance has provided effective resistance for many years, for example; resistance to diseases caused by <u>Fusarium</u> spp. and by <u>Helminthosporium</u> spp. (Johnson 1987).

By contrast, in wild species, most plants are relatively little affected by pathogens, and devastating epidemics of the type seen in crop species are rare (Clarke 1984). Why should this be the case? Knowledge of how wild species resist pathogen attack may be useful when trying to breed resistance into crop species (Clarke et al. 1987).

The search for new resistance to deploy in crop species often takes plant breeders back to the progenitor species of that crop, or to other closely related wild species (Agrios 1989). Dinoor (1977), investigated the resistance of wild oat to crown rust, caused by Puccinia coronata f.sp. avenae, which also affects cultivated oats. He found high levels of major gene resistance to the pathogen. A genetically highly diverse population contained nearly 100 physiological races of the fungus.

Major gene resistance to powdery mildew has been demonstrated in the wild relatives of cucumber (Lebeda 1984), and in the wild relatives of lettuce to downy mildew (Crute et al. 1980).

Wild relatives of crop species clearly possess resistance that is useful to plant breeders, but this does not fully explain their success in resisting pathogen attack. Do they perhaps have different types of resistance genes to crop species, which are differently deployed and with different functions (Clarke 1984). This is at present a largely unanswered question.

Tolerance has already been suggested as one means by which wild plants limit the damage caused by micro-organisms, another may be non-host resistance. Wild plant species are often not very closely genetically related to each other, and are therefore not attacked by the pathogens of other species. Johnson (1987), noted that most plants are resistant to the pathogens of genetically unrelated species. Many crops have been developed from a very narrow genetic base, and this is especially true of the cultivars of crops. Therefore crop plants may be more likely to become diseased than unrelated wild species, because they are susceptible to more pathogens that have developed on other closely related crop species.

If non-host resistance is important in wild plant pathosystems it is possible that it could be transferred to crop species. The genetic distance across which effective resistance genes could be transferred is unknown, between two species in the same genus, or even between species in different genera? It is also not known whether non-host resistance is general or specific in nature. When transferred it may provide resistance against pathogens which were non-pathogenic on the original species, or it may not. A greater knowledge of the genetic control and the possibilities of transfer between species of non-host resistance is called for.

1.5 The reasons for studying the Erysiphe fischeri/Senecio vulgaris pathosystem.

S.vulgaris is a common annual weed, with a short life-cycle of ten to twelve weeks, producing a large number of seeds which are easily germinated. It is strongly inbreeding, allowing highly homozygous true breeding lines to be maintained. The species is therefore very suitable for genetic analysis. E.fischeri is an obligate pathogen reproducing asexually only, by conidia.

The pathosystem has been previously studied by many workers, including, Ben-Kalio (1976), Harry (1980) and Bevan (1986), therfore many of the techniques, especially those for genetic analysis had already been worked out and applied.

Because S.vulgaris has no closely related crop species any changes in the pathogen population would be influenced by that species alone (and to some extent by other wild Senecio hosts, such as S.viscosus), therefore it is a closed pathosysyem and interesting to investigate.

Finally, within the Senecio genus there are a number of species closely related to S.vulgaris, which appear not to be hosts for E.fischeri. These species are capable of hybridisation with S.vulgaris (Gibbs et al. 1975), therefore the possibility exists for the transfer of non-host resistance between species, and for the experimental study of non-host resistance.

1.6 The aims of the project.

The main aims of this project were:-

1. To collect and characterise isolates of *E. fischeri*.
2. To conduct breeding experiments with resistant and susceptible lines of *S.vulgaris*, and thus determine the mode of inheritance of resistance, and look for linkage between resistance genes.
3. To electrophoretically analyse lines of *S.vulgaris* shown to possess large and small numbers of resistance genes.
4. To look for differences in resistance to *E.fischeri* between radiate and non-radiate *S.vulgaris*.
5. To attempt to transfer non-host resistance into *S.vulgaris* from a related *Senecio* species, and to genetically analyse it.

CHAPTER 2. MATERIALS AND METHODS.

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2.1 S.vulgaris

2.1.1 Collection of lines.

The locations from which seeds were collected to generate true breeding lines is shown in table 2.1. Achenes were collected from plants growing on road-sides, or in areas of disturbed urban ground, generally between March and November.

Some collections were made by Harry (1980), others by Bevan (1986). An individual plant derived from each collection was labelled as the parent of a line, and these lines were then maintained by inbreeding. Each line was expected to be true breeding for most characters; since mean levels of heterozygosity are reduced by 50% with each selfed generation.

2.1.2 Growth of plants.

To exclude the possibility of mildew infection, plants were grown in growth rooms. Seed was sown on top of S.A.I. potting compost in 11cm plastic pots. Seedlings germinated in two to four days and were transplanted into individual 11cm pots when 14 days old. At six weeks old, plants had produced sufficient leaves for use in mildew testing, and at eight to ten weeks old set seed.

S.squalidus plants were similarly grown, but were slower to mature, producing leaf tissue for testing at eight weeks and setting seed at 12 to 14 weeks. The growth rooms were maintained at 20-22^{o}C with a ten hour photoperiod, provided by mercury fluorescent bulbs, giving a P.A.R light intensity of 85 µmol photons m^{-2} s^{-1}. Skye SKP200 meter.

2.2 <u>Erysiphe fischeri</u>

2.2.1 Collection of isolates.

Mildew isolates were obtained from naturally infected plants in the field. All the isolates came from the Glasgow area; the exact locations and collection dates are given in table 2.2.

2.2.2 Isolation.

Leaves from a range of groundsel plants were cut into three segments, and the three segments placed into Petri-dishes containing a layer of 0.5% agar supplemented with 30 ppm benzimidazole. Conidial chains from the infected plants were picked off using a sharpened tungsten needle and placed on the plant segments. Petri-dishes were incubated at 18^{o}C, in a growth room fitted with warm white fluorescent tubes, giving a P.A.R light intensity of 75 µmol photons m^{-2} s^{-1}, during a ten hour photoperiod.

As conidia were formed on susceptible groundsel leaf segments, single chains were carefully picked off and transferred to leaf segments in a new Petri-dish. This procedure was repeated two more times, resulting in the isolation of genetically uniform mildew colonies. Twelve single chain isolates were obtained from 14 mildew infected plants. Once established, the 14 isolates were grown on a range of groundsel lines, (2e, 1i, 15c, 15j, 3g), to prevent adaptation to specific lines.

Table 2.1 The origin of collected lines of groundsel.

Plant Line		Area of Collection
1	c,e,f,g,h,i,m,n,p,s	Glasgow
2	a,d,e,i	Crail
3	a,f,g	Ayr
4	a,h	Dublin
5	a,c	Far Sawry
6	b,d,f	Ulverston
7	a,b,c,d,f	Coniston
8	a,d,g	Wellesbourne
9	a,c,d,g	Perth
10	c,j,s	Stranrar
11	a,e,g,i	Abington
14	h	Dumfries
15	c,i,j	Aberdeen
16	d,f	Pitlochrie
17	h	Kingussie
18	i	Inverness
19	b	Lairg
23	i,f,g	Crianlarich
24	j	Oban
25	a,b,c,d,e,f,g,h,i,j,k,l, m,n,o,p,q,r	Glasgow
26	a,b,c,d,e	Edinburgh
27	a,b,c,d,e,f,g,h,i,j,k	Cardiff
28	a,b,c,d,e,f,g,h,i,j	Swansea
29	a,b,c	Carstairs
30	a,b,c	Carstairs
31	a,b,c	Peebles
32	a,b,c	Peebles
33	a,b,c	Galashields
34	a,b,c	Galashields
35	a,b,c	Kelso
36	a,b,c	Kelso
37	a,b,c	Berwick-upon-Tweed
38	a,b,c	Berwick-upon-Tweed
39	a,b,c	Leith
40	a,b,c	Leith
41	a,b,c	Aberdeen
42	a,b,c	Aberdeen
43	a,b,c,d,e,f	Inverness
44	a,b,c,d,e,f	Inverness
45	a,b,c	Aviemore
46	a,b,c	Aviemore

Plant lines 1 to 24 were collected by I.Harry.

Table 2.2 The origin of the mildew isolates.

Date	Isolate Number	Area Collected (within Glasgow)
24:10:86	M1	Yorkhill Hospital
	M2	Kelvindale Road
	M3	Petershill Drive
	M4	Annisland Station
	M5	Broomhill School
	M11	Cowal Street
10:11:86	M6	Craigton Terrace
13:05:88	M13	Garscube Estate
	M14	Great Western Road
	M15	Duke Street
	M16	Wilton Street
07:06:88	M17	Kelvin Bridge

Plate 2.1 Groundsel plants growing in a growth room.

2.2.3 Maintenance of isolates and production of inoculum.

Mildew isolates were cultured on detached leaves maintained in Petri-dishes, and on whole plants grown in an Isolation Plant Propagator. In the propagator plant pots were covered by a clear plastic top and watered from below by a wick system. Filtered air was blown through the pots, escaping through two small holes in the pot lids, to maintain a positive pressure inside the cover. The pots were illuminated from above in a 13 hour photoperiod provided by warm white fluorescent tubes, giving a P.A.R of 85 μmol photons m^{-2} s^{-1}. Individual plants were therefore isolated from each other and could be used to culture different isolates of the fungus side by side. This system was used to bulk up inoculum for disease resistance testing. Petri-dish cultures were used as a back up, in case of mechanical failure of the propagator.

2.3 Infection tests.

2.3.1 Growing Conditions.

Testing was carried out on detached leaf segments maintained in Petri-dishes containing 0.5% agar, and as described earlier. The infection type observed on the detached leaves was similar to that from whole plants, provided the leaves were taken from plants of the same age (Bevan 1986). Plants were, where possible, tested at six weeks.

2.3.2 Inoculation.

Several methods of transferring conidia from the leaves of infected propagator plants to detached leaves, were tried. These included: using a paintbrush to brush conidia onto the leaf surface; gently rubbing the surface of the detached leaf with the infected one; and transferring conidia using a sharpened tungsten wire needle. The third method was judged to be the most efficient and economical method, and was used in all subsequent tests.

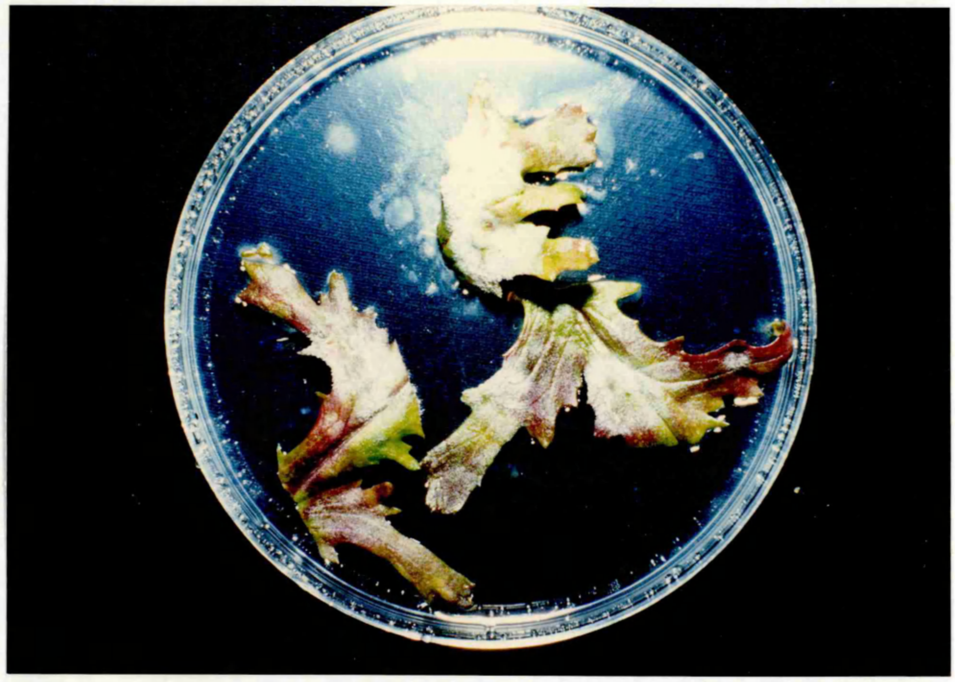
2.3.3 Scoring.

Petri-dishes containing the inoculated leaves were incubated for 14 days in the same growth room as the stock cultures, and under the same conditions. After the 14 days the leaves were scored from a visual assessment of the amount of mycelial and conidial development, and split into six infection types, using a scale adapted from that used by Harry (1980). The details of the infection scores are contained in table 2.3. Each of the three leaf segments in a Petri-dish was scored, and the average recorded. For the purpose of experiments where the inheritance of resistance to mildew was investigated, lines with mean infection scores of 1.0 or less were considered resistant to an isolate, while those with a mean infection score greater than 1.0, were susceptible.

Table 2.3 Description of infection Scores

Infection Type	Description	Infection Score
1	no conidial germination	0
2	slight mycelial development, but no conidia produced	1
3	large development of mycelium and sparse conidial production.	2
4	abundant mycelium and moderate conidial production	3
5	large colony production, abundant conidia	4
6	extensive colony covering all or most of leaf surface	5

Plate 2.2 Petri-dish culture of a leaf from line 1i inoculated with isolate M5, showing infection type 5.



2.4 Production of crossed plants

2.4.1 S.vulgaris x S.vulgaris crosses.

In general, intraspecific crosses were made between rayed and non-rayed plant lines. The resulting F1 plants possessed short stubby ray florets, due to incomplete dominance of the ray floret gene. Plate 2.3 shows the parents and hybrid of such a cross. The crossed plants could therefore be differentiated from those produced by selfing. The resulting hybrid plants were fully self-compatible and set a large amount of seed which could be used to grow up F2 populations.

Because of the very small size of groundsel florets, and their self-compatibility, (with the exception of the gynomonecious ray florets), preventing self pollination and effecting cross pollination was difficult. Several methods were tried, but the preferred method was as described by Watts (1980), for the cross pollination of composite species, and is as follows:

1. Two parent plants were grown up so that the pollen donor, usually a radiate plant, was two weeks older than the selected female, non-radiate plant.
2. An immature flower bud on the recipient plant was tagged with thread, and the nearby flower buds removed.
3. As the bud opened, and the stigmatic surfaces were pushed above the level of the corolla tube, it was sprayed with distilled water, using a syringe fitted with a narrow gauge

needle, to remove any adhering self pollen. The flower head was then blot dried and the process repeated after 30 minutes.

4. The flower head was then dusted with pollen from the donor plant, and left uncovered to set seed.

The procedure was carried out on five flower buds on each female parent. The seed from the crossed heads was immediately grown up, and any plants producing inflorescences without stubby ray florets were discarded.

2.4.2 Crosses between S.vulgaris and S.squalidus.

The crossing procedure was similar to that described above, except, because incompatibility between the two species allowed very little seed set per cross made, a greater number of flower buds were cross pollinated.

2.4.3 Production of F2 populations.

Each F1 plant was left to self naturally, and the seed collected. F2 populations were produced by sowing the seed on S.A.I. compost in 20cm by 10cm seed trays. When 14 days old, the seedlings were pricked out individually into 11cm plastic pots and placed in the growth room, under similar conditions to those of the parents. At six weeks old the plants had produced sufficient leaf tissue to be used to determine the infection types.

The size of the F2 population required for testing was determined using the formula of Mainland (1951). Assuming a

3:1 segregation of resistant to susceptible plants in the F2, a dihybrid cross in which the least frequent genotype occurs at a frequency of 1/16, would require an F2 population of 107 plants, to have a 99% chance of recovering this genotype. Populations of 107 plants were therefore used in the first tests. In the second tests the number was doubled to 214 plants, to try to differentiate more accurately between F2 ratios other than 3:1 for a single isolate and 9:3:3:1 for pairs of isolates.

2.4.3 The cytology of the interspecific hybrids

Root tip squashes were stained with lactoprop ionic orcein; essentially by the method of Ingram (1977). Young root tips were excised and pre-treated in 0.05% colchicine at 4°C for four to six hours, then fixed for five minutes in ethanol:chloroform:glacial acetic acid:formalin, 10:2:2:1, and macerated for five minutes in 1M HCl at 60°C. Roots were placed on glass slides, squashed with a brass rod and the stain applied. The slides were examined under oil immersion, and the number of chromosomes counted from mitotic metaphase plates .

2.5 Electrophoresis of soluble protein extracts.

2.5.1 Disk Electrophoresis.

Non denaturing disk polyacrylamide electrophoresis was carried out essentially by the method of Davis and Ornstein (1959). A 2.5% acrylamide large pore gel was prepared with tris-HCL buffer, pH 8.9, the running gel was cast with 7.5% acrylamide buffered in 0.05M tris, pH 6.9. Bromophenol blue was added at the top of each tube as the tracker dye, and tris-glycine, 001M pH 8.3 was used as the tank buffer.

Protein extracts were obtained from plants by grinding 20g of leaf tissue with 4g of washed sand, in a solution of 0.5% mercaptoethanol in 0.01M tris buffer, pH 9.5.

The extracts were centrifuged at 5000g for 20 minutes at 4°C. Ten microliters of the resulting supernatent was then applied to the top of each tube. A constant current of 2mA per tube was applied for 15 minutes, then increased to 5mA per tube for one hour, or until the dye front migrated to within 5mm of the end of the tube.

The gels were then removed from the tubes and stained for either peroxidase or esterase 1 activity.

Peroxidase bands were stained by the method described by Wheeler _et al._ (1979). Gels were immersed in a solution of 5mg o-dianisidine-HCL dissolved in 0.1% hydrogen peroxide in 0.05M acetate buffer pH 5.5.

Esterase 1 bands were visualised by immersing the gels in a solution containing 60mg a naphthyl acetate, 200mg fast blue RR salt, and 2ml ethanol, dissolved in 100ml of 0.05M sodium phosphate buffer, pH 7.0.

The positions of bands, visible on gels as dark gray, or dark brown lines respectively, were carefully measured with respect to the origin, and drawn to scale.

2.5.2 Vertical slab electrophoresis.

Non-denaturing slab gels were prepared using a 3% acrylamide spacer gel buffered in 0.05M tris-HCL pH 6.8, and a 12.5% acrylamide running gel buffered in tris-glycine 0.01M pH 8.3. Tris-glycine 0.01M pH 8.3 was used as the tank buffer. Leaf extracts were prepared as for disk electrophoresis, and 25µl pipetted into each well of the gel. An electric current of 35mA was applied for four to six hours, or until the dye front migrated to within 5mm of the end of the plate. Gels were removed and stained for peroxidase and esterase 1 activity as described above.

Denaturing SDS gels were prepared using the same acrylamide concentrations and buffer system. Leaf extracts were prepared as for disk electrophoresis, except that after centrifugation they were boiled for three minutes in tris buffer containing; 2% SDS, 10% glycerol, 0.001% mercaptoethanol and bromophenol blue. Once boiled, 20µl was pipetted into wells in the spacer gel and and the current applied. Gels were run at 30 mA for six to seven hours, or until the dye front approached the end of the plate.

Gels were stained for protein by immersion for one hour at 45°C in 0.1% Coomassie Brilliant Blue in methanol:acetic acid:water, 5:1:4, and destained at the same temperature in several changes of methanol/acetic acid/water, 1:1:4. Gels were photographed and stored in 8% acetic acid.

Plate 2.3 The patental and hybrid plants from an interspecific cross of S.vulgaris, plants and capitula.

Radiate parent Hybrid Non-radiate parent

CHAPTER 3. CHARACTERISATION OF THE MILDEW ISOLATES, AND THEIR COMPARISON TO PREVIOUS COLLECTIONS.

3.1 Characterisation of the mildew isolates.

3.1.1. Introduction

The mildew population of Glasgow is known to be comprised of a large number of phenotypes, all with differing combinations of virulence factors, and hence differing ability to infect groundsel lines (Bevan 1986). The total number of phenotypes is unknown, but may be several thousand, each perpetuated by asexual reproduction. With such diversity within the natural population it is likely to be rare for any two single chain isolates, derived from plants collected in Glasgow, to possess an identical set of virulence factors. However, each isolate was tested against a large number of groundsel lines, covering a large range of resistance phenotypes, to ensure that each isolate was different.

The six isolates collected in October 1986 were tested on 38 lines, and the results of these tests are given in tables 3.1 and 3.2. Figure 3.1 shows the class distributions of infection score for the six isolates.

The second set of isolates collected in 1988 were tested on a larger set of 47 lines, and these results are given in tables 3.3, 3.4 and 3.5. Figure 3.2 shows the class distribution of infection scores for these nine isolates. This test also included four of the six isolates collected in 1986, the other two isolates, M1 and M6 having been lost, due to an overheated growth room.

Differences between isolates collected in 1986 and 1988 were indicated by making pairwise comparisons of the isolates in each collection, and summing the number of times both are virulent or both are avirulent on each of the tester lines. Thus, using one line, a comparison of two isolates achieving a similar infection score gives a value of 0, whilst one of two isolates achieving very different infection scores gives a value of 1. This was repeated for all the lines used in the test, for that pair of isolates. The smaller the total value, the more similar the two isolates are, with respect to that set of test lines.

3.1.2 Results.

Table 3.1 Mean infection types produced on 38 groundsel lines by the 6 isolates collected in 1986.

Plant Line	Isolate M1	M2	M3	M4	M5	M11
1i	5.0	5.0	4.6	5.0	4.6	5.0
1n	0.3	4.0	3.3	4.0	3.6	3.3
2i	4.6	0.0	0.0	1.0	4.3	0.0
2d	3.3	3.3	2.6	3.6	4.3	4.7
2e	4.3	5.0	4.3	4.0	3.6	4.3
4a	0.0	1.0	0.3	4.3	2.0	0.3
5c	0.3	0.6	0.0	0.3	0.0	0.3
6b	5.0	0.6	0.0	0.3	0.0	4.3
7c	3.3	3.6	3.6	3.3	4.3	0.7
8d	4.6	4.0	3.0	2.0	3.0	0.3
9a	0.3	0.0	0.0	1.0	0.3	0.0
9d	4.6	0.3	0.6	2.6	0.0	3.3
10c	3.3	3.6	3.3	4.6	3.0	4.7
10s	4.3	4.6	0.3	0.6	3.0	4.3
10j	0.3	0.0	0.0	1.0	0.0	0.0
11i	1.0	0.6	0.0	0.0	0.0	0.3
11g	0.0	0.0	0.0	0.0	0.0	0.0
11e	4.3	3.6	3.6	4.3	4.0	3.7
14h	0.6	0.3	0.3	4.0	0.6	4.3
15c	3.3	3.3	3.6	4.0	2.6	0.3
16f	0.0	0.3	0.0	0.0	1.0	0.7
19b	4.3	0.6	4.0	4.3	3.3	0.0
23i	0.0	3.6	4.6	2.6	3.6	3.0
23g	5.0	5.0	5.0	4.6	1.0	3.3
25a	3.6	4.3	0.0	0.0	4.3	0.3
25g	4.3	0.3	0.3	0.0	0.0	0.0
25m	3.3	0.3	1.0	3.0	4.0	4.0
25u	4.3	0.3	1.0	0.0	0.0	0.0
27b	2.6	3.6	0.3	0.6	5.0	3.6
27f	2.6	4.3	3.0	0.0	0.3	0.0
27g	0.0	0.0	0.0	0.0	0.0	0.0
27i	4.0	1.0	0.0	0.0	0.0	2.7
27j	3.3	3.3	0.3	4.0	5.0	1.0
28a	4.6	3.6	4.0	3.6	3.6	4.3
28c	1.0	0.3	0.0	0.0	0.0	3.7
28f	4.3	3.0	0.3	0.3	1.0	4.3
28h	3.6	0.3	0.0	1.0	1.0	3.0
28j	3.0	3.0	0.3	3.3	1.0	3.0

Figure 3.1 Class distribution of the infection scores of the 1986 isolate collection

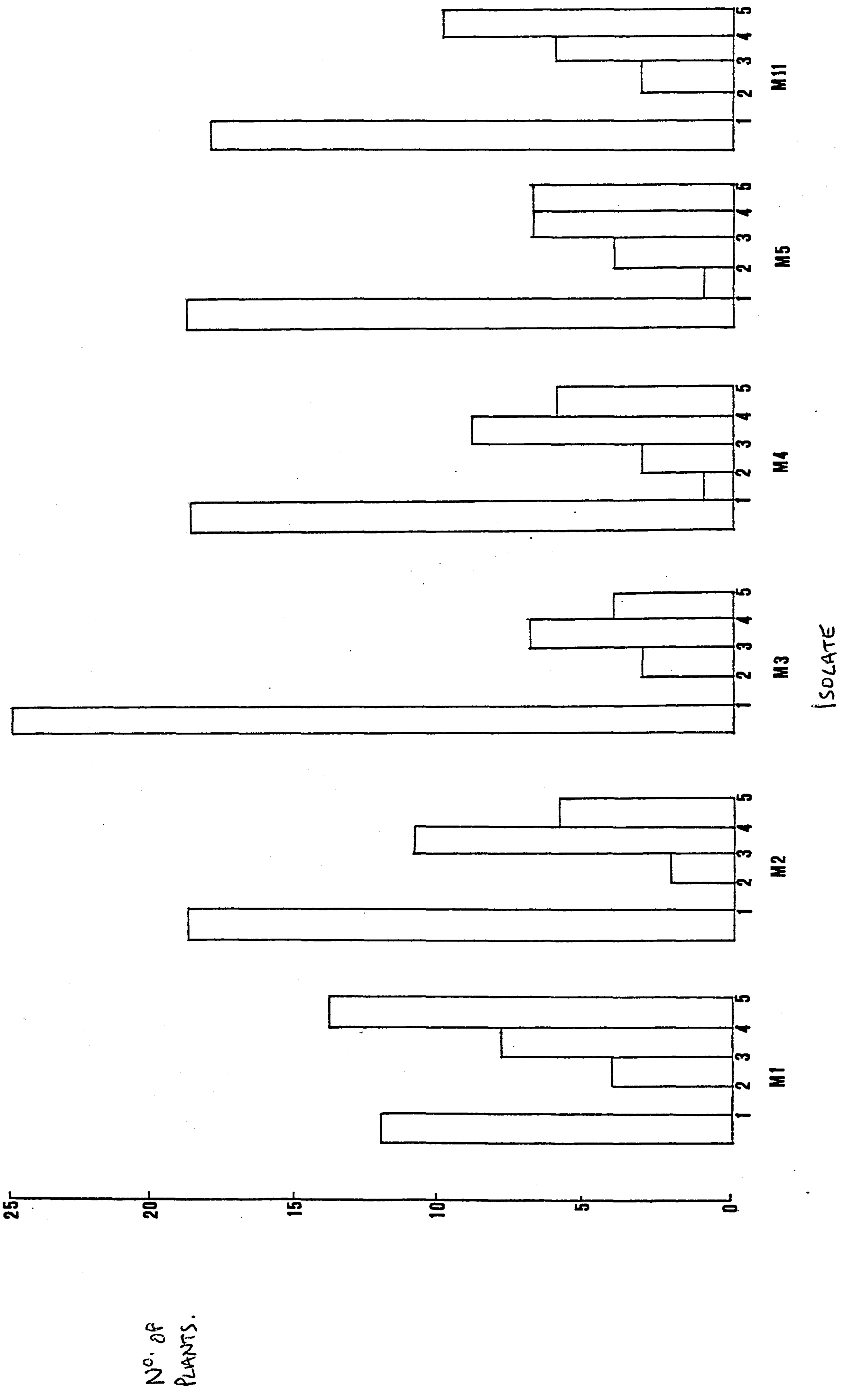

No. of Plants.
0
5
10
15
20
25
M1
M2
M3
M4
M5
M11
1
2
3
4
5
ISOLATE

Table 3.2 Differences between the 1986 isolates.

Combination of isolates	Number of differences	Combination of isolates	Number of differences
M1M2	10	M2M11	13
M1M3	15	M3M4	6
M1M4	14	M3M5	7
M1M5	13	M3M11	15
M1M11	14	M4M5	7
M2M3	7	M4M11	13
M2M4	8	M5M11	16
M2M5	7		

Table 3.3 Mean infection types produced on 47 groundsel lines by the isolates used in 1988.

Plant					Isolate				
Line	M2	M3	M4	M5	M13	M14	M15	M16	M17
1c	3.6	3.3	0.7	3.3	0.0	3.7	0.0	2.7	0.3
1e	3.7	3.3	3.7	0.3	3.3	3.7	3.0	3.0	3.7
1f	4.7	0.0	3.3	2.3	4.0	5.0	4.0	5.0	3.0
1g	3.7	3.3	3.3	5.0	5.0	4.0	4.3	3.0	0.0
1h	3.7	4.0	3.0	5.0	3.7	5.0	3.3	3.3	3.0
1i	5.0	4.7	5.0	5.0	4.0	4.7	5.0	4.3	3.7
1m	0.0	0.0	0.0	3.7	5.0	3.0	0.7	3.0	0.7
1n	4.0	3.7	3.7	3.0	0.3	4.0	4.3	3.3	3.3
1p	4.0	4.3	3.3	5.0	4.3	3.3	2.7	4.0	2.7
1s	3.0	3.3	3.0	1.0	3.7	0.7	0.0	2.7	0.0
2a	5.0	4.0	5.0	3.3	3.3	3.7	3.3	2.7	4.3
2d	3.7	3.3	4.7	5.0	3.3	5.0	3.3	4.3	3.7
2e	3.7	3.0	3.3	3.0	5.0	3.7	2.7	3.7	3.3
2i	0.0	0.7	5.0	4.0	3.3	5.0	3.7	3.0	3.7
3f	0.0	2.7	0.0	0.0	0.0	0.3	0.3	0.0	0.0
3g	5.0	4.3	3.3	3.7	4.7	3.0	3.7	3.3	3.7
4a	0.0	0.3	3.3	2.3	3.0	2.3	0.0	2.3	0.0
4h	0.3	0.3	0.0	0.7	0.0	0.0	0.0	3.7	0.0
5a	3.7	0.0	3.0	0.3	3.3	3.0	0.0	0.0	3.7
6b	0.0	0.7	0.0	0.3	0.0	0.0	0.0	0.0	0.0
6d	4.3	0.3	3.3	0.3	3.3	3.3	0.7	5.0	3.0
6f	1.0	0.0	0.0	0.0	4.0	0.0	2.3	2.7	3.7
7a	5.0	2.3	3.3	3.7	3.7	4.3	3.0	3.3	4.0
7b	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
7c	5.0	4.3	3.7	2.7	4.3	4.3	0.0	3.3	4.0
7d	0.0	0.0	0.3	0.0	0.0	0.0	0.7	0.0	3.3
7f	3.7	0.3	3.3	1.0	0.7	3.3	4.3	0.3	3.7
8a	5.0	4.3	2.7	4.0	4.7	5.0	4.3	3.3	4.0
8g	4.0	0.7	3.0	4.3	4.0	4.3	3.3	0.0	3.7
9a	0.0	0.0	0.0	0.0	0.0	0.3	0.7	0.0	0.0
9c	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	3.0
9d	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	3.7
9g	0.0	0.0	2.7	0.0	0.0	0.3	0.0	0.0	0.0
10j	0.0	0.3	0.3	0.0	2.7	0.7	0.0	0.0	0.0
11a	4.0	2.3	4.0	3.7	4.3	2.7	4.0	2.7	3.0
11e	3.3	5.0	3.7	4.7	3.0	4.3	3.3	4.7	3.7
11i	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
14h	0.0	0.7	2.7	0.3	0.0	3.3	0.3	3.3	0.0
15c	3.7	3.0	3.3	4.3	4.0	5.0	4.3	3.3	4.0
15j	5.0	3.7	3.3	5.0	4.7	4.0	5.0	4.7	5.0
16d	3.7	5.0	3.3	3.0	3.7	4.3	4.0	0.3	4.0
16f	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	3.3
17h	3.3	0.0	3.7	2.0	3.3	3.0	2.3	0.3	3.3
18i	4.3	4.3	5.0	4.3	3.7	3.7	4.7	3.7	3.7
19b	0.0	3.3	3.7	3.3	2.3	3.7	0.0	3.3	4.7
23f	0.0	5.0	3.3	4.0	0.7	4.3	4.3	3.0	3.0
23i	4.3	5.0	2.7	4.3	0.7	5.0	3.7	3.7	0.0

Table 3.4 Ordered table of the infection scores, increasing pathogen virulence, left to right, increasing plant resistance, top to bottom.

Plant					Isolate				
line	M3	M15	M2	M5	M13	M16	M17	M4	M14
1h	4.0	3.0	3.7	5.0	3.7	3.3	3.0	3.0	5.0
1i	4.7	5.0	5.0	5.0	4.0	4.3	3.7	5.0	4.7
1p	3.7	4.3	4.0	3.0	0.3	3.3	3.3	3.7	4.0
2a	5.0	3.3	5.0	3.3	3.3	2.7	4.3	5.0	3.7
2d	3.3	3.3	3.7	5.0	3.3	4.3	3.7	4.7	5.0
2e	3.0	2.7	3.7	3.0	5.0	3.7	3.3	3.3	3.7
3g	4.3	3.7	5.0	3.7	4.7	3.3	3.7	3.3	3.0
7a	2.3	3.0	5.0	3.7	3.7	3.3	4.0	3.3	4.3
8a	4.3	4.3	5.0	4.0	4.7	3.3	4.0	2.7	5.0
11a	2.3	4.0	4.0	3.7	4.3	2.7	3.0	4.0	2.7
11e	5.0	3.3	3.3	4.7	3.0	4.7	3.7	3.7	4.3
15c	3.0	4.3	3.7	4.3	4.0	3.3	4.0	3.3	5.0
15j	3.7	5.0	5.0	5.0	4.7	4.7	5.0	3.3	4.0
18i	4.3	4.7	4.3	4.3	3.7	3.7	3.7	5.0	3.7
1e	3.3	3.0	3.7	0.3	3.3	3.0	3.7	5.0	3.7
1f	0.0	4.7	4.0	2.3	4.0	5.0	3.0	3.3	5.0
1g	3.3	4.3	3.7	5.0	5.0	3.0	0.0	3.3	4.0
1n	3.7	4.3	4.0	3.0	0.3	3.3	3.3	3.7	4.0
7c	4.3	0.0	5.0	2.7	4.3	3.3	4.0	3.7	4.3
16d	5.0	4.0	3.7	3.0	3.7	0.3	4.0	3.3	4.3
2i	0.7	3.7	0.0	3.3	3.0	3.7	3.7	5.0	5.0
8g	0.7	3.3	4.0	4.3	4.0	0.0	3.7	3.0	4.3
17h	0.0	2.3	3.3	2.0	3.3	0.3	3.3	3.7	3.0
19b	3.3	0.0	0.0	3.3	2.3	3.3	4.7	3.7	3.7
23f	5.0	4.3	0.0	4.0	0.7	3.0	3.0	3.3	4.3
23i	5.0	3.7	4.3	4.3	0.7	3.7	0.0	2.7	5.0
6d	0.3	0.7	4.3	0.3	3.3	5.0	3.0	3.3	3.3
1c	0.3	0.7	4.3	0.3	3.3	5.0	3.0	3.3	3.3
1s	3.3	0.0	3.0	1.0	3.7	2.7	0.0	3.0	0.7
4a	0.3	0.0	0.0	2.3	3.0	2.3	0.0	3.3	2.3
5a	0.0	0.0	3.7	0.3	3.3	0.0	3.7	3.0	3.0
7f	0.3	4.3	3.7	1.0	3.3	0.0	3.7	3.0	3.0
1m	0.0	0.7	0.0	3.7	5.0	3.0	0.7	0.0	3.0
6f	0.0	2.3	1.0	0.0	4.0	2.7	3.7	0.0	0.0
14h	0.7	0.3	0.0	0.3	0.0	3.3	0.0	2.7	3.3
3f	2.7	0.3	0.0	0.3	0.0	3.3	0.0	2.7	3.3
4h	0.3	0.0	0.3	0.7	0.0	0.0	3.7	0.0	0.0
7d	0.0	0.7	0.0	0.0	0.0	0.0	3.3	0.3	0.0
9c	0.3	0.0	0.0	0.0	0.0	0.0	3.0	0.3	0.0
9d	0.3	0.3	0.0	0.0	0.0	0.0	3.7	0.0	0.0
9g	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.3
10j	0.3	0.0	0.0	0.0	2.7	0.0	0.0	0.3	0.7
16f	0.0	0.0	0.0	0.0	0.3	0.0	3.3	0.0	0.0
6b	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
7b	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
9a	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.3
11i	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0

Figure 3.2 Class distributions of the infection scores of the 1988 isolates.

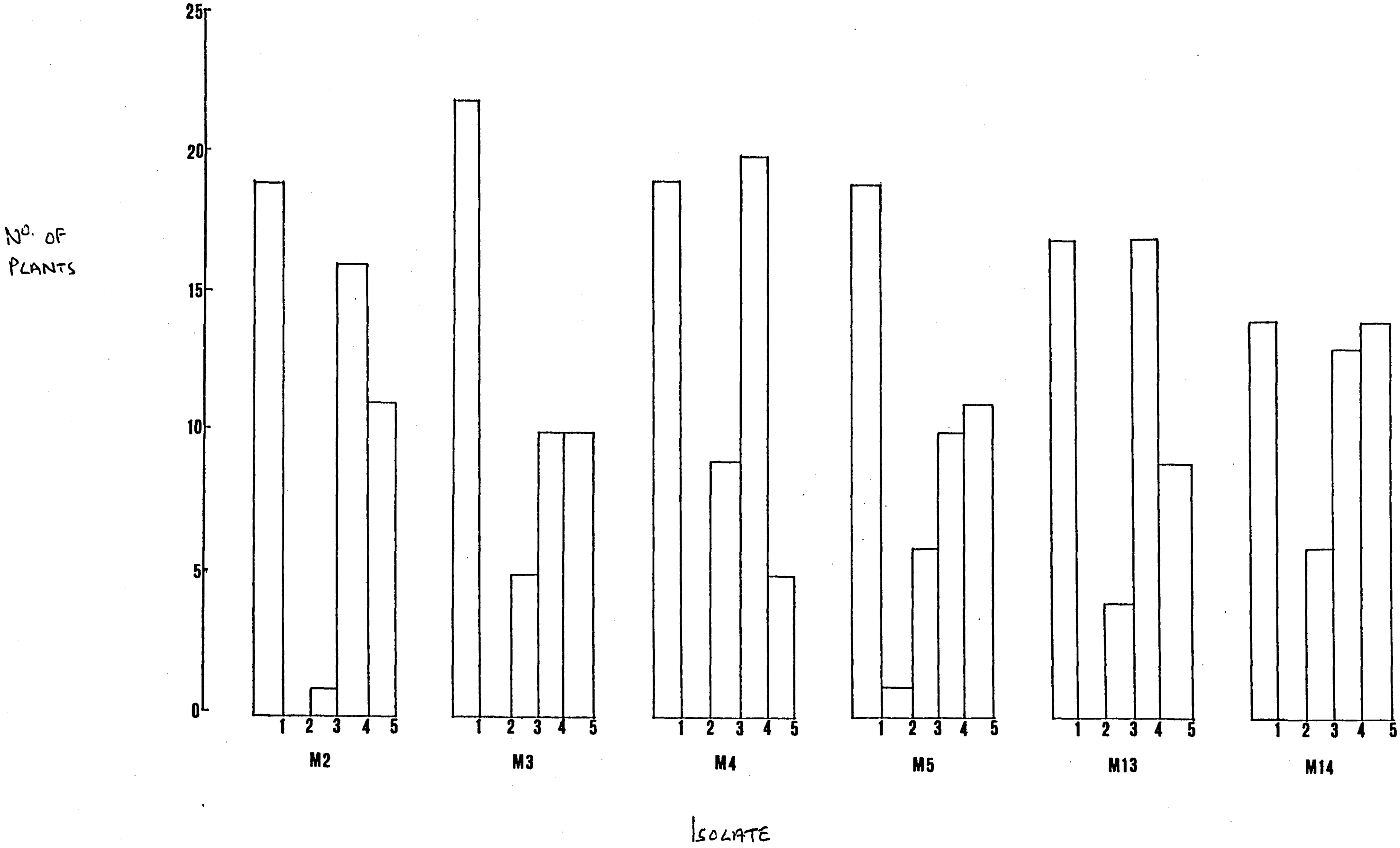

No. of Plants
25
20
15
10
5
0
1 2 3 4 5
M2
1 2 3 4 5
M3
1 2 3 4 5
M4
1 2 3 4 5
M5
1 2 3 4 5
M13
1 2 3 4 5
M14
Isolate

Figure 3.2 (cont.) Class distributions of the infection scores of the 1988 isolates.

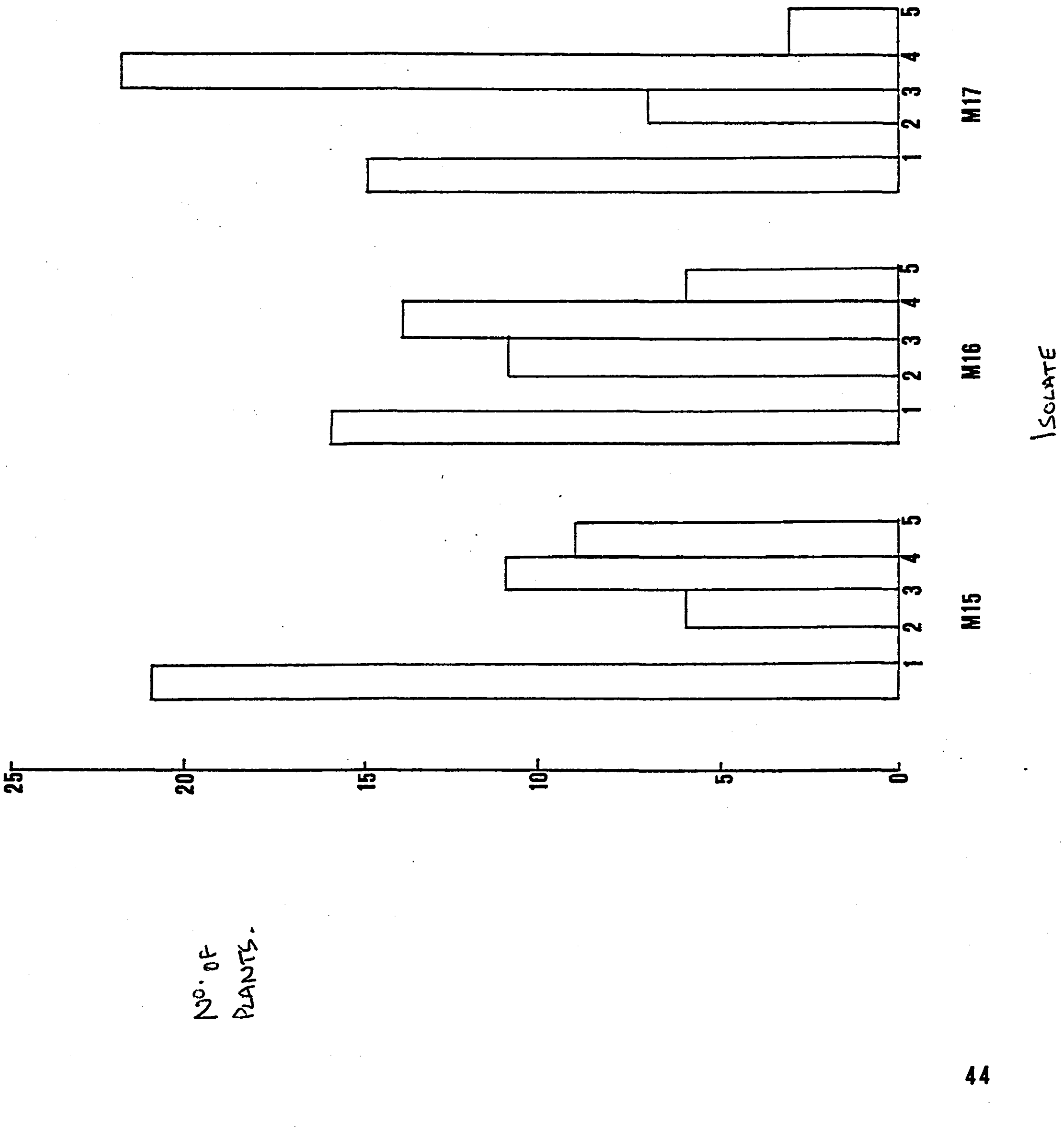

No. of plants.
0
5
10
15
20
25
1
2
3
4
5
M15
1
2
3
4
5
M16
1
2
3
4
5
M17
Isolate

Table 3.5 Differences between the 1988 isolates.

Combination of isolates	Number of differences	Combination of isolates	Number of differences
M2M3	9	M4M15	9
M2M4	7	M4M16	9
M2M5	10	M4M17	10
M2M13	10	M5M13	10
M2M14	7	M5M14	5
M2M15	8	M5M15	8
M2M16	12	M5M16	7
M2M17	13	M5M17	13
M3M4	12	M13M14	9
M3M5	9	M13M15	13
M3M13	15	M13M16	10
M3M14	11	M13M17	11
M3M15	11	M14M15	9
M3M16	9	M14M16	7
M3M17	18	M14M17	10
M4M5	9	M15M16	12
M4M13	8	M15M17	11
M4M14	4	M16M17	14

3.1.3 Discussion.

Examination of the infection scores obtained by the mildew isolates on the set of 38 groundsel lines, (tables 3.1 and 3.2), shows that the six isolates collected in 1986 are all different from each other. The most similar isolates, M3 and M4, still differ in the scores achieved on seven of the 38 lines. These differences were based on the ability to categorise interactions as producing mostly very high or very low infection scores, with few moderate infection scores in-between. The histogram of class distribution of infection score of each isolate does show a strongly bimodal distribution with the peaks in the 0.0-1.0 and 3.1-5.0 classes. For most isolates the moderate infection score class, 1.1-3.0, is not represented.

This gives confidence to the use of the calculation of differences between isolates in table 3.2, and in general when classifying the interaction between a line and an isolate in the genetics experiments.

The tests to categorise the nine isolates used in 1988, on the set of 47 groundsel lines, gave a similar result to the 1986 tests. All nine isolates were different from each other, most by their reactions on at least nine lines. The infection score class distributions again show a strongly bimodal shape, with most scores classified as very high or very low.

Isolates M2, M3, M4 and M5 were tested twice, and it is interesting to note how similar the histograms from the two tests are. This is in part to be expected, since 19 of the lines used in the first set of tests were also used in the second. Such similarity may also suggest that isolates may have a characteristic pattern of infection score distribution when tested against a large number of groundsel lines. On the present evidence this can however only be a suggestion.

In summary, the test results showed that, with respect to virulence factors, the six isolates collected in 1986, and the four collected in 1988, were all different from each other.

3.2 Comparison of the 1986-1988 mildew isolates to those collected by Harry and Bevan.

3.2.1 Introduction

The 47 line test set was originally collected by Harry (1980), and was used by both her and Bevan (1986), to characterise their collections of mildew isolates. Table 3.6 comprises the reactions of the present isolates, and those of the previous sets , on the 47 groundsel lines. All the mildew isolates were collected in the Glasgow area. Because the collections were made over a 10 year period, changes to the mildew population over time could be assessed.

3.2.3 Results

Table 3.6 A comparison of the mean infection score of all 26 isolates on 47 groundsel lines.

	1976-79					1982-85												1986-88								
Plant line.	H 1	H 2	H 3	H 4	H 5	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B 10	B 11	B 12	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9
1c	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−
1e	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
1f	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
1g	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
1h	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1i	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1m	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	+	+
1n	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
1p	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1s	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−
2a	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2e	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
2i	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
3f	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−	−
3g	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
4a	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	+	−
4h	−	−	−	−	−	−	−	−	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−	+	−
5a	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	−	+	+	−	−	+
6b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6d	−	−	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−	+	−	+	−	+	+	−	+	+
6f	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	−	+	+	+
7a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7d	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
7f	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	−	+
8a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8g	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+
9a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
9c	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
9d	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
9g	+	−	−	−	−	+	−	−	−	+	−	−	+	−	−	+	−	−	−	+	−	−	−	−	−	−
10j	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	−	−	−	−
11a	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11e	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11i	−	+	+	−	−	−	−	−	−	−	+	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−
14h	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−
15c	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
15j	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
16b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16f	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
17h	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+
18i	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19b	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+
23f	−	+	−	+	−	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+
23i	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−

"−" = infection score 0−1.0 "+" = infection score 1.1−5.0

Table 3.7 A comparison of the percentage of mildew isolates in each collection virulent, (infection score 1.1-5.0), on each of the 47 test lines.

Plant line	Isolate, collector, and number of isolates		
	Harry (5)	Bevan (12)	Campbell (9)
1c	80	92	56
1e	80	100	89
1f	100	100	89
1g	100	100	89
1h	100	100	100
1m	60	100	44
1n	80	100	89
1p	100	100	100
1s	80	100	56
2a	80	100	100
2d	100	100	100
2e	100	92	100
2i	40	0	78
3f	80	83	11
3g	100	92	100
4a	80	92	56
4h	40	42	11
5a	100	92	56
6b	0	0	0
6d	60	75	67
6f	80	92	44
7a	100	100	100
7b	0	0	0
7c	100	100	89
7d	40	25	11
7f	80	100	56
8a	100	100	100
8g	100	100	78
9a	0	0	0
9c	20	25	11
9d	20	17	11
9g	0	33	11
10j	0	100	89
11a	60	100	100
11e	80	100	100
11i	40	33	0
14h	60	0	33
15c	100	100	100
15j	100	100	100
16d	100	100	89
16f	40	0	11
17h	60	92	78
18i	80	100	100
19b	80	100	78
23f	80	100	78
23i	40	100	78

Table 3.8 A comparison of the percentage of the 47 lines that each of the isolates from the 3 collections is virulent on, (infection score 1.1-5.0).

Isolate	% lines virulent on
H1	72
H2	62
H3	83
H4	66
H5	77
B1	72
B2	66
B3	74
B4	77
B5	79
B6	83
B7	74
B8	81
B9	77
B10	77
B11	79
B12	70
C1	60
C2	56
C3	70
C4	60
C5	64
C6	70
C7	53
C8	63
C9	70

3.2.3 Discussion.

The comparison of all 26 mildew isolates in table 3.6 indicates that all the isolates have different virulence phenotypes. These 26 isolates probably represent only a small proportion of the isolates present in the natural population during the ten year period.

Over this period, seven plant lines remained susceptible to all isolates and three lines remained resistant to all isolates. It is surprising that these three lines remained resistant over such a long time, especially since plants from these lines were grown outside, during the mildew season, to deliberately try to trap a virulent mildew.

In general, the response of a particular groundsel line to each of the mildew collections was similar, whether it be very resistant or very susceptible. This characteristic was useful when selecting lines for use as parents in the crossing programme.

With such a large number of mildew isolates present in the natural population it would seem inevitable that the super-race would emerge, combining virulence factors for all groundsel genotypes, or at least for the 47 lines used in these tests. The results in table 3.8 suggest that this has not occurred, or that if it has, the isolate is very rare. The latter seems unlikely, because of the way the very resistant plants have frequently been exposed to the natural mildew population.

The most virulent of the isolates successfully attacked 83%

of the lines, or 39 out of 47. The mean value is considerably lower, in the range of 62-75%. Some isolates, such as C7, are virulent on only half of the lines, and as a result of this restricted host range, may suffer reduced fitness. The lack of virulence in some isolates and the absence of an isolate virulent on all lines may in part be due to the pathogens asexual mode of reproduction. It would be unable to accumulate virulence factors via recombination, relying instead on mutation alone to overcome new resistance factors in the host.

CHAPTER 4. GENETIC ANALYSIS OF RESISTANCE GENES.

PAGE NUMBER

4.1 Introduction

Prior to 1900 the natural laws governing the inheritance of characters, including resistance to disease in plants , were largely unknown. With the rediscovery of Gregor Mendels work in 1900, ways of experimentally investigating the inheritance of characters were established (Agrios 1989). Collectively this new field of research was named Mendelian genetics, and it was soon enthusiastically applied by plant pathologists. In essence, Mendelian genetics explained the inheritance of character traits determined by one or few genes, and predicted the segregation of these traits in the progeny of a breeding experiment. Mendel used garden peas, which posessed traits such as seed shape and seed coat colour, that appeared in two forms only. He made crosses between individuals differing in one or more of these traits, and looked at the segregation of the traits in the F1, F2 and F3 generations. From this he was able to deduce the number of genes that determined each trait, and the dominance relationships of the alleles involved (Babcock and Clausen 1927).

From this simple beginning, Mendel analysed crosses where two, three and four traits were segregating, and produced predicted segregation ratios, based on expansions of the 3:1 ratio for a monohybrid cross. Later workers added more complicated ratios to explain the inheritance of characters determined by more than one gene, and for the situations where epistasis was involved.

Armed with the methods and rules of Mendelian genetics, plant pathologists began to examine the inheritance of resistance to plant diseases, and the science of breeding for resistance was born.

One of the first plant pathologists involved was Biffen (1905, 1912), who investigated the wheat/yellow rust pathosystem. By analysing the resistance of plants in the F1,F2 and F3 generations of a cross, he found that the inheritance of resistance agreed with Mendelian laws, and that resistance was determined by a single recessive gene.
With this economically important discovery made, the search began for resistance genes in virtually all crop species, resulting in the publication of thousands of papers on the subject (Day 1974). These studies have been reviewed by Walker 1965, Hooker and Saxena 1971 and by Crute 1985.

One feature to emerge from these studies was that a single plant could possess many genes for resistance to one pathogen (Day 1974). Flor (1947) described 26 different genes for resistance to Melampsora lini assigned to five independant loci.
Using corn and its common rust, Puccinia sorghi, Saxena and Hooker (1968), showed that a single resistance locus possessed

at least 15 alleles. In addition, they showed that resistance was determined by genes at a total of three loci, and that these loci were linked.

Because the three loci were closely linked, when a cross was made between a resistant and susceptible plant the three loci did not segregate independantly in plants of the F2 generation. As a result, the proportion of plants with the parental combinations of resistance genes was greater than expected, and that of the recombinants, lower.
Using this simple test to establish linkage, a great number of characters have been shown to be determined by genes that are more or less tightly linked. Indeed this is to be expected, since every organism has only a small number of unique chromosomes and a very large number of genes. The total number of linkage groups must equal the haploid number of chromosomes.

Linkage of resistance genes in crop plants is now known to be a common occurrence, eg. Hooker and Saxena, (1971) and Mayo and Shepard (1980). Michelmore _et_ _al_. (1987), investigating the genetics of the _Lactuca_ _sativa_/_Bremia_ _lactucae_ pathosystem, found that resistance genes were present in four linkage groups. The largest of these, group one , contained four resistance genes to downy mildew, and a further one gene conditioning resistance to root aphid. Resistance to turnip mosaic virus was also shown to map into these linkage groups.

Resistance to Erysiphe fischeri in Senecio vulgaris has been shown to be conditioned by linked resistance genes. Harry (1980), showed that the genes determining resistance to four mildew pathotypes in one line of groundsel, were closely linked.

Harry found that resistance to each pathotype was generally determined by a single dominant gene, but in a few cases she obtained ratios suggesting duplicate dominant genes. Additionally she obtained apparent susceptible F1s, indicating that resistance might be determined by recessive genes. The F2s gave ratios of seven resistant to nine susceptible plants. Using recombination values calculated from F2 data she constructed a linkage map of the four resistance genes in the cross giving clear linkage.

Unlike the situation in crop plants, where multiple allelism is common, this has never been detected in S.vulgaris.

To investigate further the genetic control of resistance in S.vulgaris, and the distribution of resistance genes within the genome, three breeding experiments were conducted. Each cross was performed twice, using different plants of the same lines. Thus, two separate sets of F2 data were obtained for each cross, and where appropriate, the two sets for each cross were combined. In each of the three pairs of crosses a different resistant parent was used, to build up information from as large a number of genotypes as possible.

4.2 Results

4.2.1 Cross 1.

This cross was made between two non-radiate lines 2e and 9a. Line 2e was susceptible to all 12 virulence phenotypes, and line 9a resistant to all of them. In this, and all subsequent crosses the female parent is referred to as the P1, and the male parent the P2. An F1 plant from the cross was allowed to self, and a population of 107 F2 plants was raised. The reactions of both parents and F1 and F2 progeny plants were tested with mildew isolates M2,M3,M5, and M11. The reciprocal cross was also made. A summary of the results is given in table 4.1, and figure 4.1, and the full results are given in appendix table 4.1.

The F1 plants from both crosses were resistant to all five mildew isolates, indicating that resistance was determined by nuclear and not cytoplasmic genes. Because of the similarity of the results of the F1 s, only one of the pair of reciprocal crosses was tested.

The segregation of resistance to each isolate in the F2 population agreed well with a 3:1 ratio, suggesting that resistance to each isolate was determined by a single dominant gene.

Although the resistance genes to each isolate showed some degree of assortment, the parental types were the commonest, indicating some degree of association between the genes. In order to investigate this association, the F2 segregations of the resistance genes were examined in pairwise combinations, for deviation from a 9:3:3:1 ratio, the ratio expected if all the genes segregated independently from each other.

Table 4.1 Cross 1. (2ex9a) Reaction of the parents and F1 to the first 4 isolates, and the segregation of resistance in the F2.

Isolate	Infection Score			No.of plants in F2				X^2	P
	P1	P2	F1	Obs.		Exp. (3:1)			
				R :	S	R :	S		
M2	3.7	0.6	0.8(0.0)	79	28	80.3	26.7	0.08	NS
M3	4.2	0.0	0.3(0.1)	81	26	80.3	26.7	0.03	NS
M5	3.3	0.6	0.6(0.5)	83	24	80.3	26.7	0.46	NS
M11	3.3	0.8	0.1(0.0)	84	23	80.3	26.7	0.42	NS

Figures in brackets are for the F1 plant produced in the reciprocal cross.

Table 4.2 Cross 1. Association between pairs of resistance genes.

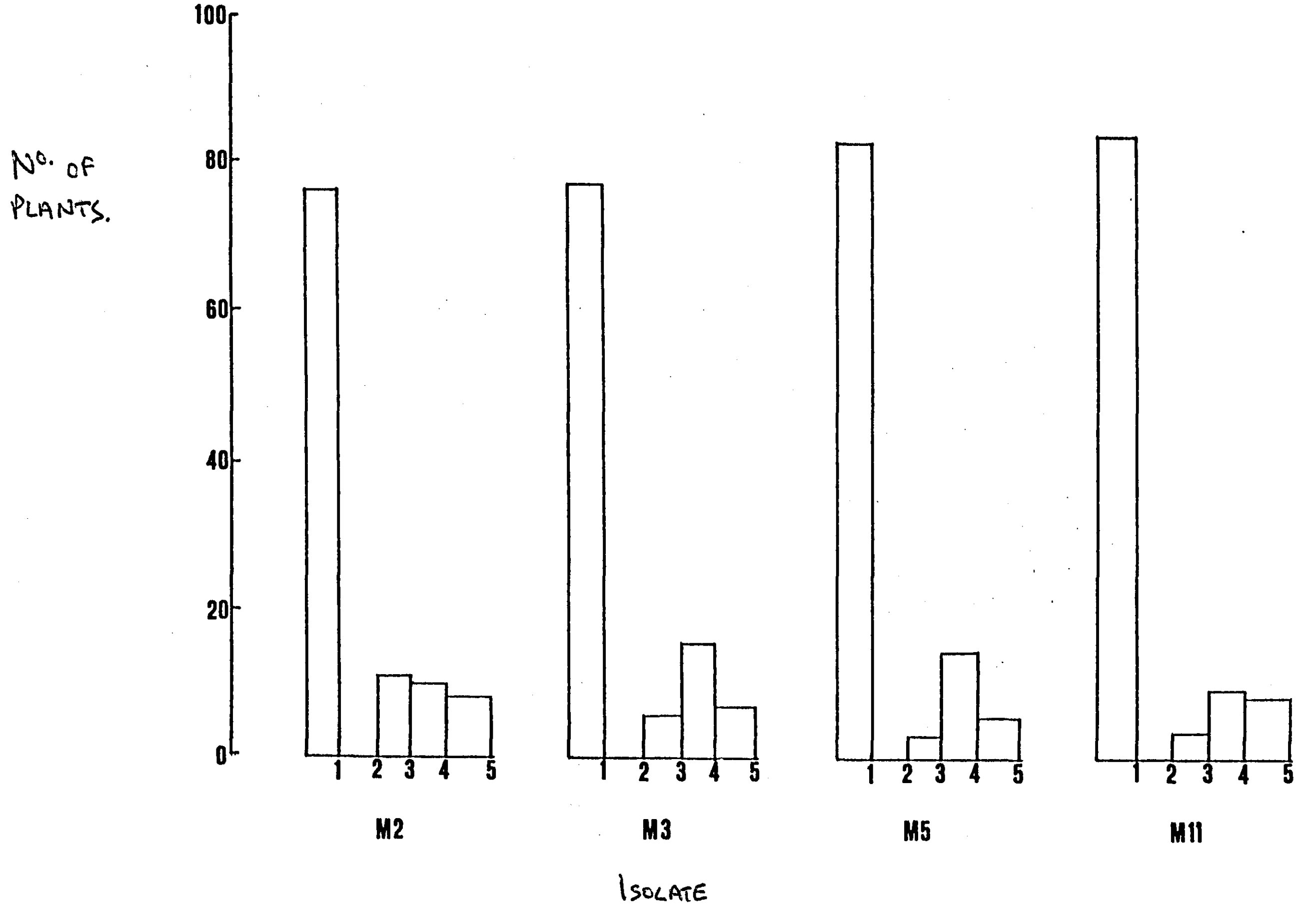
Resistance genes	No. of Plants Exp.	Obs.	X^2 (9:3:3:1) P	Recombination value
R2R3	60.2	70		
r2R3	20.1	8	52.3	14.5 ± 2.5%
R2r3	20.1	7	<0.001	
r2r3	6.6	22		
R2R5	60.2	68		
r2R5	20.1	8	43.9	17.5 ± 2.7%
R2r5	20.1	10	<0.001	
r2r5	6.6	21		
R2R11	60.2	72		
r2R11	20.1	5	68.1	11.5 ± 2.2%
R2r11	20.1	7	<0.001	
r2r11	6.7	23		
R3R5	60.2	65		
r3R5	20.1	11	26.1	25.0 ± 3.3%
R3r5	20.1	13	<0.001	
r3r5	6.7	18		
R3R11	60.2	67		
r3R11	20.1	10	27.4	23.0 ± 3.1%
R3r11	20.1	13	<0.001	
r3r11	6.7	17		
R5R11	60.2	80		
r5R11	20.1	2	66.2	4.8 ± 1.4%
R5r11	20.1	4	<0.001	
r5r11	6.7	21		

R = Dominant resistance gene conferring resistance to a particular isolate e.g. R2/M2

r = Recessive resistance gene conferring susceptibility to particular isolate e.g. r2/M2.

Recombination values calculated by Allard's method (1956).

Figure 4.1 Class distributions of the infection scores of the 4 isolates used in cross 1, first test.


No. of PLANTS.
100
80
60
40
20
0
1
2
3
4
5
M2
M3
M5
M11
ISOLATE

The large deviations from the expected ratio were due to a shortage of non-parental combinations of resistance genes, and a corresponding over representation of the parental combinations. This shows linkage between the genes determining resistance to each mildew isolate. Allard's (1956), method was used to calculate the linkage values in table 4.2. and the linkage map constructed from them is given below.

```
                      ¦------- 11.5--------------¦¦---4.7--¦
R3--------------------R2------------------------R11-------R5
¦-------14.5----------¦¦--------------17.5-----------------¦
¦-------------------------23.0-------------------¦
¦-----------------------------25.0-------------------------¦
```

4.2.1 Analysis of the second set of F2 plants from cross 1.

To verify the results of the first cross, a second set of F2 plants were raised from the seed produced by the original F1 hybrid. These plants were tested with seven isolates, three used in the first test, M2,M3 and M5 and additionally with isolates M4,M13,M14 and M16. Isolates M4,M13,M14 and M16, were not available to test the reactions of the original F1 plant, but they were tested on the parental lines, as shown in table 4.3. One of the isolates used in the first test, M11, was unfortunately lost and therefore could not be included. A total of 214 plants were tested with five isolates: M2,M4,M13,M14 and M16; and 154 tested with two isolates, M3 and M5. The detailed results are given in appendix table 4.2, with a summary in tables 4.3 and 4.4, and figure 4.2.

Table 4.3 Cross 1. (2ex9a), Reaction of the parents and F1 to 7 mildew isolates, and the segregation of resistance in the F2.

Isolate	Infection Score			No.of plants in F2				X^2	P
	P1	P2	F1	Obs.		Exp.(3:1)			
				R	S	R	S		
M2	3.7	0.6	0.8	165	49	161	53	0.5	>0.30
M3	4.2	0.0	0.3	122	32	116	39	1.5	>0.20
M4	3.3	0.0	+	173	41	161	53	3.6	>0.05
M5	3.8	0.6	0.6	125	29	116	39	3.1	>0.05
M13	5.0	0.0	+	159	55	161	53	0.1	>0.70
M14	3.7	0.3	+	163	51	161	53	0.2	>0.50
M16	2.7	0.0	+	174	40	161	53	4.2	<0.05

\+ The F1 was not tested against these isolates.

Table 4.4 Association between pairs of resistance genes.

Resistance genes	Obs.no. Plants	X^2	P	Recombination Value
R2R3	114			
r2R3	9	72.2	<0.001	10.2 ± 2.3%
R2r3	5			
r2r3	26			
total	154			
R2r4	158			
r2R4	28	50.3	<0.001	18.0 ± 3.5%
R2r4	8			
r2r4	26			
total	214			
R2R5	112			
r2R5	13	52.2	<0.001	14.0 ± 3.1%
R2r5	7			
r2r5	22			
total	154			
R2R13	156			
r2R13	9	102.1	<0.001	8.3 ± 2.3%
R2r13	10			
r2r13	39			
total	214			
R2R14	158			
r2R14	13	87.4	<0.001	10.1 ± 2.5%
R2r14	8			
r2r14	35			
total	214			
R2R15	163			
r2R15	16	67.5	<0.001	12.2 ± 2.8%
R2r15	8			
r2r15	27			
total	214			
R3R4	114			
r3R4	11	50.8	<0.001	14.0 ± 3.1%
R3r4	8			
r3r4	14			
total	154			
R3R5	111			
r3R5	13	26.6	<0.001	19.0 ± 3.6%
R3r5	11			
r3r5	19			
total	154			

Table 4.4 Association between pairs of resistance genes. (cont.)

Resistance genes	Obs.no. Plants	X^2	P	Recombination Value
R3R13	111			
r3R13	4	79.5	<0.001	10.6 ± 2.6%
R3r13	11			
r3r13	28			
total	154			
R3R14	113			
r3R14	9	57.0	<0.001	16.2 ± 3.3%
R3r14	9			
r3r14	23			
total	154			
R3R16	113			
r3R16	12	42.9	<0.001	13.0 ± 2.8%
R3r16	9			
r3r16	20			
total	154			
R4R5	112			
r4R5	13	45.0	<0.001	21.0 ± 3.8%
R4r5	12			
r4r5	17			
total	154			
R4R13	156			
r4R13	9	52.3	<0.001	16.0 ± 3.3%
R4r13	23			
r4r13	26			
total	214			
R4R14	156			
r4R14	9	52.3	<0.001	16.0 ± 3.3%
R4r14	23			
r4r14	26			
total	214			
R4R16	161			
r4R16	13	41.2	<0.001	17.0 ± 3.4%
R4r16	22			
r4r16	18			
total	214			
R5r13	109			
r5R13	8	46.3	<0.001	21.0 ± 3.8%
R5r13	15			
r5r13	22			
total	154			

Table 4.4 Association between pairs of resistance genes. (cont.)

Resistance genes	Obs.no. Plants	X^2	P	Recombination Value
R5R14	109			
r5R14	13	31.1	<0.001	19.0 ± 3.6%
R5r14	14			
r5r14	18			
total	154			
R5R16	114			
r5R16	11	36.6	<0.001	11.0 ± 2.7%
R5r16	12			
r5r16	17			
total	154			
R13R14	154			
r13R14	15	72.1	<0.001	16.5 ± 3.3%
R13r14	11			
r13r14	34			
total	214			
R13R16	155			
r13R16	23	44.5	<0.001	26.0 ± 4.2%
R13r16	10			
r13r16	26			
total	214			
R14R16	160			
r14R16	14	74.9	<0.001	17.0 ± 3.4%
R14r16	9			
r14r16	31			
total	214			

Figure 4.2 Class distributions of the infection scores of the 7 isolates used in cross 1, second test

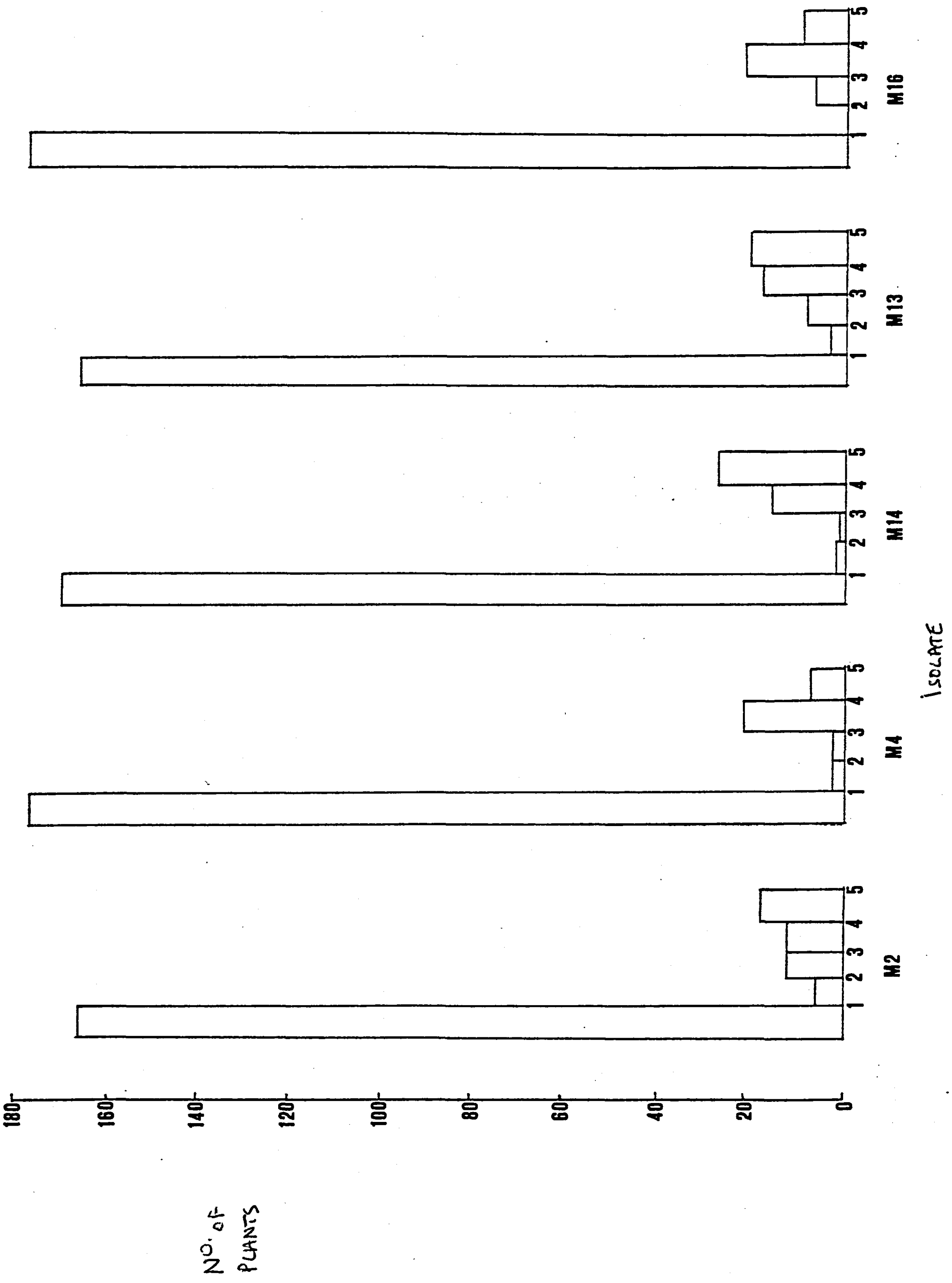
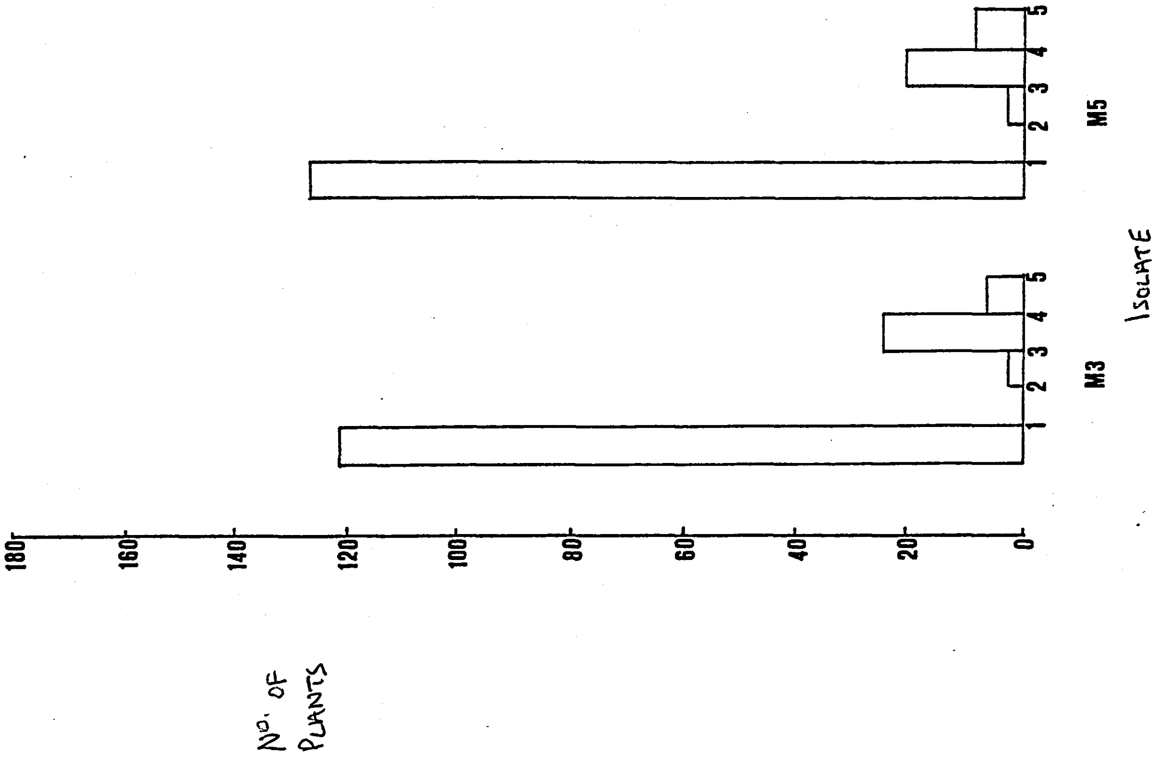

No. of Plants
180
160
140
120
100
80
60
40
20
0
M2
M4
M14
M13
M16
1
2
3
4
5
Isolate

Figure 4.2 (cont.). Class distributions of the infection scores of the 7 isolates used in cross 1, second test


No. OF PLANTS
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
M3
1
2
3
4
5
M5
ISOLATE

Because of the variability in the recombination values with the larger number of isolates used in this test, the values for the 21 possible combinations of resistance genes are more difficult to interpret. The diagram below shows a best-fit arrangement of resistance genes in a linkage map. It does not include all 21 map distances calculated, but does show the general pattern of resistance genes linked in 3 groups.

```
                              |----11.0----|
|--------16.2------------||--10.4--||-10.1-|
R16---------------------R3---R13--R2------R14--------R5--R4
                                   |-----------18.0--------|
|--------------------17.0-----------------|
|-----------------------19.0--------------------------|
|--------------------------------26.0---------------------|
```

The two sets of data for the reaction of plants to mildew isolates M2, M3 and M5 were examined for homogeneity as described by Parker (1979), and a X^2 test performed to assess if the results were sufficiently similar to be combined (table 4.5). Highly significant X^2 values showed that the data could be combined (table 4.5), and an overall linkage map was constructed as follows:-

```
|-----------------------------23.0 ± 3.0%-----------------|
R3-----------------------R2-------------------------------R5
|------14.0 ± 2.3%------||----------17.0 ± 2.5%-----------|
```

Table 4.5 Test of homogeneity of the F2 results from the two repeats of cross 1 and the combined F2 results.

	Isolate					
	M2		M3		M5	
	R :	S	R :	S	R :	S
First Test	79	28	81	26	83	24
Second Test	165	49	122	32	125	29
X^2	0.41		0.48		0.47	
P	>0.9		>0.9		>0.9	

Resistance genes	No. of plants Exp. (9:3:3:1)	Obs.	X^2	P	Recombination value
R2R3	146.8	185			
r2R3	48.9	16	120.8	<0.001	14 ± 2.3%
R2r3	48.9	12			
r2r3	16.4	48			
total	261.0	261			
R2R5	146.8	180			
r2R5	48.9	21	89.4	<0.001	17 ± 2.5%
R2r5	48.5	17			
r2r5	16.4	43			
total	261.0	261			
R3R5	146.8	176			
r3R5	48.9	24	57.0	<0.001	23 ± 3.0%
R3r5	48.0	24			
r3r5	16.4	37			
total	261.0	261			

4.2.2 Cross 2.

Cross 2 was made between radiate line 25p, and non-radiate line 1i, with the intention of examining the genetic control of resistance genes in a further resistant line. Line 25p is very resistant to mildew, and 1i very susceptible. However, in contrast to cross 1, the F1 hybrid was not resistant to the five mildew isolates used, M2,M3,M4,M5 and M11. The results are set out in tables 4.6 and 4.7, and in figures 4.3 and 4.4, with the detailed results in appendix tables 4.3 and 4.4.

Two separate F2 populations, each of 107 plants were raised and tested . The first population was tested with isolates M2,M3,M5 and M11, the second population with M2,M3 and M5, with M4 replacing M11.

The two sets of data were examined for homogeneity, as described previously, and a X^2 test was performed to assess whether the results were sufficiently similar to be combined (Table 4.8).

A highly non-significant value from the X^2 test, showed that the two sets of results could be combined and analysed together (Table 4.9).

The segregation ratio for susceptibility to isolate M2 very closely fits a 15:1 ratio, suggesting 2 dominant genes coding for susceptibility. Susceptibility to the remainder of the isolates segregates to give ratios which are close to 55 susceptible to 9 resistant, although for M4, the observed values fall just outwith the 0.05 significance level. The ratio of 55:9, susceptible : resistant, would result if two dominant genes are required to confer resistance to one mildew isolate, and their expression is suppressed by a dominant inhibitor gene. Figure 4.5 contains an explanation of the genetic model involved.

This model explains the segregation of resistance genes segregating independently. If two resistance genes are considered together, in pairwise combinations, as was done in the previous cross to calculate linkage, five genes would be segregating, two sets of two resistance genes and an inhibitor gene (Six if a separate inhibitor influences each pair of resistance genes). Clearly the genetics of this type of system become very complicated and the number of plants in the progeny required to analyse for association between genes very large.

Table 4.6. Cross 2. (25pX1i), Reaction of the parents and F1 to 4 isolates, and the segregation of resistance in the F2 progeny.

Isolate	Infection Score			Obs.ratio in F2 (R:S)		X^2 for exp.ratio in F2		
	P1	P2	F1			1:3	9:55 (R:S)	1:15
M2	0.7	3.7	4.7	4	103	25.82	9.38	1.16*
M3	0.6	4.0	3.7	14	93	4.26	0.07**	8.48
M5	0.4	4.5	4.7	14	93	4.26	0.07**	8.48
M11	0.5	4.1	4.0	12	95	6.40	0.69**	4.47

Table 4.7. Cross 2. (25pX1i), Reaction of the parents and F1 to 4 isolates, and the segregation of resistance in the F2 progeny.

Isolate	Infection Score			Obs.ratio in F2 (R:S)		X^2 for exp.ratio in F2		
	P1	P2	F1			1:3	9:55 (R:S)	1:15
M2	0.7	3.7	3.4	10	97	14.12	1.94**	1.73**
M3	0.6	4.0	4.2	17	90	4.87	0.31**	16.89
M4	0.0	5.0	3.8	8	99	17.60	3.79	0.27**
M5	0.4	4.5	4.5	13	94	9.54	0.31**	6.32

* $0.05 < p < 0.01$

** $0.10 < p < 0.50$

*** $0.50 < p < 0.90$

Table 4.8 Test of homogeneity of the results of the two repeats of cross 2.

	Isolate					
	M2		M3		M5	
	R	S	R	S	R	S
First Test	4	103	14	93	14	93
Second Test	10	97	17	90	13	94
X^2	1.37		0.08		0.02	
P	>0.1		>0.5		>0.5	

Table 4.9 Cross 2, combined F2 results.

Isolate	Obs.ratio in the F2 (R:S)	X^2 for exp. ratio (R:S) in F2		
		1:3	9:55	1:15
M2	14 : 200	39.65	9.93	0.01***
M3	31 : 183	13.14	0.04***	26.53
M5	27 : 187	16.80	0.35***	16.05

* 0.05 < p < 0.01

** 0.10 < p < 0.50

*** 0.50 < p < 0.90

Figure 4.3 Class distributions of the infection scores of the 4 isolates used in cross 2, first test.

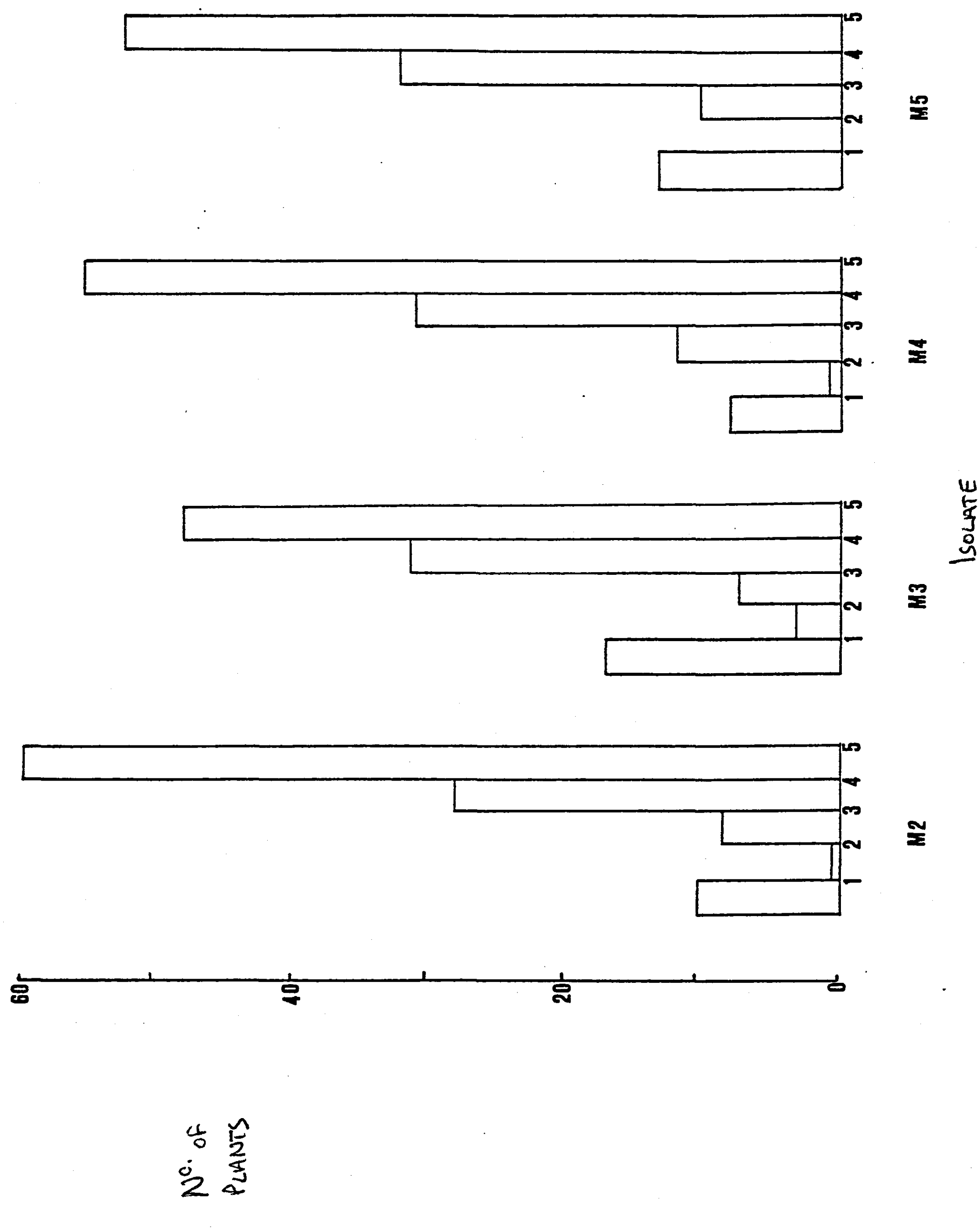

0
20
40
60
1
2
3
4
5
M2
M3
M4
M5
Isolate
No. of Plants

Figure 4.4 Class distributions of the infection scores of the 4 isolates used in cross 2, second test.

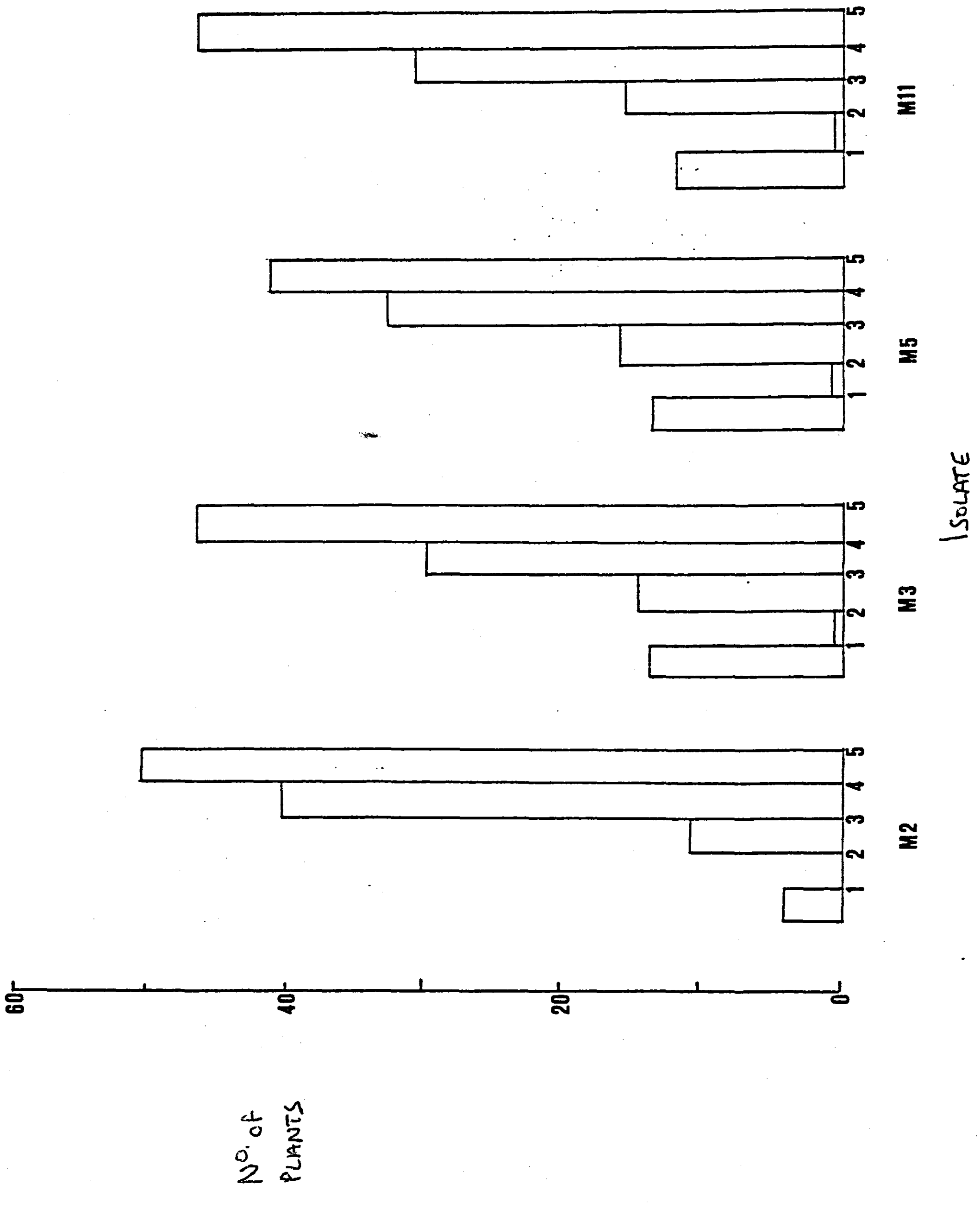

N°. of PLANTS
60
40
20
0
1
2
3
4
5
M2
M3
M5
M11
ISOLATE

Figure 4.5 Genetic model of the 55:9, susceptible to resistant ratio.

Two dominant resistance genes are both necessary to confer resistance to one mildew isolate

P1 $R_aR_aR_bR_bii$ * P2 $r_ar_ar_br_bII$

F1 $R_ar_aR_br_bIi$

F2

	RaRbI	RaRbi	raRbI	RarbI	Rarbi	raRbi	rarbI	rarbi
RaRbI	RaRbI	RaRbI	RaRbI	RaRbI	RaRbI	RaRbI	RaRbI	RaRbI
RaRbi	RaRbI	RaRbi	RaRbI	RaRbI	RaRbi	RaRbi	RaRbi	RaRbi
raRbI	RaRbI	RaRbI	raRbI	RaRbI	RaRbI	raRbI	raRbI	raRbI
RarbI	RaRbI	RaRbI	RaRbI	RarbI	RarbI	RaRbI	RarbI	RarbI
Rarbi	RaRbI	RaRbi	RaRbI	RarbI	Rarbi	RaRbi	RarbI	Rarbi
raRbi	RaRbI	RaRbi	raRbI	RaRbI	RaRbi	raRbi	raRbI	raRbi
rarbI	RaRbI	RaRbI	raRbI	RarbI	RarbI	raRbI	rarbI	rarbI
rarbi	RaRbI	RaRbi	raRbI	RarbI	Rarbi	raRbi	rarbI	rarbi

Ra & Rb = Dominant resistance genes, conferring resistance to isolate M3.

I = Dominant inhibitor gene.

Genotypes underlined are resistant to isolate M2, those not are susceptible.

55 susceptible : 9 resistant.

If five genes are segregating independently, then 6000 represents the smallest F2 population required to obtain one of the least frequent genotype, whereas the population size tested here was only 214. It is therefore not possible to draw any conclusions from an analysis of the segregation ratios from this test. However as a guide to the accuracy of the 55:9 ratio, for resistance to one mildew isolate, the F2 data can be analysed as pairwise combinations of resistance genes, with the 55:9 ratio expanded to give the expected values for the population size used, but with only one gene influencing resistance to both isolates. Only resistance to mildew isolates M3 and M5 was tested with the larger population size of 214, and found to segregate according to a 55:9 ratio, therefore only these results were analysed in this way. Figure 4.6 contains the results of this analysis. Because of the complicated genetics of the inheritance of resistance suggested by these results, the calculation of linkage between the loci involved is not possible.

Figure 4.6 Dihybrid segregation ratio with two 55:9 ratios.

If resistance to each mildew isolate segregates as 55 susceptible plants to 9 resistant plants, with a population size of 214, and considering resistance to each isolate, M3 and M5, determined by two genes, with one dominant inhibitor gene influencing resistance to both isolates, then the following F2 results are predicted :

	Exp. proportion	Exp. number
R3R5	77/1024	16.1
r3R5	65/1024	13.5
R3r5	65/1024	13.5
r3r5	817/1024	170.8
total	1024	214.0

R3 = resistance gene to mildew isolate M3

R5 = resistance gene to mildew isolate M5

Results from Cross 2.

	Exp.no.	Obs. no.	X^2	P
R3R5	16.1	7		
r3R5	13.5	20	11.3	<0.05
R3r5	13.5	20		
r3r5	170.5	167		
total	214.0	214		

4.2.3 Cross 3.

The third cross was made between the radiate line 28a, susceptible to all isolates, and the non-radiate line, 10j, resistant to all isolates. The results are detailed in table 4.10.

The F1 was resistant to all the isolates it was tested against (M1, M2, M5 and M11).

The F2 was again analysed on two occasions, the first time using isolates M1, M2, M5 and M11, and the second time using isolates M2, M3, M4, M5, M13, M14, M15 and M16. The results of the first test are summarised in table 4.10 and figure 4.7, with the detailed results in appendix table 4.5.

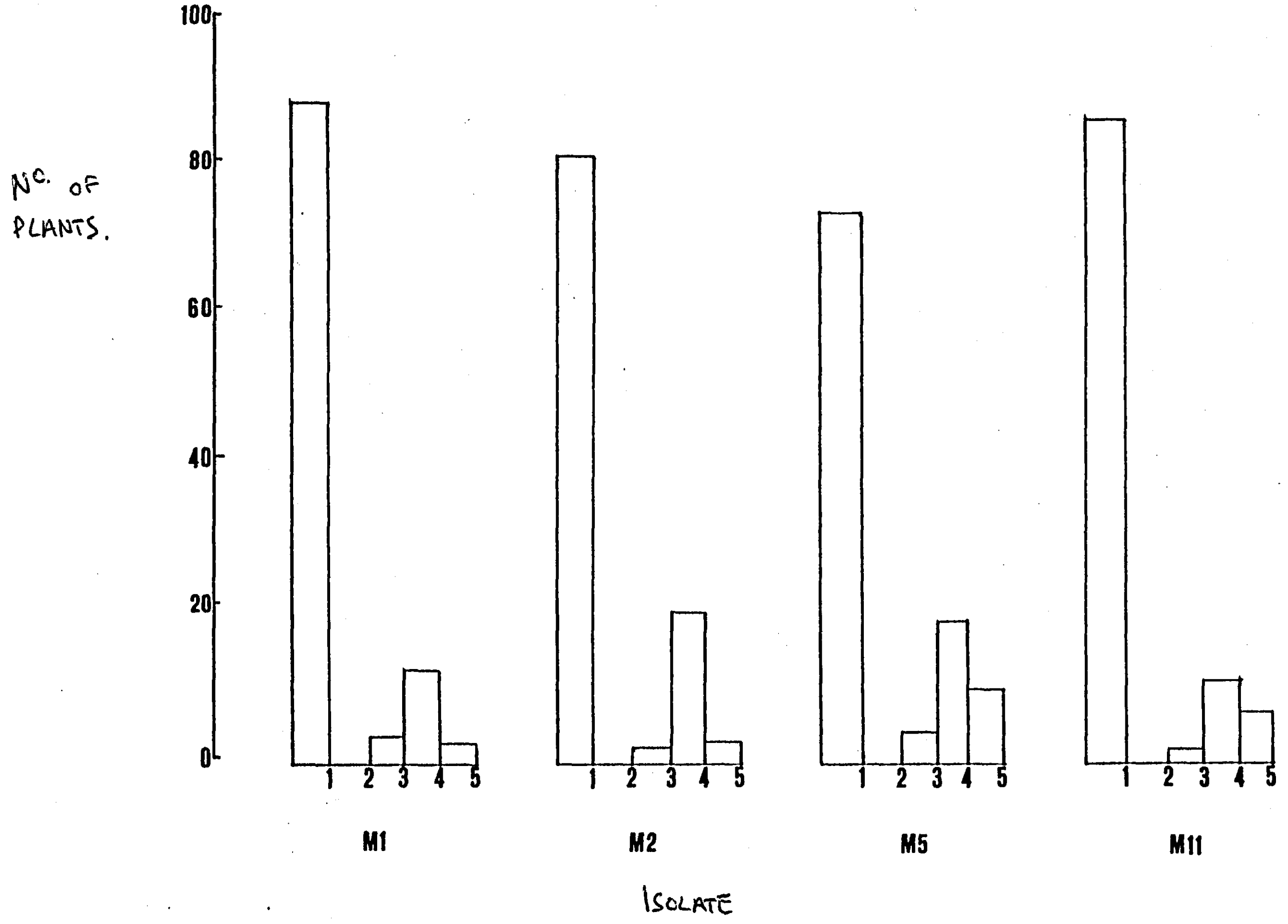
Table 4.10 Cross 3, (28aX10j), Reaction of the parents and the F1 to 4 isolates and the segregation of resistance in the F2.

Isolate	Infection Score			No.of plants in F2				X^2	P
	P1	P2	F1	Obs.		Exp. (3:1)			
				R	S	R	S		
M1	4.3	0.3	0.3	87	20	80.3	26.7	2.2	>0.1
M2	4.1	0.1	0.7	79	28	80.3	26.7	0.1	>0.5
M5	3.8	0.8	1.0	76	31	80.3	26.7	0.9	>0.1
M11	4.3	0.8	1.0	86	21	80.3	26.7	1.5	>0.1

Table 4.11 Cross 3, Association between pairs of resistance genes.

Resistance genes	Exp.no. Plants	Obs.no. Plants	X^2	P
R1R2	60.2	68		
r1R2	20.1	13	3.6	>0.1
R1r2	20.1	19		
r1r2	6.6	7		
total	107.0	107		
R1R5	60.2	63		
r1R5	20.1	13	3.5	>0.1
R1r5	20.1	24		
r1r5	6.6	7		
total	107.0	107		
R1R11	60.2	71		
r1R11	20.1	17	5.1	>0.1
R1r11	20.1	16		
r1r11	6.6	3		
total	107.0	107		
R2R5	60.2	56		
r2r5	20.1	19	1.5	>0.5
R2r5	20.1	24		
r2r5	6.6	8		
total	107.0	107		
R2R11	60.2	66		
r2R11	20.1	21	2.4	>0.1
R2r11	20.1	16		
r2r11	6.6	4		
total	107.0	107		
R5R11	60.2	59		
r5R11	20.1	27	3.8	>0.1
R5r11	20.1	17		
r5r11	6.6	4		
total	107.0	107		

Figure 4.7 Class distributions of the infection scores of the 4 isolates used in cross 3, first test.


N^c. of PLANTS.
100
80
60
40
20
0
1 2 3 4 5
M1
1 2 3 4 5
M2
1 2 3 4 5
M5
1 2 3 4 5
M11
ISOLATE

When the association of resistance genes was analysed (table 4.11), close fits to a 9:3:3:1 ratio were found in all cases, indicating that none of the genes determining resistance to each of the mildew isolates was closely linked.

Given the results from cross 1, where linkage was indicated, this was a surprise. The test was therefore repeated, with an F2 population of 214 plants and eight test isolates. These results are summarised in table 4.12 and figure 4.9, with the full results in appendix table 4.6.

From these results, resistance to mildew isolates M2, M4, M5 and M15 segregate to give ratios of 9 resistant to 7 susceptible. Resistance to isolates M14 and M16 segregates to give ratios of 9 susceptible to 7 resistant. Resistance to isolate M3 segregates to give a ratio of 55 susceptible to 9 resistant, as it did in cross 2 (2e X 9a).

A segregation ratio of 9:7 can occur in the F2 when two dominant genes are both required to determine a character. In the case of isolates M2, M4, M5 and M15 both dominant genes determine resistance, in isolates M14 and M16, they determine susceptibility. Figure 4.3 gives an explanation of the genetic model involved. Unfortunately, because most of the isolates were collected after the F1 plant had been produced, the reaction of the F1 to them was never tested. An F1 test would have been most useful in the case of M14 and M16 where reversal of dominance was shown.

Only two isolates were common to both tests of this cross, M2 and M5. The F2 results were compared for homogeneity as described previously, and only those for isolate M5 were sufficiently similar to be combined (table 4.13).

When the two sets of F2 data were combined and analysed together they still agreed with a segregation ratio of 9 resistant to 7 susceptible (table 4.14).

Figure 4.8 A genetic model to explain the segregation ratio of 9:7 in the F2.

P1 RaRaRbRb P2 rararbrb

F1 RararaRbrb

F2

	RaRb	Rarb	raRb	rarb
RaRb	RaRb	RaRb	RaRb	RaRb
Rarb	RaRb	Rarb	RaRb	Rarb
raRb	RaRb	RaRb	raRb	raRb
rarb	RaRb	Rarb	raRb	rarb

a) 9 resistant to 7 susceptible

Ra and Rb = two genes coding for resistance to one mildew isolate, both genes must be present, resistance is dominant to susceptibility.

The 9 genotypes underlined are therefore resistant, and the 7 not underlined are susceptible.

b) 9 susceptible to 7 resistant

ra and rb = two recessive genes coding for resistance to one mildew isolate, if either or both genes are present as homozygous recessives the genotype is resistant.

The 9 genotypes underlined are now susceptible, and the 7 not underlined are resistant.

Table 4.12 Cross 3,(28aX10j), The reaction of the parents and F1. to eight isolates and the segregation of resistance in the F2.

Isolate	Infection Score			Obs. No.		X^2 for exp. ratio in F2				
	P1	P2	F1	in F2		3:1	1:3	9:55	9:7	7:9
				R :	S			R : S		
M2	4.1	0.1	0.7	115	99	50.1	--	--	0.5 **	8.4
M3	4.0	0.3	+	37	177	--	7.2	1.9 **	--	61.6
M4	3.6	0.3	+	131	86	20.8	--	--	2.3 **	--
M5	3.8	0.4	0.6	120	94	39.6	--	--	0.0 ***	--
M13	4.0	2.7	+	3	211	$$$				
M14	3.8	0.0	+	91	123	--	33.9	--	--	0.2 ***
M15	4.3	0.0	+	111	103	84.8	--	--	1.5 **	--
M16	4.3	0.3	+	86	128	--	25.3	--	--	1.2 **

\+ The F1 was not tested with these isolates.

$$$ M13 was found to be virulent on both parental lines after being used in the test. The results were discarded.

** $0.10 < p < 0.50$

*** $0.50 < p < 1.00$

Figure 4.9 Class distributions of the infection scores of the 8 isolates used in cross 3, second test.

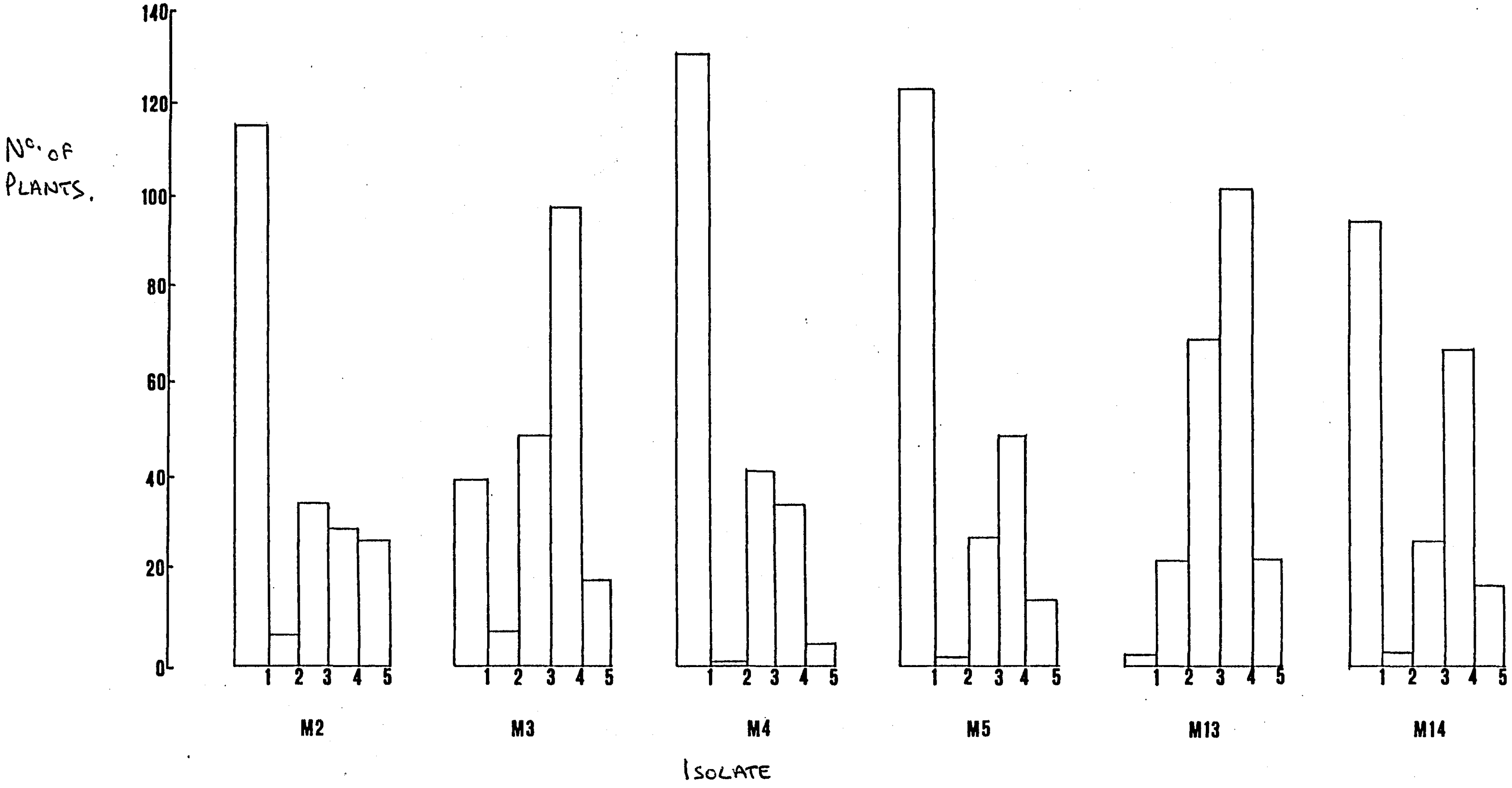

No. of Plants.
140
120
100
80
60
40
20
0
1
2
3
4
5
M2
M3
M4
M5
M13
M14
Isolate

Figure 4.9 (cont.). Class distributions of the infection scores of the 8 isolates used in cross 3, second test.

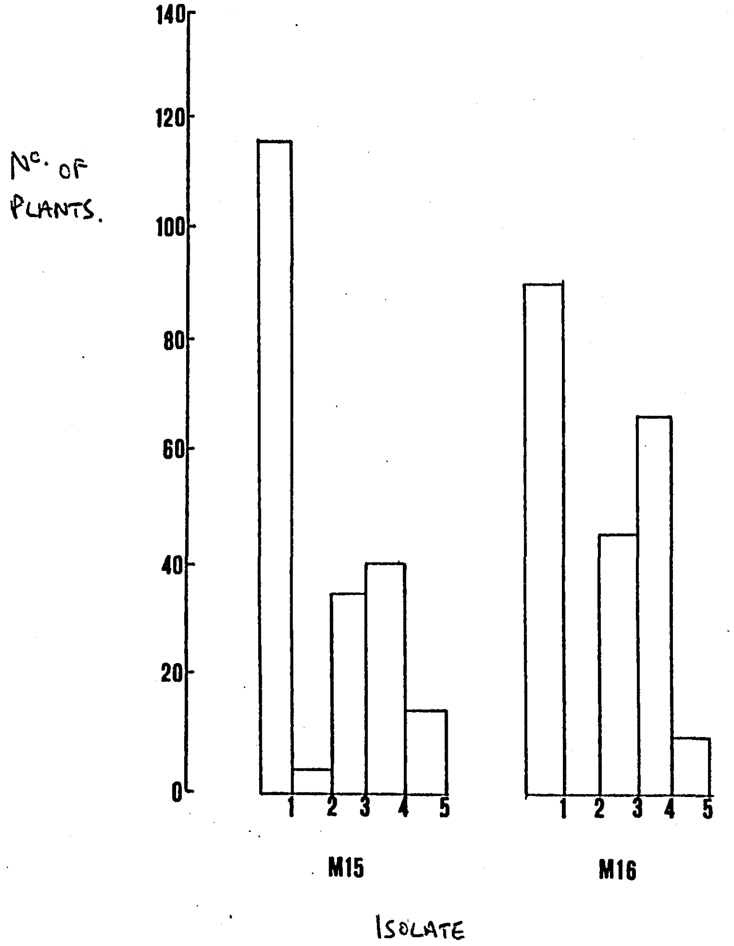

N^o. OF PLANTS.
140
120
100
80
60
40
20
0
1
2
3
4
5
M15
1
2
3
4
5
M16
ISOLATE

Table 4.13 Homogeneity of results from cross 3, first and second test

	Isolate			
	M2		M5	
	R	S	R	S
First Test	79	28	76	31
Second Test	115	99	120	94
X^2	12.1		6.7	
P	$0.01<p<0.001$		$0.1<p<0.05$	

Table 4.14 Combining the results of M5 from both tests.

Isolate	Expected no. R : S 9 : 7	Observed No. R : S	X^2	P
M5	180.6:140.4	196:125	3.2	>0.05

4.3 Discussion.

The results of the three crosses were varied and demonstrated the range of genetic control determining resistance to mildew in groundsel. This was not unexpected since Harry (1980), found that resistance was under three different types of genetic control in only four crossing experiments. However in each case examined, resistance was shown to be determined by a small number of major genes. Each resistance gene determined resistance to only one isolate of the pathogen, and her results were in agreement with the gene-for-gene concept.

The results from cross 1 indicate that resistance in line 9a was determined by single genes, with a separate dominant gene present for each isolate tested. When the data from the two samples of F2 plants were combined and tested for linkage between resistance genes, the seven resistance genes clearly mapped into three groups. The genes in each group - three in one group being tightly linked - probably represent gene families. There was no evidence of multiple allelism, with some plants in the F2 possessing all seven of the resistance genes, and this was to be expected since the parent plants were highly homozygous.

The results are in agreement with the gene-for-gene concept, with the pathogen probably possessing avirulence genes specific to each of the resistance genes in the host.

Segregation for resistance to the four mildew isolates agreed with the ratio of 55 susceptible to 9 resistant. As explained in figure 4.5, this ratio can result when resistance is determined by either one of two dominant genes whose expression is inhibited by a dominant suppressor gene at a separate locus. In cross 2 the dominant allele of the suppressor gene was present in line 1i, the mildew susceptible parent, and the recessive allele in the mildew resistant line, 25p. As discussed in chapter one, the gene-for-gene concept has been extended to cover the situation where resistance to one isolate of pathogen is determined by two genes in the host. The pathogen then possesses two corresponding avirulence genes. The results of cross two can therefore still be explained in terms of the gene-for-gene concept.

A second explanation for the results of cross 2 is that groundsel, being a polyploid species, may have duplicate sets of resistance genes. If this were the case, it is possible that there is only one avirulence gene in the pathogen which is matched to two duplicate resistance genes in the host. Until the genetics of the pathogen have also been investigated, both explanations are possible.

The occurrence of suppressors of resistance genes has been reviewed by Christ et al. (1987), especially in situations where resistance genes from distant wild relatives have been incorporated into the host species.

An example of a dominant suppressor gene was discussed by Dyck and Samborski (1968), concerning resistance of wheat to Puccinia recondita. They reported that the cultivar Prelude, which possessed a dominant gene for resistance to rust, gave susceptible progeny when crossed with the cultivar Thatcher. They suggested that Thatcher possessed one or more genes that suppressed the functioning of the rust resistance gene. Later, Kerber and Dyck (1977), presented evidence that resistance genes to Puccinia graminis in wheat, were inactivated by actions of a suppressor gene at a second locus, the suppressor gene possibly derived from a progenitor species. Both of these examples show the action of a non-allelic inhibitor, similar to that demonstrated to occcur in S.vulgaris. In S.vulgaris four separate pairs of genes determining resistance to four mildew isolates were found to be inhibited by a suppressor gene. Whether the same suppressor gene operated against all four pairs of resistance genes, or whether there was a separate one for each, is not known. It is difficult to test the two possibilities, because the size of the F2 population required to investegate the segregation of five or six genes would be prohibitively large.

One test that was done was to compare the segregation of resistance in the F2 of a dihibrid cross, with that expected by an expansion of the 55:9 ratio, with the expression of both pairs of resistance genes inhibited by the same suppressor gene (figure 4.6). The results of the X^2 test were just outwith the 0.05 significance level. However, the sample size was too small for rigorous statistics and therefore the results do no seriously contradict the 55:9 genetic model.

Where line 28a was used as the resistant parent in cross 3, there was again evidence for more than one gene determining resistance to a single isolate of the pathogen, although the results from the first and second sample of the F2 were different. In the first, the results were similar to those obtained in cross 1, with ratios close to 3 resistant to 1 susceptible. However, in the second sample, the results were closer to a 9 to 7 or 7 to 9 ratio. This progeny ratio could result when two dominant genes are both required to determine

a character (figure 4.8). The 9 to 7 ratio has previously been reported by Baker (1966), who showed that resistance to crown rust in oats was determined by two complementary resistance genes. He suggested that the two resistance genes originated, one each in the two parental lines of the cultivar he used.
This ratio is however, difficult to reconcile with the gene-for-gene concept, in terms of the matching avirulence genes that the pathogen is required to possesss. Crute (1985) has suggested that in some cases apparent 9 to 7 ratios may in fact be miss- classified 3 to 1 ratios. If dominance in resistance genes is only partially expressed it is possible that some of the heterozygotes could be classified with the homozygous recessives. The effect of this would be to skew the F2 ratio in the direction of the 9 to 7 ratio.

If this has occurred in cross 3, it may explain why the results appear to deviate from the more simple ratios obtained in the first two crossses, and provide results which are more easily reconciled with the gene-for-gene concept.

The apparent reversal of dominance seen in the F2 segregation ratios of line 28a, is not uncommon, Lupton and Macer (1962) and Hooker and Saxena (1967), have both shown its occurrence in wheat/yellow rust interactions.
In general, however, host resistance determined by recessive genes is less common than that determined by dominant genes (Hooker and Saxena 1971).

Taken as a whole, the results demonstrate that resistance to isolates of E.fischeri was determined by one or few genes, with resistance usually dominant to susceptibility. The results could be shown to be in agreement with the gene-for-gene concept, although in the case of cross 3 the results were ambiguous.

It is interesting that the linkage of resistance genes demonstrated in the first cross was not detected in cross 3. (The unusual mode of genetic control of resistance in cross 2 precluded testing for linkage.) It is possible that the frequency of recombination between resistance loci is controlled by genes at a separate locus (Whitehouse 1972, and Simpchen and Stamberg 1969). If the different parental lines used in the three crosses had different alleles of genes controlling recombination frequency, the calculation of linkage between resistance loci would be upset. It is not however, possible to tell whether this has occurred in any of these three crosses.

There is a variety of modes of genetic control of the inheritance of resistance to E.fischeri, but this is not surprising, many other species demonstrate complicated genetic interactions with pathogens, involving multiple and complementary resistance genes. S.vulgaris is capable of hybridisation with a number of other *Senecio* species (Gibbs *et al.* 1971), which may explain the origin of some of its complicated resistance to mildew. What is clear is that further studies of larger populations of *S.vulgaris* and an analysis of the genetics of the pathogen are needed to verify and extend these findings.

CHAPTER 5. ELECTROPHORETIC CHARACTERISATION OF THE PARENTAL LINES USED IN INTRASPECIFIC CROSSES

PAGE NUMBER

5.1 Introduction

Since its introduction in the 1950's by Davis and Ornstein (1959), electrophoresis has been developed into a powerful technique for the analysis of proteins extracted from all classes of organism from virus to mammal. It was originally developed to examine the variability of individual enzyme proteins, but is now routinely used to resolve hundreds of proteins on a single polyacrylamide gel (Torp and Andersen 1982).

One of the most useful applications for electrophoresis is to compare the similarity of individuals in terms of certain proteins. Quinn (1982), used electrophoresis to investigate the parentage of goslings in the nests of the lesser snow goose. Within plant pathology, comparisons have been made between plant lines resistant or susceptible to a specific pathogen. Gabriel and Ellingboe (1982), bred near isogenic lines of wheat, differing in a single major gene for resistance to rust. They attempted to correlate differences in rust resistance to differences in protein patterns resolved on two dimensional gels.

Electrophoresis can also be used in the construction of detailed genetic maps, when used in conjunction with molecular biology techniques. One such example is the digestion of DNA extracts by restriction enzymes to produce restriction fragments of different lengths which are then run on a gel to produce different banding patterns. The patterns are generally unique for each individual. This technique was used by Michelmore *et al.* (1987), to construct restriction fragment length polymorphism maps of the genomes of *Lactuca sativa*, (lettuce), and *Bremia lactucae*, (downy mildew).

Within the *S.vulgaris*/*E.fischeri* pathosystem the host plant is the easier to study electrophoretically. The powdery mildew fungus produces only conidia, which are viable for only a short time, preventing bulking-up and storage prior to analysis. *S.vulgaris* has been used previously in electrophoresis studies by Hull (1974). He investigated the distribution of esterase isoenzymes in several *Senecio* species growing in central Scotland. He was able to differentiate between species, and between populations of the same species, on the basis of their enzyme variation.

In the present study electrophoresis was used to examine the differences between lines of groundsel resistant and susceptible to powdery mildew. An examination of both total protein and of specific enzymes was undertaken. Ideally this would have been done for isogenic lines of groundsel, differing only in the possession of resistance genes to mildew, but the production of such lines using groundsel is almost impossible, given the strongly inbreeding nature of the species. Instead the lines possessing the greatest known and smallest known number of resistance genes were compared. These lines, in general, corresponded to the parental lines of the intraspecific crosses, produced for genetic analysis of resistance genes, in chapter four.

5.2 Results

5.2.1 Disk-gel disk electrophoresis

Electrophoresis was first carried out using disk-gels cast in tubes, because of the relative simplicity of the method, and its value in developing a staining protocol for specific enzymes. Preliminary experiments indicated that the enzyme proteins esterase 1 (Est.1), and peroxidase (Per.), could be satisfactorily resolved on the gels.

The banding patterns of proteins from a number of radiate and non-radiate lines of groundsel were compared; mostly those of the parental lines used to generate intra-specific crosses.

The results for a number of lines are shown in figures 5.1 and 5.2, for Est.1 and Per. respectively.

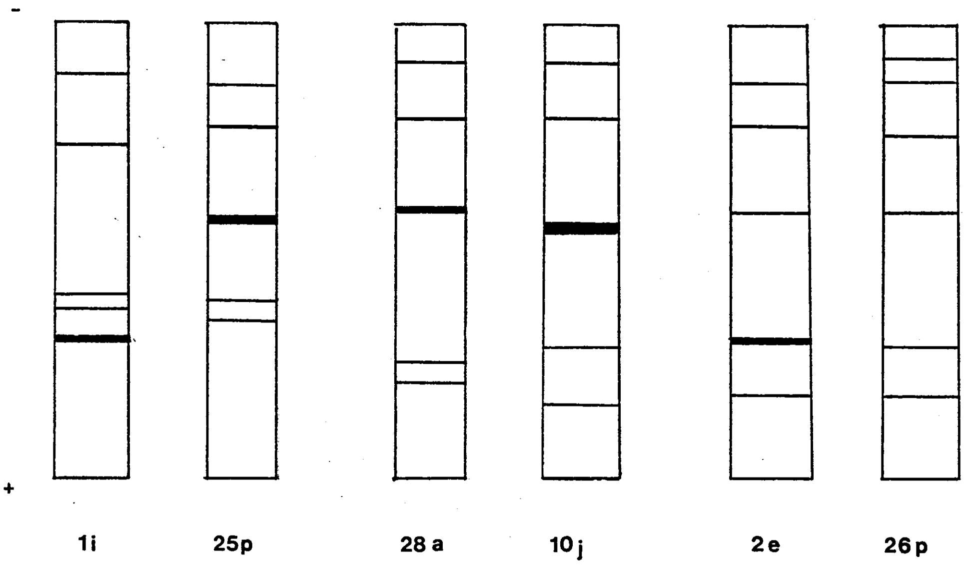
Because the gels were difficult to photograph the results are presented diagramatically . The diagrams are drawn to scale from careful measurements of the bands on the gels after staining.

Examination of the banding patterns of both enzymes shows no definite and reproducible differences between any of the plant lines tested. All differences were small, and within the normal levels of variability found between different separations.

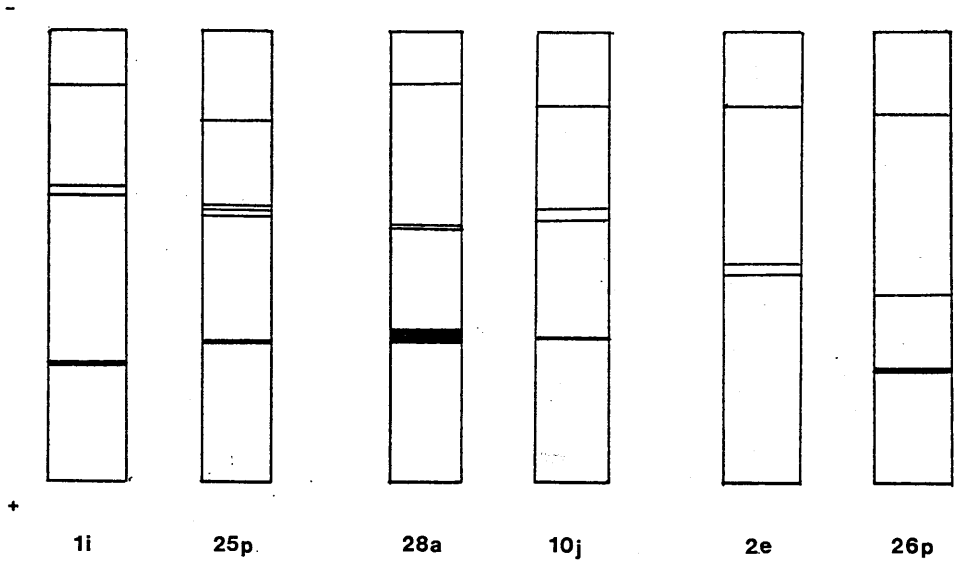
Because of these problems, and the general lack of resolution, slab-gels were used in all subsequent experiments.

Figure 5.1 Est.1 and Per. banding patterns of proteins from 6 groundsel lines.

Est.1



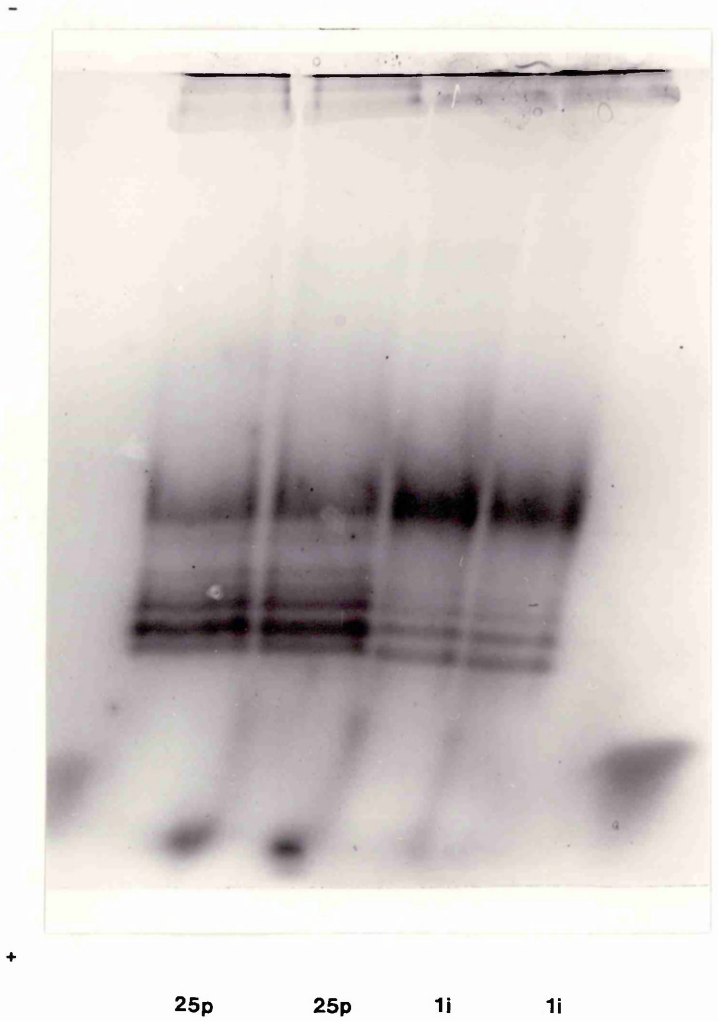
Per.



5.2.2 Non-denaturing vertical slab electrophoresis.

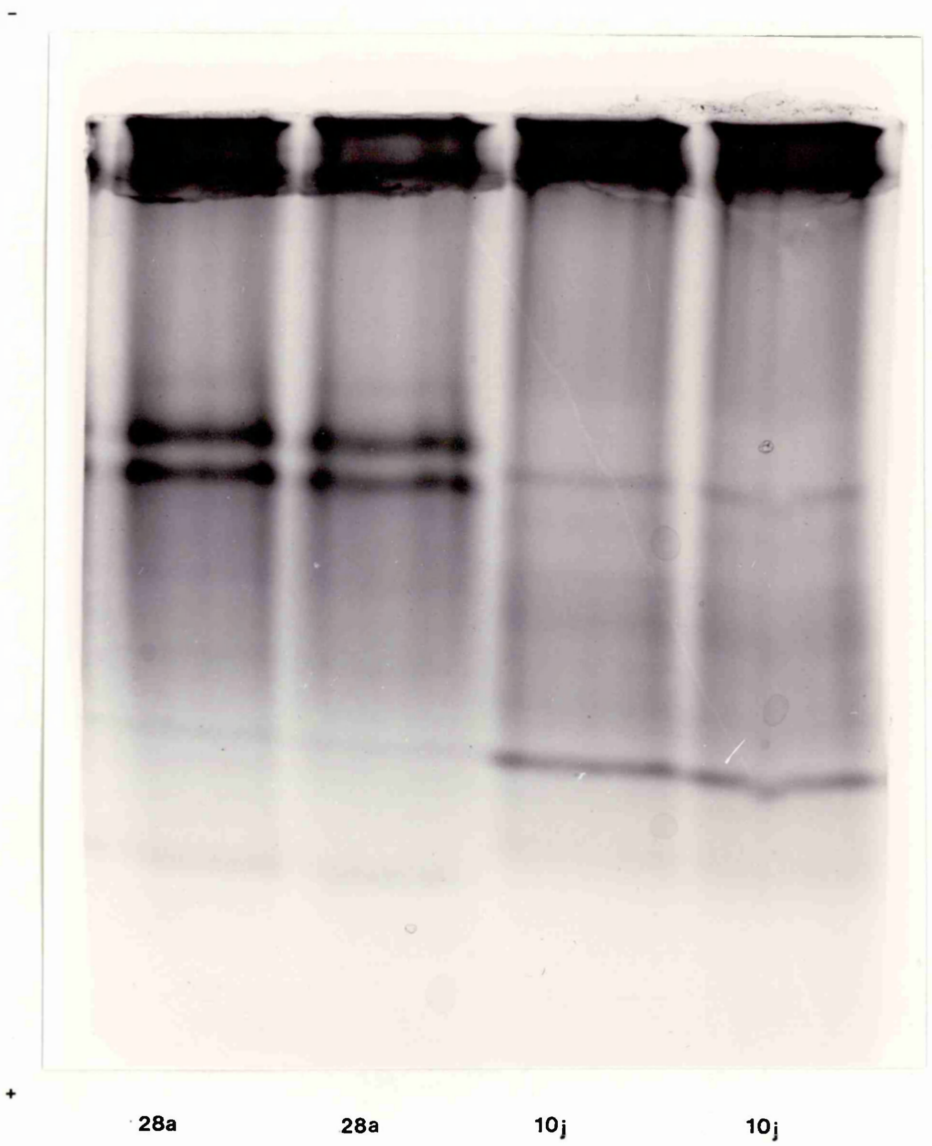
Electrophoresis of the soluble proteins from a number of groundsel lines was performed, and the gels stained for either Est.1, or Per. activity. Plate 5.1 shows the Per. bands resolved from lines 25p and 1i, and plate 5.2 shows the Est.1 bands resolved from lines 28a and 10j. These pairs of lines were used as the parents of crosses 2 and 3 respectively.

Plate 5.1 Peroxidase banding patterns of proteins from groundsel lines 25p and 1i.



(Protein samples from each line were run in two tracks, adjacent to each other.)

Plate 5.2 Esterase 1. banding patterns of proteins from groundsel lines 28a and 10j.

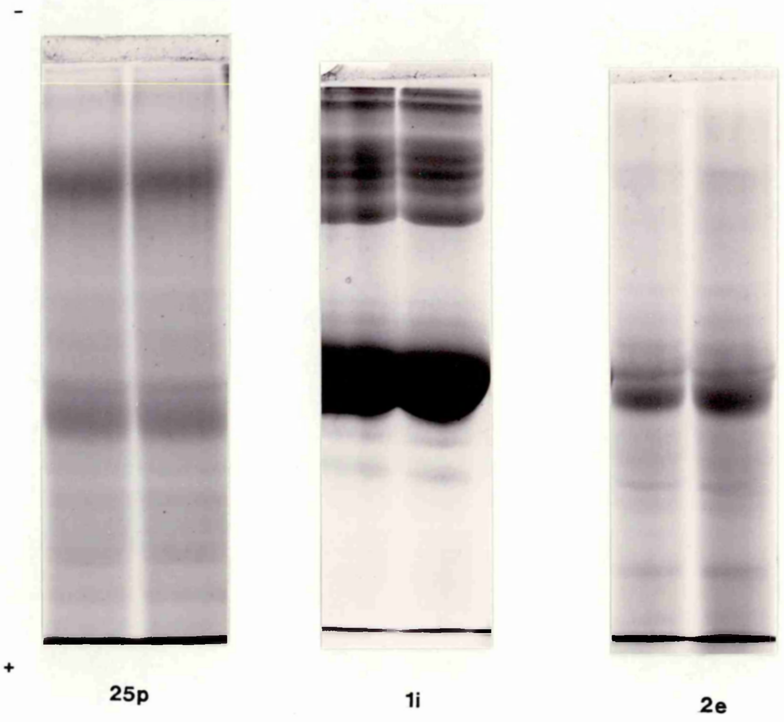


(Protein samples from each line were run in two tracks, adjacent to each other.)

5.2.3 Denaturing slab-gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed to determine differences in overall protein composition, between lines. The banding patterns of proteins from three groundsel lines, 25p, 1i and 2e are shown in plate 5.3.

Plate 5.3 Coomassie blue banding patterns of proteins from groundsel lines 25p, 1i and 2e.



(Protein samples from each line were run in two tracks, adjacent to each other.)

5.3 Discussion

The results obtained from disk-electrophoresis experiments were disappointing. Esterase 1. and peroxidase, both easily resolved enzymes, produced banding patterns that contained few bands, and these were poorly differentiated. In the case of esterase 1. this was surprising since Hull (1974), had performed a similar analysis and reported the presence of up to 40 different esterase 1. protein bands for S.vulgaris alone.

When the enzymes were separated using slab-gels, the results improved, and differences in both enzymes were seen in the zymograms of plant lines 25p and 1i, and of plant lines 28a and 10j. The bands were rather faint, probably due to the protein sample not being sufficiently concentrated. Nevertheless the differences between these two sets of parental lines were reproducible, and so reflect isozyme variation that could be examined further in the future.

SDS gel electrophoresis produced many more protein bands than isozyme analysis, and therefore was potentially more useful for detecting differences between groundsel lines. The banding patterns of the three plant lines shown in plate 5.3 are all different. Line 25p possesses a large number of resistance factors, whilst none have so far been identified in lines 1i and 2e. An examination of the zymograms of these, and other lines, revealed no consistent similarities or differences between resistant lines or between susceptible lines. The differences in protein banding patterns appeared random with respect to resistance phenotype.

In conclusion, neither isozyme nor total protein analysis revealed differences that were correlated with resistance phenotype. An analysis of a larger number of lines, using more sensitive staining techniques may have revealed more significant differences, but this was not possible here.

CHAPTER 6. COMPARISON OF THE RESISTANCE FACTORS POSSESSED BY RADIATE AND NON-RADIATE GROUNDSEL, AND THE INTROGRESION BETWEEN *S.VULGARIS* VAR *HIBERNICUS* AND *S.SQUALIDUS*.

PAGE NUMBER

6.2 Comparison of the resistance factors possessed by radiate and non-radiate groundsel.

6.3 Introgression between *S.vulgaris* var *hibernicus* and *S.squalidus*.

6.1 Introduction

Senecio vulgaris L. occurs in Great Britain as two distinct varieties, *S. vulgaris* var *vulgaris*, possessing disk florets only, and *S. vulgaris* var *hibernicus*, possessing eight to twelve ray florets in addition to disk florets (Allen 1967). These are commonly referred to as the non-radiate and radiate varieties respectively (see plate 6.1). Trow, in 1913, was the first to investigate the genetics of the two varieties of the species. He showed that the radiate character was determined by a single major gene with incomplete dominance. This gene he designated R. Thus, radiate plants were termed RR, non-radiate NN, and the heterozygote, which possessed stubby ray florets, RN. These designations were later revised by Hull in 1974 to TrTr, TnTn and TrTn, for the three genotypes respectively.

Both varieties show a similar large range of variation in morphological characters, with the exception of the type of flower head, and it is not usually possible to distinguish between the two until flowering occurs. The possession of ray florets does however affect the breeding system of that variety. Unlike the non-radiate variety which inbreeds at 99% or more (Gibbs *et al*. 1975), the radiate is capable of outcrossing at up to 35% (Haskell 1953). This is partly because the ray florets attract insect pollinators not normally seen on non-radiate plants, and partly because the ray florets are gynomonecious and mature before the disk florets on the same flower head (Ingram and Taylor 1982). These last two factors combine to make the outer ray florets obligately outcrossing. Because this difference in breeding system is under single gene control, that of the radiate gene, the species has been of great interest to population geneticists. Abbott and his co-workers have produced a long series of papers on this subject (1976, 1980, 1982, 1985, 1986, 1988).

Two theories have been advanced to explain the origin of the radiate variant in groundsel (Ingram *et al.* 1980).

1. The radiate allele has arisen through introgression between *S.vulgaris* and the related species *S.squalidus*.

2. The radiate allele has arisen by mutation of *S.vulgaris*.

The second theory is thought equally possible by Stace (1978), who claims that there is a lack of evidence of the occurrence of the hybrid between *S.vulgaris* and *S.squalidus*, termed *S.baxteri*. Such a plant would be a triploid hybrid, and as such, very infertile. There are only six cytologically confirmed reports of naturally occurring hybrids. The most comprehensive of these by Crisp and Jones in 1970. Additionally, there are large areas of southern England where the two species co-exist, but the radiate variety of *S.vulgaris* has never been recorded.

There is support for the introgression theory from a number of workers, including Hull (1974, 1976), and Monaghan and Hull (1976). They argued, firstly, that in terms of vegetative characters, groundsel plants growing near large populations of S.squalidus are more similar to that species than are populations not growing close to S.squalidus. Furthermore, isozyme analysis of plants of both species showed that the radiate variety of groundsel was more similar to S.squalidus than was the non-radiate. From these, and other studies, they concluded that introgressive hybridisation between the two species was a continuing and frequent occurrence.

Whilst agreeing with the introgression theory, Ingrams et al. (1980), disputed that introgression was an ongoing or frequent event. Rather, it was likely to have been an ancestral or very rare event, owing to the low level of hybrid fertility. She was able to synthesise the hybrid under laboratory conditions, and did recover tetraploid groundsel plants that were morphologically very similar to naturally occurring radiate groundsel.

If the origin of radiate groundsel is indeed via introgression with S.squalidus, it is possible that other genes have also been transferred between the two species (Abbott 1986). S.squalidus is not a host for E.fischeri (Junnel 1963, Blumer 1967), so therefore appears completely resistant to the pathogen. Were the genes for non-host resistance to have been transferred into groundsel during introgression, the resulting radiate plants could be more resistant to E.fischeri than the corresponding non-radiate plants. This would depend on the resistance genes being expressed, and functioning in the new genetic background. To test this possibility, the large collection of groundsel lines amassed over the last ten years, was used to look for differences in resistance to E.fischeri between the two groundsel varieties.

In addition to the above, an attempt was made to artificially introgress the two species, under laboratory conditions. If a hybrid could be produced between S.squalidus and a very susceptible line of groundsel, any resistance subsequently recorded would have come from S.squalidus alone, i.e. it would be non-host resistance. The segregation of such resistance could be analysed in test crosses to determine how it was inherited. The analysis of non-host resistance is not possible using S.squalidus alone, because all individuals are resistant to all known isolates of the mildew and so individual resistance genes would never be seen to segregate. The results of these experiments are presented in the second half of this chapter.

Plate 6.1 Radiate and non-radiate S.vulgaris.

S.vulgaris var. vulgaris

S.vulgaris var. hibernicus

6.2 Comparison of the resistance factors possessed by radiate and non-radiate groundsel.

6.2.1 Materials and methods

The first comparison of the numbers of resistance factors possessed by the two varieties of groundsel, was done in 1987, using the existing collection of radiate lines, and a random selection of non-radiate lines. These results are given in tables 6.1 and 6.2. A larger comparison was carried out in 1988 using pairs of lines, one of each variety collected from 30 sites around Scotland. At each site seed was collected from one radiate and one non-radiate plant, growing in close proximity to each other. By this sampling procedure 30 lines of each variety were collected, making possible a more reliable comparison between the two. The results of the second test are given in tables 6.3, 6.4 and 6.5.

6.2.2 Results

Table 6.1 Comparison of the number of resistance factors possessed by 22 radiate and 22 non-radiate lines.

Plant Line	Isolate					No.of R.F.s
	M1	M2	M3	M4	M5	
Non-Radiate						
1i	5.0	5.0	4.6	5.0	4.6	0
1n	0.3	4.0	3.3	4.0	3.6	1
2i	4.6	0.0	0.0	1.0	4.3	3
2d	3.3	3.3	2.6	3.6	4.3	0
2e	4.3	5.0	4.3	4.0	3.6	0
4a	0.0	1.0	0.3	4.3	2.0	3
5c	0.3	0.6	0.0	0.3	0.0	5
7c	3.3	3.6	3.6	3.3	4.3	0
8d	4.6	4.0	3.0	2.0	3.0	0
9a	0.3	0.0	0.0	1.0	0.3	5
9d	4.6	0.3	0.6	2.6	0.0	3
10c	3.3	3.6	3.3	4.6	3.0	0
10j	0.3	0.0	0.0	1.0	0.0	5
11i	1.0	0.6	0.0	0.0	0.0	5
11g	0.0	0.0	0.0	0.0	0.0	5
11e	4.3	3.6	3.6	4.3	4.0	0
14h	0.6	0.3	0.3	4.0	0.6	4
15c	3.3	3.3	3.6	4.0	2.6	0
16f	0.0	0.3	0.0	0.0	1.0	5
19b	4.3	0.6	4.0	4.3	3.3	1
23i	0.0	3.6	4.6	2.6	3.6	1
23g	5.0	5.0	5.0	4.6	1.0	1
Radiate						
6b	5.0	0.6	0.0	0.3	0.0	4
10s	4.3	4.6	0.3	0.6	3.0	2
25a	3.6	4.3	0.0	0.0	4.3	2
25f	4.3	0.3	0.3	0.0	0.0	4
25m	3.3	0.3	1.0	3.0	4.0	2
25p	4.3	0.3	1.0	0.0	0.0	4
27a	0.0	0.3	0.0	1.0	0.3	5
27b	3.0	4.0	0.0	0.0	4.3	2
27f	5.0	2.6	0.6	0.0	0.3	3
27g	4.3	2.3	0.0	0.0	0.0	3
27h	0.6	1.0	0.3	0.0	0.3	5
27i	4.3	0.6	3.0	0.3	0.0	3
27j	4.3	0.3	0.0	0.6	0.0	4
27k	0.0	1.0	0.3	0.6	0.0	5
27l	3.3	3.0	0.0	4.6	5.0	1
28a	4.3	3.0	3.7	3.0	4.3	0
28b	4.3	4.0	0.0	0.0	0.6	3
28c	0.6	0.0	0.3	0.0	0.3	4
28d	4.6	3.0	4.0	0.3	1.0	2
28e	4.0	3.6	0.6	0.0	0.3	3
28f	3.6	3.0	0.0	1.0	0.6	3
28h	2.6	0.6	0.0	0.6	0.3	4

Table 6.2 Average number of resistance factors in 22 radiate and non-radiate lines.

No. of factors	Non-radiate	Radiate
0	8	1
1	3	1
2	1	5
3	3	6
4	1	6
5	6	3
Mean	2.2	3.2

Table 6.3 Average number of resistance factors in 30 radiate and non-radiate lines.

No. of factors	Non-radiate	Radiate
0	7	3
1	3	3
2	6	4
3	8	5
4	3	6
5	3	9
Mean	2.2	3.1

Table 6.4 Comparison of resistance factors in 30 sets of radiate and non-radiate lines from the same site.

Number of times radiate line has fewer resistance factors than non-radiate line - 6

Number of times radiate line has equal resistance factors to the non-radiate line - 5

Number of times the radiate line has more resistance factors than the non-radiate line - 19

Table 6.5 Comparison of the number of resistance factors possessed by 30 radiate and 30 non-radiate lines

Plant Line	Isolate M1	M2	M3	M4	M5	No. of R.F.s
Non-radiate						
29a	4.0	3.3	4.3	3.0	4.0	0
29b	0.3	3.4	0.3	0.7	3.0	3
29c	3.3	0.3	5.0	1.0	2.7	2
31a	0.0	3.3	0.0	0.0	3.0	3
31b	3.3	3.0	4.3	5.0	3.7	0
31c	0.3	0.3	0.3	3.7	0.3	4
33a	3.7	4.3	3.7	3.3	3.0	0
33b	0.7	0.3	0.0	0.0	0.0	5
33c	4.7	4.3	1.0	2.7	3.0	1
35a	1.0	2.7	2.3	2.7	0.3	2
35b	4.0	3.0	4.3	3.3	3.3	0
35c	0.3	3.3	3.3	0.3	1.0	3
37a	0.7	0.3	4.0	1.0	3.7	3
37b	3.7	3.3	4.3	3.3	3.0	0
37c	1.7	0.7	2.3	1.0	0.3	3
39a	2.7	2.3	0.3	0.3	2.3	2
39b	3.0	0.7	4.7	3.7	3.7	0
39c	0.3	0.7	5.0	3.0	0.3	3
41a	3.0	3.7	4.7	3.3	0.7	1
41b	2.3	0.3	1.0	4.3	4.7	2
41c	0.0	0.0	0.0	0.3	0.3	5
43a	4.0	3.0	0.0	4.0	2.3	1
43b	3.3	3.7	3.7	1.7	2.0	0
43c	0.0	0.0	3.0	0.0	0.0	4
43d	4.0	0.7	3.3	0.0	2.3	2
43e	5.0	0.3	3.0	0.7	0.3	3
43f	0.7	5.0	4.3	0.3	0.7	3
43a	1.0	0.0	3.7	0.0	0.3	4
45b	1.0	0.3	0.3	0.3	0.3	5
45c	0.3	2.7	2.3	0.7	2.3	2

Table 6.5 (Cont.) Comparison of the number of resistance factors possessed by 30 radiate and 30 non-radiate lines

Plant Line			Isolate			No.of
	M1	M2	M3	M4	M5	R.F.s
Radiate						
30a	3.3	4.3	3.3	3.7	5.0	0
30b	2.7	2.7	0.3	4.0	3.7	1
30c	0.7	0.0	0.0	0.0	0.7	5
32a	0.0	0.0	0.3	1.0	0.7	5
32b	0.7	1.0	4.3	3.7	0.0	3
32c	3.0	0.0	4.3	0.0	0.0	3
34a	4.5	0.7	0.0	0.3	0.0	4
34b	0.0	2.7	0.0	0.0	4.0	3
34c	0.0	0.7	0.7	0.0	0.0	5
36a	1.0	0.0	1.0	0.7	4.3	4
36b	0.0	0.3	0.0	0.7	2.3	4
36c	0.0	0.3	0.0	3.7	0.0	4
38a	0.0	0.0	0.3	0.0	0.0	5
38b	3.0	5.0	5.0	0.5	3.3	1
38c	0.3	0.0	0.3	0.0	0.0	5
40a	0.3	0.0	0.7	0.3	0.3	5
40b	4.0	0.0	0.7	1.0	0.7	4
40c	0.0	0.3	0.0	0.7	4.0	4
42a	3.0	3.0	2.7	2.3	0.3	1
42b	0.0	0.0	0.0	0.0	0.0	5
42c	0.0	0.0	0.3	0.0	0.0	5
44a	2.7	3.3	0.3	0.0	0.0	4
44b	0.3	2.3	0.7	0.3	4.3	3
44c	0.7	2.7	5.0	0.0	0.0	3
44d	2.7	4.0	3.7	3.3	4.0	1
44e	1.0	0.0	3.7	0.0	0.7	4
44f	0.0	0.0	0.0	0.3	0.0	5
46a	3.3	2.7	2.0	4.7	4.3	0
46b	3.0	4.0	0.0	0.7	3.7	2
46c	0.7	0.7	4.0	0.0	0.0	4

6.3 Introgression between *S.vulgaris* var *hibernicus* and *S.squalidus*.

6.3.1 Materials and methods.

S.vulgaris var *hibernicus* was introgressed with *S.squalidus* by first crossing the two, and then backcrossing the hybrid with *S.vulgaris* var *hibernicus* through several generations, according to the procedure set out in figure 6.1.

The production of hybrids between *S.vulgaris* and *S.squalidus* was done essentially by the method of Watts (1980), as described in Chapter 2.

Ingram (1977), successfully synthesised the hybrid by emasculating *S.vulgaris* buds at an early stage of development, using a razor blade, and then cross pollinating, with a camel hair brush. This method was tried, but abandoned, because of the very high failure rate of emasculated buds to develop.

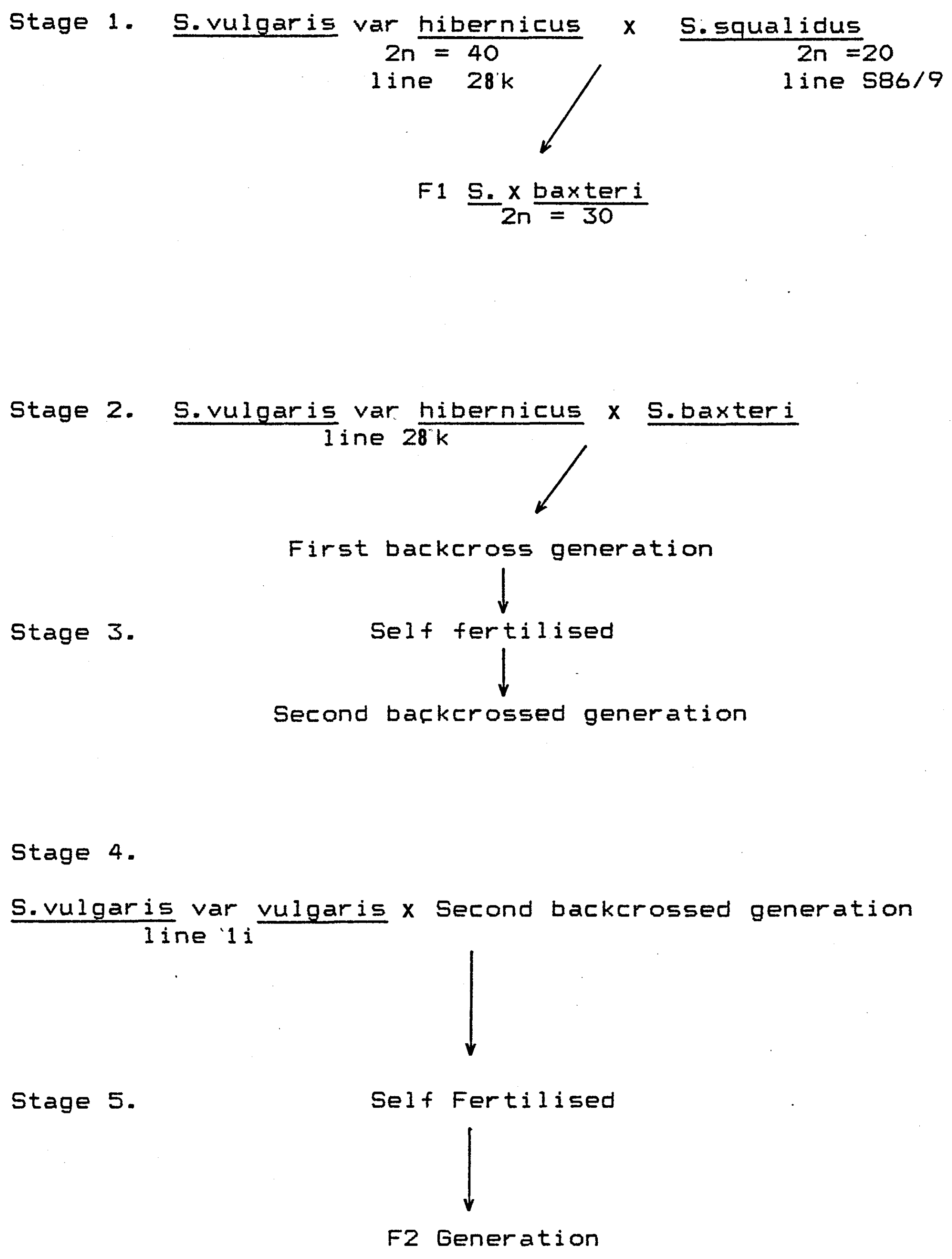
The crosses were made between the radiate line 27k, susceptible to mildew isolates M1, M2, M3, M4 and M5, and the *S.squalidus* line S86/9, *S.vulgaris* var *hibernicus* the female parent, *S.squalidus* the male.

Previous work (Ingram 1977), suggests the cross is more likely to succeed when *S.squalidus* is used as the male parent, and with a radiate as opposed to non-radiate line of *S.vulgaris* as the female parent. S86/9 was grown from the seed of a single head from a cross between two *S.squalidus* lines, S86/2 and S86/4, both collected in Leith, Edinburgh. (Because S.squalidus is obligately outcrossing, the maintenance of inbred lines is not possible.) Both S.squalidus lines were tested against the five mildew isolates, and as expected, were found to be resistant to them all.

Ten 27k plants (S.vulgaris var hibernicus), were raised for the cross, along with 20 S86/9 (S.squalidus) plants as the pollen donor plants, the latter planted five weeks before the former. Five inflorescences on each female plant were marked and sprayed with distilled water to remove any self-pollen, pollen from the donor plants was then liberally applied, using a paint brush. Each morning on the four subsequent days, more pollen was brushed on, to ensure a plentiful supply of forgein pollen.

Plants were covered with muslim and left to set seed, which was subsequently collected, the seed from each flower head placed in a separate bag.

Figure 6.1 The production of backcrossed *S.vulgaris* by introgression.



6.3.2 Results.

From the 50 crossed flower heads, only six contained fertilised seed. This was sown, and the resulting plants examined for hybrids.
Since the correct identification of hybrid plants was of importance, care was taken not to wrongly classify a self as an F1. Rosser (1955), gives a full description of a wild hybrid plant he collected; and Ingram (1977), describes the hybrids she synthesised. Hybrid plants differ from the groundsel parent in having more vigorous vegetative growth, achieving a larger final size, and with fewer lateral branches. The flower heads are larger, possessing longer and broader non-overlapping ray florets. Plants take longer to mature, and because they are triploids never set selfed seed. Only plants possessing all these characters were retained. The number of hybrid plants recovered from each inflorescence is shown in table 6.6. Plate 6.2 shows the parent and hybrid plants.

Leaf tissue from the hybrid plants was tested for resistance to the five isolates, and the results are shown in table 6.7.

All four hybrid plants showed high levels of resistance to the five mildew isolates, and line F1.3, which showed the lowest mean infection score, was chosen to backcross to the parental groundsel line (27k).

Plate 6.2 Parents and hybrid from the cross S.vulgarisx S.squalidus, plants and capitula.

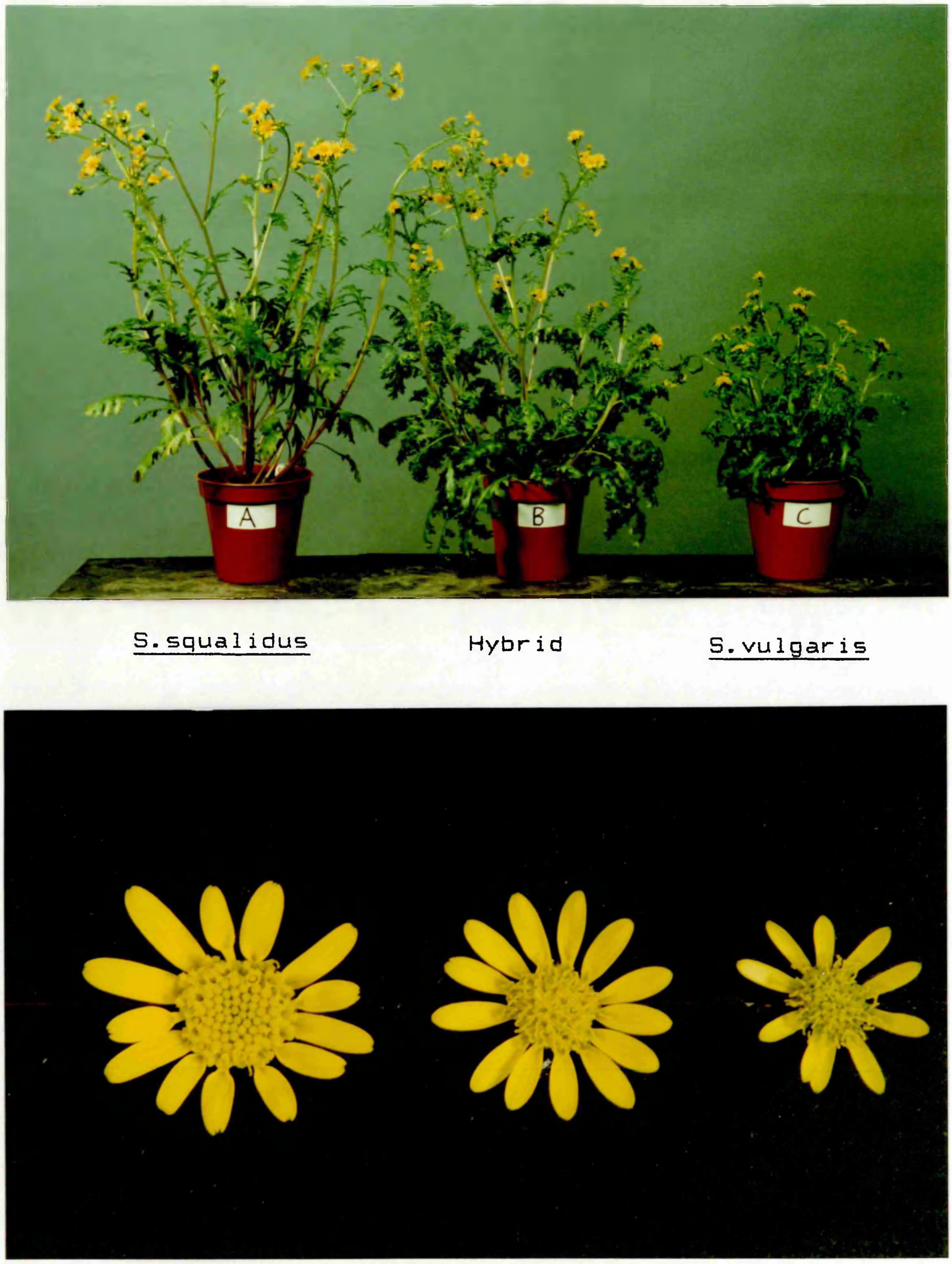


Table 6.6 Number of hybrid plants from each of the fertilised inflorescences.

Flower Head	Number of fruits	Number of Hybrids	Number of Selfs	Failed to Germinate
1	6	0	5	1
2	3	0	3	0
3	2	0	2	0
4	3	1	1	1
5	5	0	5	0
6	3	3	0	0

Table 6.7 Mean infection scores of the parents and F1s.

Plant			Isolate		
Line	M1	M2	M3	M4	M5
P1. 28k	3.7	4.0	4.0	4.3	4.7
P2. S86/9	0.0	0.0	0.0	0.0	0.0
F1.1	0.3	1.0	0.0	0.0	0.0
F1.2	0.3	0.0	0.3	0.3	0.0
F1.3	0.0	0.0	0.0	0.0	0.0
F1.4	0.7	0.3	0.0	0.0	0.3

Table 6.8 Mean infection scores of the first generation of backcrossed plants (B.1).

Plant			Isolate		
Line	M1	M2	M3	M4	M5
B.1	0.3	0.0	0.3	0.0	0.7
B.2	0.0	0.0	0.0	0.0	2.7
B.3	0.0	0.0	0.5	0.3	0.0
B.4	0.3	0.3	0.0	0.0	0.0
B.5	0.3	0.0	0.0	0.3	0.0
B.6	0.7	0.3	0.0	0.3	0.3
B.7	0.3	0.3	0.3	0.3	0.0
B.8	0.0	0.0	0.7	0.0	0.0
B.9	0.0	0.3	0.0	0.3	0.3

Achenes from the backcrossed plants were collected, sown immediately, and the resulting plants screened for backcrosses. These plants were tested for resistance to the mildew isolates and only plants which remained resistant to all five isolates were retained. In total, nine backcrossed plants were recovered, and their mean infection scores to the mildew isolates are shown in table 6.8.

The non-host resistance to E.fischeri possessed by the S.squalidus parent had clearly been transferred into the backcrossed line of S.vulgaris.

The backcrossed line, B.1.5 was then selfed, and a sample of the seed sown, and eight of the resulting plants tested for mildew resistance. Plant B.2.4 was selected for use in further experiments. The results are given in table 6.9.

The final stage of the programme was to cross the backcross groundsel line with the very susceptible groundsel line, 1i. Plant B.2.4 was used as the female parent, and line 1i as the male pollen donor.

Line 1i is susceptible to all mildew isolates, and non-radiate. The inflorescences of the crossed generation were then covered and allowed to set self seed. This seed was collected and a population of 24 plants raised, which were tested for resistance to each of the five mildew isolates. Because of space limitations the production of a larger population was not possible. The results from this cross are contained in tables 6.10 and 6.11 and figure 6.2.

6.3.2.1 The cytology of interspecific hybrids.

One of the surest methods of checking the products of the introgression experiment for hybridity was a chromosome count of the plants. Hybrids from a cross between *S.vulgaris* (2n=4x=40) and *S.squalidus* (2n=2x=20), would be expected to be 2n=3x=30. When the hybrid is then backcrossed to the parental groundsel, the expected chromosome number would be expected to range between 40 and 50.

Lactoproprionic orcein was used to stain root tips of the F1 and backcrossed plants, as described in Chapter 2. Difficulties were encountered with the F1 plants, mainly because they were old plants by the time staining was possible. Because there were only four hybrid plants these were too valuable to be destructively sampled at an earlier stage. The 20 to 40 chromosomes were also very small, and do not take up stain easily (Ingram 1977), so that mitotic figures are difficult to interpret and photograph. Root tips from young seedlings of the backcrosses were successfully stained, and the results are given in table 6.12. The chromosomes were not successfully photographed.

Table 6.9 Mean infection scores of the second backcrossed generation (B.2, selfs of B.1.5).

Plant	Isolate			
Line	M2	M3	M4	M5
B.2.1	0.0	0.3	0.0	0.7
B.2.2	0.3	0.7	0.3	0.3
B.2.3	0.0	0.7	0.3	0.3
B.2.4	0.0	0.0	0.3	0.3
B.2.5	0.3	0.0	0.3	1.0
B.2.6	2.7	0.3	0.7	1.0
B.2.7	0.0	0.3	0.3	0.3
B.2.8	0.7	0.3	0.3	1.0

Table 6.10 Mean infection scores of the cross B.2.4 X 1i.

Plant	Isolate			
Line	M2	M3	M4	M5
P1. B.2.4	0.0	0.0	0.3	0.3
P2. 1i	5.0	4.7	5.0	5.0
F1.	2.3	0.7	2.7	1.0
F2.1	0.3	0.7	0.0	0.0
F2.2	4.3	0.7	4.0	2.3
F2.3	3.3	2.7	0.3	1.3
F2.4	0.0	2.7	4.3	0.7
F2.5	2.3	0.0	3.7	1.7
F2.6	2.3	0.3	0.6	3.7
F2.7	3.0	2.7	4.0	0.3
F2.8	2.7	3.3	3.0	4.7
F2.9	2.0	0.0	0.3	2.7
F2.10	4.7	4.0	3.0	3.7
F2.11	1.7	0.0	0.0	2.7
F2.12	2.3	0.0	0.3	1.0
F2.13	0.3	2.3	3.3	3.7
F2.14	2.3	0.3	1.0	0.3
F2.15	0.0	1.3	0.7	4.0
F2.16	1.7	0.0	2.0	4.0
F2.17	1.7	2.7	2.3	1.3
F2.18	0.3	2.3	0.0	2.0
F2.19	3.0	0.3	4.3	3.7
F2.20	0.0	4.7	1.7	3.0
F2.21	2.7	2.7	2.3	0.0
F2.22	0.0	3.0	0.7	2.0
F2.23	4.7	0.3	0.3	3.3
F2.24	0.0	3.3	3.0	0.7
S:R	16:8	13:11	14:10	17:7

Resistant = infection score of 0.0-1.0
Susceptible = infection score of 1.1-5.0

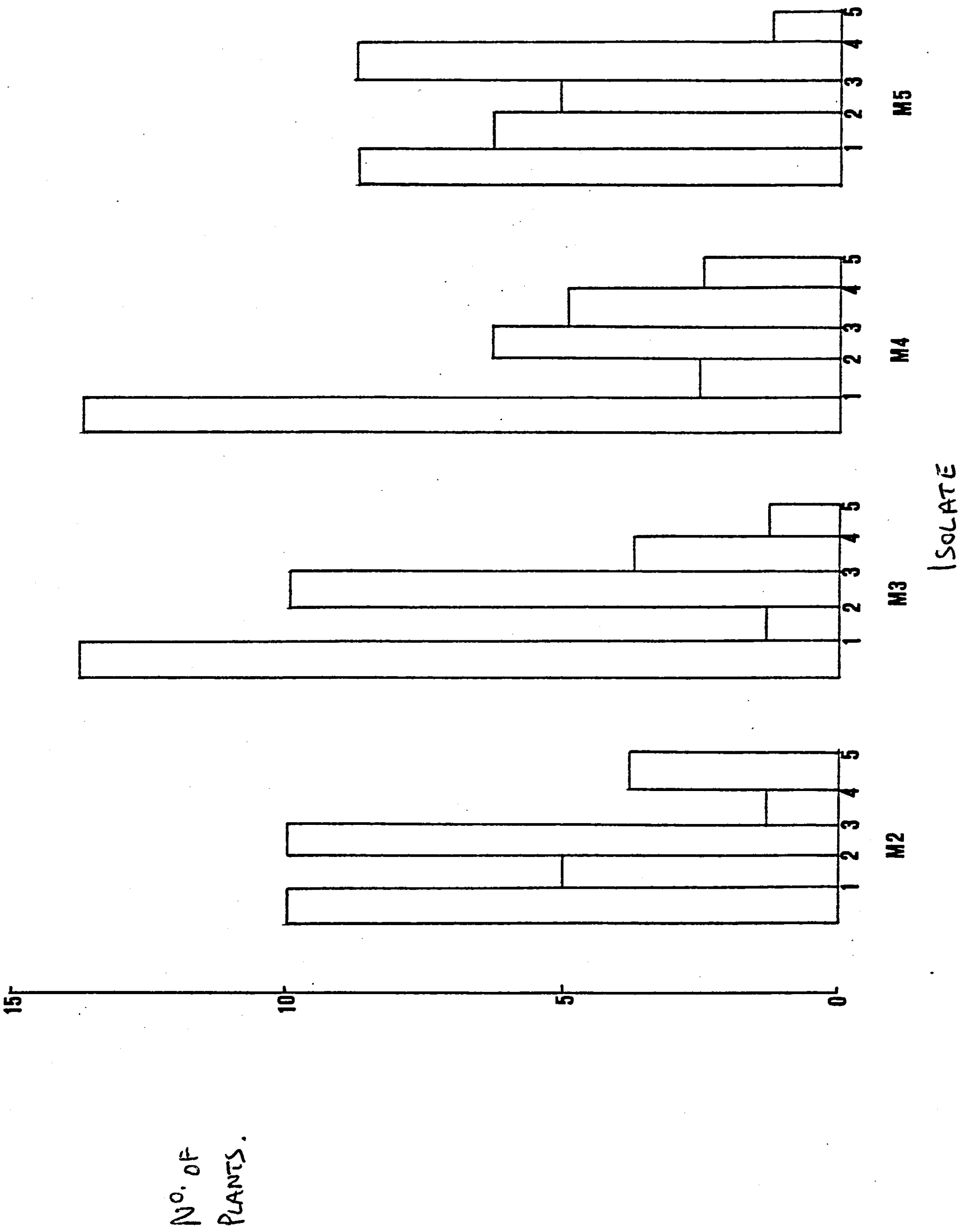
Table 6.11 Results of the cross, B.2.4 x 1i.

Isolate	Obs.no plants R : S	Expected No.(3:1) R : S (1:3) (8 : 16) (16 : 8)	X^2	P
M2	10 :14	6 : 18	3.55	>0.050
M3	11 :13	18 : 6	5.55	<0.020
M4	11 :13	6 : 18	5.55	<0.020
M5	7 :17	18 : 6	26.88	<0.001

Table 6.12 The cytology of interspecific hybrids

Plant Line	Number of Chromosomes
B.1.1	43
B.1.3	41
B.1.5	44
B.1.8	43
B.2.1	42
B.2.2	42
B.2.4	43

Figure 6.2 Class distributions of the infection scores of the F2 plants in cross B.2.4 x 1i.


15
10
5
0
No. of PLANTS.
1
2
3
4
5
M2
M3
M4
M5
ISOLATE

6.4 Discussion

The results of the two experiments comparing the relative resistance to mildew of the radiate and non-radiate varieties of groundsel, both suggest the former to be more resistant. The second experiment, which used radiate and non-radiate plants from the same location (table 6.3), showed mean resistance to the five isolates used of 3.1 and 2.2, respectively.

These results agree with those of Ingrams _et al_. (1980), Hull (1974), and Madden (1989), in establishing differences between the groundsel varieties, other than capitulum type. Ingrams _et al_. found differences in growth rates, Hull, isozyme differences, and Madden differences in resistance to herbicides. Additionally, because the radiate variety shows greater resistance to mildew, it is more like _S.squalidus_, and may therefore be more closely related to it, than is the non-radiate variety. This gives support to the theory that radiate groundsel arose through introgressive hybridisation with _S.squalidus_. In the process, mildew resistance genes could have been transferred between the species.

To investigate this possibility, the two species were introgressed by backcrossing, and a new backcrossed line of tetraploid groundsel was established. This line displayed the same mildew resistance phenotype as the _S.squalidus_ parent. The plants were highly resistant to all the mildew isolates they were tested against, with infection scores of 1.0 or less in all cases (table 6.8). When the plants were selfed and the next generation tested, they too were resistant to all the mildew isolates, indicating the stability of the introgressed resistance (table 6.9). Thus non-host resistance had been transferred from one species to another. The resistance functioned in the new host, and when passed on to the next generation, functioned there too. However, when these resistant plants were then crossed with a second line of groundsel, resistance appeared to segregate (see tables 6.10 and 6.11).

These results confirm two things, firstly, that introgression can occur between S.vulgaris and S.squalidus, at least under laboratory conditions, and that when it does occur, genes other than those coding for capitulum type are transferred. Secondly, and perhaps more importantly, non-host resistance genes can be transferred between species, and be effective in the new host. If this holds true for other pairs of related species, it will be of great interest to plant breeders, as a source, and a means to transfer new resistance into crop species. Many crop species are derived from, or are closely related to, wild species, that do not suffer from severe attack from fungal pathogens. This may be due to tolerance of the pathogen on the part of the wild species (Clarke 1984), i.e. it is attacked by the pathogen but does not suffer serious injury, or it may be that the species is a non-host for that pathogen, and is not attacked at all. If it is the latter, that non-host resistance could be exploited by plant breeders to improve the resistance of related crop species.

Non-host resistance is defined as " all individuals of a species are unaffected by any member of a particular pathogenic species." (Fraser 1985). Non-host resistance can be thought of as a number of barriers, each of which the pathogen has to surmount to cause infection. These barriers are can be pre-infection or constitutitive in type, or may be post-infection, such as the hypersensitive response (Crute et al. 1985, Fraser 1985). It is the number of these barriers that protects a species from pathogenic attack, and it seems unlikely that a single, or even a few genes, could control the variety of barriers to infection. Non-host resistance is also very durable and non-specific in nature (Day 1972). Newton and Crute (1989), have also discussed non-host resistance, quoting Niks's (1988), concept that it may be termed a poor adaptation of the micro-organism to the general defence mechanisms in the plant. They state, that as a consequence of the poor adaptation of the micro - organism, the expression of virulence in the pathogen is low, this being a feature of non-host resistance.

Many of these factors suggest that genetic control is by a large number of genes.

When a line of groundsel from the second backcrossed generation was crossed with a second groundsel line, very susceptible to mildew, segregation of resistance and susceptibility occurred (stages 4 and 5 of figure 6.1, table 6.10 and figure 6.2). The F1 plant was resistant to only two of the four mildew isolates it was tested with, and the F2 plants showed infection scores from very resistant to very susceptible. In previous intraspecific crosses (chapter four), the F2s did not show such variation of infection scores, and the ratio of resistant to susceptible plants agreed with simple mendelian segregation ratios. This was not the case in this cross, where the results do not agree with resistance to each mildew isolate being determined by single genes (table 6.11). The variation of infection scores and the lack of evidence for single gene control in the F2, suggest that resistance may be determined by several genes. It is however, unlikely to be under polygenic control, this being difficult to reconcile with the gene-for-gene interactions shown to occur in non-radiate groundsel. It is important to note however, that this conclusion is based on the analysis of only 24 F2 plants. A much larger sample would be necessary to confirm these results.

In conclusion, the results of the experiments discussed in this chapter suggest that, introgressive hybridisation can occur between S.squalidus and S.vulgaris, and may explain the emergence of the radiate variety of *S.vulgaris* in Britain. Non-host resistance may be transferred from *S.squalidus* into *S.vulgaris* and may be determined by a number of genes.

CHAPTER 7. THE MILDEW RESISTANCE PHENOTYPES OF TRIAZINE RESISTANT GROUNDSEL LINES.

PAGE NUMBER

7.1 Introduction

As a result of the wide-spread deployment of certain agro-chemicals, to control plant and animal pests, their usefulness has been undermined by the development of resistance in target species. Resistance in plant communities, to herbicides previously successful in controlling them, is one such example.

A herbicide which is able to kill most individuals in a target population imposes very strong selection pressure for the development of resistant lines within that population. Because the target species are often weed species, predominately inbreeding, with short generation times, and capable of great fecundity, natural selection can produce changes rapidly. Therefore, if a single herbicide is used over a wide area, unless it achieves a 100% kill, which seems unlikely, those individuals which have resistance are capable of producing further generations of weed plants. With each repeated application of the herbicide the selection pressure for resistant lines increases. This applies equally if resistance is achieved by a new gene mutation, or by the selection of a gene already present in the gene pool, but present in the population at a very low level.

One of the first reports of herbicide resistance concerned the use of two triazine compounds, simazine and atrazine, to control S.vulgaris (Ryan 1970). In 1968 he found biotypes of

S.vulgaris growing in a nursery site in America, where triazines were heavily used, which were resistant to both simazime and atrazine. Since then, an increasing number of species have been reported to possess biotypes resistant to triazines, and by 1985 the number had risen to 38 (Gressel 1985, quoted in Madden 1989).

Triazines are semi-persistent herbicides, which are selective against broadleaf plants, and which are frequently used to control weed species in crop fields. They act by disrupting photosynthesis, and so reduce the fixation of CO_2. Herbicide molecules bind competitively with a 32kDa protein in the thylakoid membrane of the chloroplast. This protein is the normal binding site of plastoquinone, the electron acceptor for the reaction centre of photosystem two (Kyle and Ohad 1986).

Resistance to triazine is thought to result from gene mutations within the chloroplast DNA of the plant, preventing the binding of herbicide molecules (Radosvich and Appleby 1973). Resistant plants are usually smaller and less leafy than susceptible plants, have a lower average biomass, and are less competitive in competition trials with other species (Madden 1989). In the absence of herbicide application these features appear to lower the fitness of the resistant plant and so it would be expected to be present in the population at low levels.

Because resistance to triazine is now widespread in populations of S.vulgaris in some areas, the question arises as to whether the mutation to resistance occurred relatively few times within a population, and was maintained in a small number of lines and spread by occasional cross pollination, or whether it occurred many times in a number of different plants in a population. One way to investigate this would be to compare the zymograms of the proteins from a sample of resistant plants. If the mutation was very infrequent, and subsequently spread by inbreeding, resistant plants should be related and their zymograms show similarities. Alternatively, if the mutation occurs frequently, it would occur in a number of different genetic backgrounds, and this would be reflected in their different zymograms.

As an alternative to direct electrophoretic evidence, resistance to mildew isolates can be assessed. If all the herbicide resistant plants from one sample area have a similar mildew resistance phenotype, then it could be assumed that the herbicide resistant mutation occurred infrequently, and was then maintained in one line. If however, the plants have a range of mildew resistance phenotypes, the herbicide resistant mutation probably arose separately in a number of different lines.

To test this hypothesis, seed was collected from a number of triazine resistant populations of S.vulgaris growing in England. Populations of plants were established from this seed, and tested for resistance to a range of mildew isolates.

7.2 Materials and methods

Seed collected from groundsel plants, in populations resistant to triazine, was supplied by A.D.Madden, of the Botany Department at Glasgow University. Seed from five different populations was obtained, each collected in 1982, from the following areas:-

TRI.1 Heathlands Farm, Berks.
TRI.2 Mortimar Hill, Berks.
TRI.3 Springells Farm, Berks.
TRI.4 Jealott's Hill, Berks.
TRI.5 Reading University.

The seed from each population was not from individual lines, but was a bulked sample from several plants. In previous experiments (A.D.Madden 1989), plants grown from the seed collections were found to have very high levels of triazine resistance. Since collection, each bulk sample has been maintained over several generations by allowing the constituent lines to inbreed.

Fifty seeds from each collection were sown, and when 14 days old, ten seedlings from each were selected at random, and transplanted into 11cm plastic pots. Three weeks after transplanting, detached leaves from the ten plants from each collection were inoculated with mildew isolates M1, M2, M3, M4, M5, and M15, and their reaction to each isolate recorded.

7.3 Results

The results of the inoculation test (table 7.1), show that each of the populations consists of a number of different resistance phenotypes, with respect to the six mildew isolates used, five in TRI.1, 7 in TRI.2, three in TRI.3, 6 in TRI.4, and three in TRI.5.

Many of the different resistance phenotypes were therefore represented several times within each population. The different plants of each resistance phenotype may have been derived from a single parent plant in the original collection. However, since the lines were only tested with six mildew isolates, it is possible that plants within each group may differ for resistance to mildew isolates not tested for. Several phenotypes were present in more than one population. For instance, plants susceptible to all isolates occurred in four out of five populations, while plants resistant to all isolates except M15, occurred in three out of the five. There were however, clear differences between the populations .

To investigate if triazine resistant plants had similar levels of resistance to the mildew isolates as do triazine susceptible plants, the resistance of the 50 triazine resistant plants, and of the 47 test lines, was compared. (table 7.2).

With the exception of reactions to isolate M3, both sets of plants had very similar reactions to the mildew isolates. This suggested that there was little difference in mildew resistance between triazine resistant and susceptible plants, in the two samples studied.

Table 7.1 Mean infection scores of triazine resistant plants.

Plant line	M1	M2	M3	Isolate M4	M5	M15	Resistance factors
TRI.1 1	3.7	4.0	4.0	4.0	2.0	3.3	none
TRI.1 2	3.7	2.7	4.3	3.7	3.0	3.3	none
TRI.1.3	3.3	4.0	2.3	3.3	3.3	0.3	R15
TRI.1.4	3.0	0.0	5.0	2.3	4.0	3.7	R2
TRI.1.5	0.0	0.0	2.3	3.0	3.3	4.0	R1,R2
TRI.1.6	3.7	2.7	3.3	3.0	5.0	4.3	none
TRI.1.7	3.3	0.3	4.0	4.3	3.0	3.3	R2
TRI.1.8	4.0	3.3	4.7	3.0	2.7	3.0	none
TRI.1.9	4.0	4.0	3.3	0.3	3.0	1.0	R4,R15
TRI.1.10	4.3	4.3	2.7	3.3	3.3	4.0	none
TRI.2.1	0.7	3.0	3.3	1.0	2.7	3.3	R1,R4
TRI.2.2	4.0	0.0	0.0	0.3	0.0	0.0	R2,R3,R4,R5,R15
TRI.2.3	3.3	0.0	0.3	0.0	0.7	0.0	R2,R3,R4,R5,R15
TRI.2.4	4.0	0.3	0.0	0.7	1.0	1.0	R2,R3,R4,R5,R15
TRI.2.5	0.0	0.0	0.0	0.0	0.0	0.0	R1,R2,R3,R4,R5,R15
TRI.2.6	4.7	0.3	4.7	5.0	5.0	5.0	R2
TRI.2.7	0.0	0.3	0.3	0.0	0.0	0.0	R1,R2,R3,R4,R5,R15
TRI.2.8	4.0	3.3	3.3	3.7	4.0	5.0	none
TRI.2.9	4.7	3.0	0.3	0.7	0.0	0.7	R15,R3,R4,R5
TRI.2.10	3.3	0.0	1.0	3.3	4.3	0.3	R2,R3,R15
TRI.3.1	3.7	4.3	3.3	3.0	3.3	4.0	none
TRI.3.2	4.0	3.7	3.7	5.0	4.7	5.0	none
TRI.3.3	4.3	1.0	3.7	3.7	1.0	0.3	R2,R5,R15
TRI.3.4	0.3	0.0	3.0	3.7	3.3	4.3	R1,R2
TRI.3.5	4.3	3.3	3.7	5.0	4.7	4.7	none
TRI.3.6	3.7	3.7	3.3	4.0	4.3	3.7	none
TRI.3.7	3.7	2.7	3.3	3.3	4.3	4.3	none
TRI.3.8	3.7	3.7	3.7	2.7	3.0	3.7	none
TRI.3.9	3.7	5.0	2.3	3.0	4.3	4.0	none
TRI.3.10	4.3	3.7	4.7	2.7	3.7	2.3	none

Table 7.1 (cont.) Mean infection scores of triazine resistant plants.

Plant line	M1	M2	M3	M4	M5	M15	Resistance factors
TRI.4.1	4.0	0.7	0.3	0.0	0.3	0.7	R2,R3,R4,R15
TRI.4.2	0.7	0.3	0.0	0.0	3.7	4.0	R1,R2,R3,R4
TRI.4.3	0.0	0.3	1.0	1.0	0.0	3.3	R1,R2,R3,R4,R5
TRI.4.4	3.3	4.0	4.0	3.7	3.7	4.0	none
TRI.4.5	4.0	3.7	4.0	3.7	0.3	0.0	R5,R15
TRI.4.6	5.0	4.0	3.3	3.3	3.3	0.0	R15
TRI.4.7	3.3	3.0	4.0	3.3	3.7	3.3	none
TRI.4.8	4.0	3.7	5.0	4.7	2.3	4.0	none
TRI.4.9	3.3	3.3	3.7	4.7	5.0	4.0	none
TRI.4.10	3.0	4.7	3.3	5.0	3.7	0.7	R15
TRI.5.1	1.3	0.0	2.3	0.3	4.3	0.3	R2,R4,R15
TRI.5.2	3.3	0.3	3.3	0.7	0.0	0.3	R2,R4,R5,R15
TRI.5.3	2.3	0.0	2.0	1.0	1.0	0.0	R2,R4,R5,R15
TRI.5.4	3.3	0.0	2.7	0.0	0.0	0.0	R2,R4,R5,R15
TRI.5.5	3.3	0.0	0.7	1.0	0.0	0.0	R2,R3,R4,R5,R15
TRI.5.6	2.0	0.0	4.0	0.0	0.0	0.0	R2,R4,R5,R15
TRI.5.7	3.0	0.0	3.3	0.0	0.3	0.7	R2,R4,R5,R15
TRI.5.8	2.7	0.0	4.0	0.0	0.0	0.0	R2,R4,R5,R15
TRI.5.9	2.3	1.0	3.0	1.0	0.3	0.3	R2,R4,R5,R15
TRI.5.10	4.3	0.0	4.3	0.3	0.0	0.0	R2,R4,R5,R15

Table 7.2 Comparison of the virulence of isolates on triazine resistant plants, and on the 47 test lines.

Isolate	% of plants virulent on	
	47 test lines	50 triazine resistant lines
M1	+	86
M2	60	50
M3	50	78
M4	70	58
M5	64	64
M15	53	52

+ These plants were not inoculated with isolate M1.

7.4 Discussion

The diversity of mildew resistance phenotype, both within and between herbicide resistant populations, suggests a high rate of mutation to triazine resistance in many lines within the population, rather than a single or few events. This has resulted in the establishment of resistance in a large number of different genetic backgrounds. Because of the strongly inbreeding nature of groundsel these genotypes would then be perpetuated. In the present sample of only 50 plants the mutation was present in 16 different resistance backgrounds.

This result was surprising, since the mutation to triazine resistance occurs in chloroplast DNA, believed to evolve very slowly in higher plants. Zurawski and Clegg (1987), estimated the mutation rate of chloroplast DNA at only $1.5\ 10^{-9}$ substitutions per site per year, as opposed to a much higher rate for nuclear DNA. It is, however, possible that the protein involved in triazine resistance has a much higher turnover rate than chloroplast DNA as a whole, because of frequent damage caused by high light levels (Kyle and Ohad 1986). This would allow for the possibility of increased mutation.

If triazine resistance is determined by a frequently occurring mutation in maternally inherited DNA, it will spread much faster in the population than if it were determined by nuclear genes. This must pose serious problems for the control of weed species using single herbicides only.

Frequent changes of herbicides to control weeds would be effective, providing the level of resistance to each individual herbicide decreases with a decline in its use. This will in part depend on the method of inheritance of resistance, but also may depend on the selective advantage of the herbicide susceptible plant over the resistant plant, in the absence of herbicide.

There is evidence, however, that there may be no such advantage. Radosevich and Appleby (1973), found that in the absence of simazine, resistant biotypes were able to photosynthesise more efficiently than susceptible ones. They suggested this was due to an increased rate of turnover of the chlorophyll molecule in the resistant biotype. Watson _et al_. (1987), found that triazine resistant groundsel lines had better seed survivorship, and shorter germination times, than did triazine susceptible lines. Madden (1989), also observed shorter germination times for triazine resistant groundsel lines, as opposed to susceptible ones. All three characters are important features of a weed species survival strategy, and would be likely to improve its overall fitness.

These findings suggest that even when a herbicide is used less frequently, or in combination with others, resistant biotypes may persist because they possess physiological advantages over susceptible ones, possibly due to pleiotropic effects. Whether these advantages outweigh the disadvantages of lower biomass, discussed earlier, is uncertain. Other factors may be involved in determining the overall fitness of herbicide resistant plants, otherwise they might be expected to have increased in frequency much more than they have done. Long term studies of the relative frequencies of the resistant and susceptible biotypes, in areas which have a history of triazine use, and in areas where they have never been used, are needed to resolve this question.

CHAPTER 8. GENERAL CONCLUSIONS.

8.1 General conclusions.

The enormous variation of both host and pathogen within this pathosystem, detected by previous workers, has been confirmed by the work of this project. *S.vulgaris* appears to possess a very large number of resistance genes, to which there must be correspondingly large numbers of virulence genes in the pathogen. How such variation can be maintained in the population is unclear; certainly the predominance of inbreeding in the host and asexual reproduction in the parasite will perpetuate individual genotypes, but must there not be some selection pressure against possessing so many functioning resistance genes, a physiological load?

The breeding experiments confirmed that resistance to isolates of *E.fischeri* in *S.vulgaris* is determined by one or few major genes, and may involve complicated epistatic relationships with genes at other loci. Again there is complication where a simpler explanation might have been expected. Resistance genes were linked in some of the plant lines, and within these linkage assemblages there were smaller groups of more tightly linked gene families. These may have arisen through tandem duplication of one gene, or they may be genetically unrelated loci. An answer to this question and many others awaits a molecular genetics analysis.

On the question of the origin of radiate *S.vulgaris* in this country, the results in chapter six support the view that this varient has arisen through introgression with *S.squalidus*. The suggestion is also made that non-host resistance, when incorporared into *S.vulgaris*, is effective against *E.fischeri*, and may be determined by several genes. This raises interesting possibilities for the use of non-host resistance in crop breeding programmes, where it may prove to be more durable than host resistance.

Finally, the use of resistance genes to genetically differentiate plants from populations of triazine resistant *S.vulgaris* revealed that mutations to herbicide resistance have possibly occurred at a relatively high frequency in a large number of separate individuals, rather than as a single rare event in one genotype, which then increased by selection. This was unexpected, it had previously been thought that such mutations would be rare events, because they occurred in chloroplast DNA where the mutation rate of DNA is relatively low (Radosevich and Appleby 1973).

In common with other workers such as Abbott (1976 1986), Ingram (1977) and Hull (1974, 1976), *S.vulgaris* has been found to be a very useful "laboratory plant". It may already be one of the best understood of all wild plant species, in terms of its physiology (Ben-Kalio 1979), reproductive strategy (Abbott 1985) and genetics of mildew resistance

(Harry 1980 and Clarke _et al_. 1987). As molecular biology techniques are developed which enable the analysis of complex genomes, _S.vulgaris_ may have many attractions as a model species, for research on many aspects of the genetics of host pathogen interactions.

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APPENDIX

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Table 4.1 Cross 1, Mean Infection score of 107 F2 Plants tested against 4 isolates.

Plant	Isolate				Plant	Isolate			
no.	M2	M3	M5	M11	no.	M2	M3	M5	M11
1	0.3	3.3	0.0	0.0	46	4.3	3.7	4.0	3.3
2	0.0	0.0	0.0	0.0	47	0.0	0.3	0.3	0.7
3	3.0	3.0	5.0	2.7	48	0.0	0.0	0.0	0.3
4	3.0	3.3	0.3	4.3	49	3.0	5.0	5.0	4.7
5	0.0	0.3	0.3	0.7	50	0.3	0.3	0.7	0.0
6	0.0	2.7	0.0	0.0	51	0.3	0.7	0.0	0.0
7	0.0	0.0	0.0	0.3	52	4.3	0.3	3.3	3.7
8	0.0	0.7	0.3	0.3	53	3.7	3.3	0.3	0.3
9	0.3	0.3	0.0	0.0	54	3.3	3.7	3.7	3.0
10	0.0	0.0	0.3	0.3	55	0.3	0.3	0.3	0.0
11	4.0	4.3	0.3	0.3	56	0.0	0.3	0.7	0.0
12	3.7	0.3	3.0	4.3	57	0.3	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	58	0.0	0.3	0.3	0.0
14	0.3	0.0	0.0	0.0	59	4.0	3.7	3.7	3.0
15	0.0	0.0	0.3	0.0	60	0.3	0.0	0.0	0.0
16	3.0	0.3	3.7	3.7	61	0.0	0.0	0.0	0.0
17	2.7	3.0	0.0	0.3	62	0.3	0.0	5.0	4.3
18	0.0	0.3	0.7	0.3	63	2.3	3.3	0.3	0.7
19	0.0	0.3	0.0	0.0	64	0.0	0.0	0.0	0.0
20	0.0	0.3	0.3	0.3	65	0.3	0.3	0.0	0.0
21	0.3	0.7	0.3	0.0	66	0.0	0.3	0.0	0.0
22	0.3	0.0	0.0	0.0	67	0.0	0.0	0.0	0.0
23	0.3	0.3	0.0	0.0	68	0.3	0.7	0.3	0.0
24	2.3	3.3	3.7	3.3	69	4.3	0.0	3.3	3.0
25	0.3	0.0	0.0	0.0	70	0.0	0.0	0.3	0.3
26	0.3	0.0	0.0	0.0	71	0.3	0.3	0.0	0.0
27	3.7	3.3	0.3	0.3	72	0.0	4.3	0.0	0.0
28	3.0	0.0	3.3	3.7	73	0.0	0.0	0.0	0.0
29	0.3	0.0	0.0	0.0	74	0.0	0.3	3.3	3.7
30	0.7	1.0	0.3	1.0	75	0.3	0.3	0.0	0.0
31	0.3	0.0	0.0	0.0	76	1.0	0.0	0.3	0.0
32	4.3	3.3	0.3	0.3	77	1.0	0.3	0.0	0.0
33	4.3	0.0	1.0	3.3	78	4.3	3.7	4.0	1.0
34	0.0	2.3	0.0	0.0	79	0.3	0.0	0.7	0.0
35	0.0	0.3	0.3	0.3	80	4.3	5.0	4.7	4.3
36	0.0	1.0	0.3	0.3	81	0.0	0.0	0.3	0.0
37	2.3	2.7	0.0	4.3	82	0.0	0.0	0.3	0.7
38	0.0	0.3	0.0	0.0	83	4.0	3.3	3.7	3.3
39	0.0	0.0	0.3	0.3	84	3.3	3.0	3.7	3.3
40	3.0	3.7	3.7	3.7	85	0.0	0.0	0.0	0.3
41	0.3	4.7	0.3	0.0	86	2.3	0.0	0.0	0.0
42	4.0	4.3	5.0	5.0	87	0.0	0.7	0.0	0.0
43	0.0	0.3	0.0	0.0	88	0.0	0.3	0.3	0.3
44	0.3	0.0	0.0	0.0	89	0.0	4.0	0.3	0.7
45	4.3	4.3	4.0	4.7	90	0.0	0.3	3.0	0.0

Table 4.1 (cont.) Cross 1, Mean Infection score of 107 F2 plants tested against 4 isolates.

Plant		Isolate			Plant		Isolate		
no.	M2	M3	M5	M11	no.	M2	M3	M5	M11
91	0.0	0.0	0.3	0.0	100	3.3	0.0	0.3	0.3
92	0.0	0.0	0.0	0.0	101	0.3	1.0	1.0	0.7
93	0.0	0.3	0.3	0.0	102	0.3	0.3	0.7	0.3
94	0.3	1.0	0.3	0.0	103	0.3	0.0	0.0	0.0
95	0.0	0.0	0.0	0.7	104	4.3	4.0	4.7	4.7
96	0.0	0.3	0.3	0.7	105	0.0	0.3	0.0	0.3
97	0.3	4.0	2.3	0.0	106	0.0	0.0	0.0	0.0
98	0.0	0.0	1.0	1.0	107	0.0	1.0	4.0	0.3
99	1.0	0.3	0.3	0.3					

Table 4.2 Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant	Isolate						
Line	M2	M3	M4	M5	M13	M14	M16
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.7	0.0	0.0	0.0
3	0.0	0.3	0.3	0.7	0.0	0.0	0.3
4	0.0	0.3	0.3	0.7	0.0	0.0	0.0
5	3.0	3.7	3.3	3.3	3.7	5.0	3.7
6	1.0	3.7	4.0	0.0	3.3	5.0	1.0
7	0.3	0.0	0.0	0.3	0.0	0.0	0.7
8	4.0	5.0	5.0	0.0	0.0	4.3	4.3
9	0.0	0.0	0.0	1.0	0.0	0.0	0.3
10	0.3	0.0	0.0	0.7	0.0	0.0	0.3
11	3.0	0.0	0.0	0.7	0.7	0.0	0.0
12	0.3	0.0	0.3	0.3	0.0	0.0	0.7
13	0.0	0.0	0.0	0.0	1.0	0.0	0.7
14	0.0	0.3	0.0	0.0	0.0	0.0	0.0
15	0.0	3.3	4.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	0.7	0.0	2.3	0.0	0.0	0.0	0.0
18	2.3	0.0	0.3	0.3	2.3	4.3	3.6
19	0.0	0.0	0.0	0.0	0.7	0.0	0.3
20	3.3	3.7	3.7	4.3	2.6	3.6	3.3
21	0.0	0.3	0.7	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	3.3	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	3.3	0.0	0.0	0.0	0.3
25	0.0	0.3	0.0	0.7	0.0	0.0	0.7
26	4.3	3.3	4.3	3.6	4.0	0.0	3.3
27	3.0	3.7	4.0	3.3	0.0	0.0	0.0
28	0.0	0.3	3.3	0.7	0.0	0.0	0.0
30	5.0	3.7	4.0	4.3	3.0	3.7	4.3
31	4.0	4.6	3.6	4.0	3.6	5.0	0.0
32	2.0	0.0	0.0	0.0	0.3	0.0	0.3
33	3.0	0.0	3.7	4.6	4.0	3.6	0.3
34	4.0	3.6	3.6	4.3	2.3	3.3	3.7
35	0.7	0.3	0.0	0.0	1.0	1.0	0.0
36	0.7	0.0	0.0	0.0	0.0	0.3	0.0
37	4.0	3.3	4.0	5.0	5.0	3.3	4.0
38	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	0.3	0.0	0.7	0.0	0.0	0.0	0.0
40	0.0	4.0	4.3	4.0	4.3	0.0	0.3
41	0.0	0.0	0.0	0.0	2.3	0.0	1.0
42	3.3	4.0	3.6	0.0	3.0	3.6	0.7
43	0.7	0.0	0.3	0.3	0.3	3.3	0.0
44	0.0	0.0	0.0	0.0	0.0	0.0	0.0
45	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4.2 (cont.) Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant Line	Isolate						
	M2	M3	M4	M5	M13	M14	M16
46	0.7	0.0	0.0	0.0	0.0	0.0	0.7
47	3.7	3.3	0.0	3.6	4.0	4.7	3.7
48	0.0	0.0	0.0	0.0	0.0	0.3	0.7
49	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50	0.0	0.3	0.0	0.0	0.0	0.0	0.3
51	0.7	4.3	4.7	0.0	5.0	0.0	2.7
52	0.3	0.0	0.0	0.0	0.0	0.0	0.0
53	0.3	0.0	0.0	0.3	0.0	0.0	0.0
54	0.0	0.0	0.0	1.0	1.0	0.0	0.3
55	0.0	0.0	0.0	0.0	0.7	0.0	0.3
56	3.0	0.3	0.0	0.0	0.7	0.0	0.0
57	0.0	0.0	0.0	0.0	0.0	0.0	0.0
58	0.0	0.0	1.0	0.0	0.0	0.0	0.3
59	3.7	4.0	3.7	2.7	3.0	4.5	3.0
60	5.0	4.0	0.0	3.7	3.3	3.3	3.7
61	0.0	0.0	0.0	0.0	0.0	0.7	0.3
62	0.0	0.3	0.0	3.3	0.0	0.3	0.7
63	0.0	0.0	0.0	0.0	0.7	0.7	0.0
64	0.0	0.0	0.0	0.7	0.3	0.3	0.3
65	0.3	0.0	0.0	0.3	0.6	0.0	0.0
66	0.0	0.0	0.0	0.0	0.0	0.0	0.3
67	0.0	0.0	0.0	0.3	0.3	0.0	0.0
68	4.7	5.0	3.7	3.3	4.3	5.0	0.0
69	0.0	0.0	0.0	0.0	0.0	0.0	0.0
70	0.0	0.0	0.3	0.0	0.0	0.0	3.0
71	0.0	0.0	0.0	0.0	0.0	0.0	0.3
72	5.0	4.0	4.3	0.0	4.3	4.7	0.0
73	0.0	0.0	0.0	0.0	0.0	0.7	0.0
74	0.0	0.0	0.3	0.0	0.0	0.7	0.0
75	5.0	3.7	0.3	4.3	0.0	4.3	3.7
76	2.7	4.0	3.7	3.7	4.0	0.0	3.7
77	0.7	0.0	0.0	5.0	0.0	0.3	0.0
78	0.0	0.0	0.0	0.0	0.0	0.0	0.0
79	0.0	0.0	0.0	0.0	0.3	1.7	0.0
80	0.7	0.0	0.0	0.7	0.0	0.3	0.0
81	0.0	0.0	0.0	0.0	0.0	0.0	0.3
82	0.0	0.0	0.3	0.7	0.0	0.0	0.3
83	0.0	0.0	0.3	0.0	0.0	1.0	0.3
84	4.3	3.7	0.3	5.0	5.0	5.0	3.3
85	4.7	3.0	4.3	5.0	3.7	4.7	4.3
86	0.0	0.0	3.3	3.3	0.3	0.0	0.7
87	3.0	0.0	0.0	3.7	4.7	0.0	4.7
88	0.0	0.3	0.0	0.0	0.0	0.0	0.0
89	0.0	0.0	0.0	0.7	0.0	0.0	0.0
90	0.3	0.0	0.3	3.0	0.0	0.0	0.0

Table 4.2 (cont.) Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant	Isolate						
Line	M2	M3	M4	M5	M13	M14	M16
91	0.0	0.3	0.0	0.0	1.0	0.0	0.3
92	0.0	0.0	0.3	0.3	0.3	1.0	3.0
93	0.0	0.3	0.0	0.0	3.3	1.7	0.0
94	0.0	0.0	0.0	0.0	0.0	0.7	0.0
95	0.0	0.0	0.0	0.0	0.0	0.3	0.0
96	0.0	0.0	0.0	0.0	0.3	0.0	3.7
97	0.3	0.0	0.3	0.0	0.0	0.0	0.7
98	0.0	0.0	0.3	0.3	0.7	0.3	0.7
99	0.0	0.0	0.0	3.7	0.0	0.0	0.0
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
101	0.3	0.0	0.3	0.7	0.0	0.7	0.3
102	0.7	0.0	0.0	0.0	0.0	0.0	0.3
103	0.0	0.0	0.0	0.7	0.0	0.0	0.0
104	3.7	3.3	0.3	3.3	4.3	0.0	3.3
105	0.0	0.3	0.0	0.0	0.3	0.7	0.0
106	4.3	3.6	4.3	3.7	3.7	5.0	5.0
107	0.0	0.3	0.3	0.7	0.0	0.0	0.7
108	0.0	0.0	0.3	0.0	0.0	0.0	0.7
109	0.7	0.0	0.0	0.0	0.0	0.0	0.7
110	3.0	0.3	0.0	0.0	4.3	4.3	4.3
111	0.7	0.0	0.3	0.0	0.0	1.0	0.3
112	0.0	0.3	0.0	0.0	0.0	0.0	0.0
113	0.3	0.0	0.0	2.3	0.0	0.0	0.0
114	0.0	0.0	0.0	0.0	1.0	0.3	0.0
115	1.0	0.0	0.7	1.0	1.0	0.0	0.0
116	0.7	0.0	0.0	0.7	0.7	0.0	0.3
117	0.0	0.0	0.0	0.0	0.0	0.0	0.0
118	0.0	0.0	0.3	0.0	0.0	0.0	0.0
119	4.0	3.0	0.0	4.3	5.0	4.0	3.7
120	0.7	0.0	0.3	0.0	0.0	0.7	0.7
121	0.3	0.0	0.7	0.0	0.0	0.0	0.0
122	3.3	4.6	4.0	4.0	4.7	4.7	0.0
123	0.0	0.0	0.0	0.7	0.0	0.0	0.0
124	0.3	0.0	0.3	0.7	0.0	0.0	0.0
125	0.7	0.0	0.0	0.0	0.0	0.0	0.3
126	0.0	0.0	0.0	0.0	0.0	0.0	0.0
127	4.3	0.0	3.7	3.3	4.3	4.3	4.0
128	0.0	0.0	0.0	0.0	0.0	0.0	0.0
129	0.0	0.0	0.0	0.0	0.0	0.0	0.0
130	0.0	0.0	0.3	0.0	0.0	0.0	0.0
131	0.7	0.0	0.0	0.0	0.7	0.0	0.3
132	0.0	0.0	0.0	0.0	0.0	0.0	0.3
133	0.0	0.0	0.7	0.0	0.0	0.0	0.0
134	0.0	0.0	0.3	0.0	0.0	0.0	0.7
135	0.0	0.0	0.0	0.0	0.0	0.0	3.7

Table 4.2 (cont.) Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant Line	Isolate M2	M3	M4	M5	M13	M14	M16
136	0.3	0.0	0.0	3.3	0.0	0.0	0.3
137	5.0	3.7	0.0	0.0	3.7	4.3	0.3
138	0.0	0.0	0.0	0.0	0.0	0.7	0.0
139	0.0	0.3	0.0	0.0	0.0	0.0	0.3
140	0.0	0.0	0.0	0.0	0.0	0.0	1.0
141	0.0	3.3	0.0	0.0	4.7	4.0	0.0
142	0.0	0.0	0.3	0.7	0.0	0.0	0.0
143	0.3	0.0	0.0	0.0	0.0	0.0	0.0
144	0.0	0.0	0.0	0.3	0.0	0.0	2.3
145	0.3	3.7	0.0	0.3	0.0	1.0	0.0
146	4.3	4.7	3.7	0.0	4.3	3.7	3.3
147	0.0	0.0	0.0	0.7	0.0	0.0	0.7
148	0.0	0.0	0.3	0.0	0.0	0.0	0.0
149	4.7	4.7	0.7	0.0	4.0	4.3	4.5
150	5.0	4.0	0.0	0.0	5.0	0.0	0.7
151	0.0	0.7	0.0	0.3	5.0	0.7	0.0
152	0.3	0.0	0.7	0.0	0.0	0.0	0.0
153	0.0	0.0	0.3	0.0	0.0	0.0	0.0
154	0.0	0.0	0.3	0.0	0.0	0.0	0.0

Table 4.2 (cont.) Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant Line	Isolate M2	M4	M13	M14	M16
155	0.3	0.0	0.0	0.3	0.0
156	0.3	0.0	0.0	0.0	0.3
157	2.0	2.0	3.0	4.3	3.0
158	3.3	0.0	1.6	3.6	0.7
159	0.0	0.0	0.0	4.3	0.0
160	0.0	0.0	0.3	0.3	0.0
161	1.6	0.0	1.0	5.0	0.3
162	0.0	0.0	0.3	0.0	0.3
163	0.0	0.0	0.3	0.0	0.0
164	0.0	0.0	0.7	0.0	0.0
165	0.3	0.0	0.0	0.0	0.0
166	1.0	0.0	1.0	0.0	0.0
167	1.3	1.7	0.3	4.7	4.5
168	0.0	0.3	0.0	0.0	0.0
169	0.0	0.0	0.0	0.3	0.0
170	0.0	0.0	0.0	0.0	0.0

Table 4.2 (cont.) Cross 1, Mean Infection scores of 214 F2 plants tested against 5 isolates, and 154 F2 plants tested against 2 isolates.

Plant			Isolate		
Line	M2	M4	M13	M14	M16
171	0.0	0.0	0.0	0.0	0.0
172	0.3	0.7	0.0	3.0	2.3
173	0.3	0.0	0.0	0.0	0.0
174	0.0	0.0	0.0	0.0	0.0
175	0.0	0.0	0.7	0.0	0.0
176	0.0	0.0	0.0	0.0	0.0
177	0.0	0.0	0.0	0.0	0.7
178	0.7	0.0	0.0	0.0	0.0
179	0.3	0.7	0.3	0.0	0.0
180	0.0	0.0	0.0	0.0	0.0
181	0.0	0.0	0.3	0.3	0.0
182	0.0	0.0	0.0	0.0	0.0
183	2.7	3.0	4.7	3.7	4.7
184	0.0	0.0	0.0	0.7	0.3
185	3.0	2.3	3.5	0.0	0.0
186	0.0	0.0	0.0	0.3	0.0
187	4.7	0.0	4.7	0.7	0.0
188	0.0	0.3	0.0	0.0	0.3
189	0.0	0.0	1.0	0.0	0.0
190	0.0	0.0	0.0	0.0	0.7
191	0.0	0.0	0.0	0.0	0.3
192	0.3	0.0	0.0	0.0	0.0
193	0.3	0.0	0.0	0.3	0.0
194	4.7	3.3	4.7	4.7	3.7
195	0.0	0.0	0.0	0.0	0.3
196	4.3	5.0	4.3	3.7	3.7
197	0.3	0.7	0.0	0.0	0.3
198	0.0	0.0	0.0	0.0	0.3
199	3.0	2.3	2.0	4.7	0.0
200	0.0	0.3	1.0	0.0	0.0
201	5.0	1.7	3.7	4.7	3.3
202	0.0	0.0	0.0	0.7	0.0
203	0.3	0.0	2.0	0.0	0.0
204	0.7	1.7	0.3	4.7	0.0
205	1.7	0.0	0.0	0.0	0.0
206	1.0	0.3	0.3	5.0	4.0
207	1.3	0.3	0.0	0.3	0.7
208	0.0	0.0	0.3	0.3	0.7
209	0.3	0.0	3.7	0.0	0.0
210	0.3	0.0	0.3	0.0	0.0
211	0.0	0.0	3.3	0.0	0.0
212	0.0	0.0	0.0	0.0	0.0
213	0.0	0.0	0.0	0.0	0.3
214	0.0	0.0	0.7	0.0	0.0

Table 4.3 Cross 2. Mean infection score of 107 plants tested against 4 isolates.

Plant		isolate			Plant		isolate		
no.	M2	M3	M4	M5	no.	M2	M3	M4	M5
1	4.6	5.0	3.6	4.6	51	4.3	4.6	4.6	4.3
2	4.0	4.6	4.3	4.6	52	3.3	4.3	3.3	3.6
3	4.0	4.0	4.0	5.0	53	3.0	4.0	5.0	4.6
4	4.0	4.6	3.0	3.0	54	4.0	4.0	3.0	4.3
5	5.0	4.6	0.0	4.6	55	4.3	4.3	2.6	4.0
6	4.3	5.0	4.0	4.3	56	5.0	4.6	3.3	4.0
7	3.3	2.3	5.0	3.0	57	4.6	4.3	4.6	3.0
8	3.0	4.3	2.3	4.3	58	4.6	4.0	5.0	0.0
9	4.3	5.0	4.3	5.0	59	3.0	4.3	0.3	4.6
10	3.6	3.0	4.6	4.6	60	2.3	4.3	4.6	5.0
11	3.0	4.3	4.3	4.0	61	5.0	2.6	2.3	4.0
12	4.0	4.6	4.6	3.6	62	4.6	3.6	4.0	4.6
13	4.3	2.6	4.3	2.3	63	3.6	3.0	4.0	3.3
14	4.6	5.0	3.6	4.6	64	3.6	0.3	4.0	3.6
15	3.0	4.6	2.0	4.6	65	3.0	4.3	0.3	4.6
16	0.0	0.0	0.3	0.0	66	5.0	3.6	4.0	3.3
17	4.3	0.0	0.0	4.3	67	0.6	3.6	4.6	4.6
18	4.0	3.3	4.6	3.6	68	2.6	3.3	4.3	3.6
19	3.6	0.3	0.0	3.3	69	4.6	4.0	3.0	3.6
20	4.3	2.6	4.0	4.6	70	0.0	0.0	4.6	4.6
21	4.0	4.3	0.6	2.6	71	5.0	0.6	2.6	0.0
22	3.6	4.6	5.0	5.0	72	4.6	4.0	5.0	4.6
23	4.6	1.6	2.6	4.3	73	4.0	3.6	4.3	5.0
24	4.0	3.0	4.3	4.6	74	3.6	4.0	2.6	5.0
25	4.6	4.6	4.6	4.0	75	4.0	4.0	5.0	2.6
26	4.3	5.0	4.0	4.0	76	3.6	0.0	4.3	0.3
27	3.0	4.6	4.6	4.0	77	4.3	3.0	3.3	4.6
28	4.0	5.0	0.0	2.3	78	4.3	0.0	4.0	0.0
29	4.6	3.0	3.6	4.0	79	3.6	0.0	4.3	4.3
30	4.6	4.0	4.3	4.3	80	5.0	4.3	4.0	2.0
31	4.3	4.0	4.3	4.6	81	4.6	4.3	4.0	3.0
32	3.0	4.3	2.3	4.6	82	3.6	4.0	3.6	5.0
33	5.0	4.3	0.0	3.0	83	4.3	0.0	4.3	4.3
34	4.0	3.3	5.0	3.3	84	2.3	0.0	4.3	4.0
35	0.3	5.0	0.3	4.6	85	4.0	4.6	4.0	4.0
36	4.3	0.6	4.0	4.3	86	5.0	3.0	0.6	0.0
37	4.0	5.0	3.6	3.0	87	5.0	3.0	3.6	4.3
38	4.6	0.0	0.0	3.3	88	4.0	4.6	4.3	4.0
39	3.3	5.0	3.3	3.6	89	4.3	4.0	4.6	4.6
40	4.0	5.0	4.3	3.0	90	5.0	5.0	3.3	4.6
41	4.3	4.3	4.0	4.6	91	4.3	4.0	4.3	5.0
42	4.6	4.6	3.6	4.0	92	3.6	2.6	3.0	0.0
43	4.6	4.0	4.6	3.6	93	4.0	4.0	4.6	4.3
44	4.0	4.0	3.0	4.6	94	3.6	0.3	3.3	4.0
45	4.0	4.0	4.6	4.3	95	4.0	4.3	2.3	0.3
46	3.6	4.0	4.3	4.0	96	5.0	3.3	2.3	4.6
47	4.3	3.3	0.3	3.6	97	3.6	5.0	3.3	0.0
48	4.6	5.0	5.0	3.3	98	3.6	3.0	4.0	2.3
49	5.0	4.3	3.0	3.0	99	3.6	3.0	4.0	2.3
50	4.6	4.3	4.6	4.6	100	4.0	4.0	0.3	0.0

Table 4.3 Cross 2,(continued).

Plant no.	isolate M2	M3	M4	M5	Plant no.	isolate M2	M3	M4	M5
101	4.6	4.3	4.0	4.0	105	4.6	5.0	4.6	3.0
102	4.0	3.3	2.6	4.6	106	4.3	4.0	4.0	0.0
103	3.3	2.3	5.0	0.0	107	4.3	5.0	3.3	4.0
104	4.3	4.3	4.6	2.6					

Table 4.4 Cross 2. Mean infection scores of 107 plants tested against 4 isolates.

Plant	isolate				Plant	isolate			
no.	M2	M3	M4	M5	no.	M2	M3	M4	M5
1	4.3	4.0	3.6	4.3	51	4.0	5.0	4.3	0.3
2	3.6	4.6	4.0	4.0	52	4.3	0.0	3.6	3.0
3	0.3	0.0	4.3	4.0	53	0.3	3.3	5.0	4.6
4	4.6	4.0	4.6	4.6	54	3.3	3.0	4.6	3.6
5	5.0	4.3	5.0	3.3	55	5.0	4.0	4.0	0.0
6	5.0	5.0	5.0	3.3	56	0.6	5.0	4.6	4.0
7	5.0	5.0	4.6	5.0	57	4.0	4.6	4.0	0.0
8	0.0	0.3	3.6	0.0	58	4.3	3.3	4.3	4.3
9	4.6	4.3	3.6	0.0	59	5.0	4.0	3.3	4.6
10	4.3	4.6	3.6	4.6	60	5.0	5.0	5.0	4.3
11	5.0	5.0	5.0	4.0	61	5.0	4.0	5.0	3.6
12	5.0	3.0	4.6	3.6	62	0.0	4.3	4.3	0.3
13	4.6	4.6	4.6	3.3	63	2.0	4.6	4.0	4.6
14	3.6	4.0	5.0	4.6	64	5.0	2.0	4.0	4.0
15	4.0	4.6	3.3	0.0	65	4.6	0.0	3.0	5.0
16	5.0	5.0	5.0	3.6	66	4.0	4.3	3.3	2.6
17	4.6	0.0	0.0	4.0	67	5.0	2.0	4.0	5.0
18	0.3	0.3	0.0	0.0	68	2.6	2.6	5.0	3.3
19	4.0	0.0	0.0	2.6	69	5.0	5.0	4.6	5.0
20	5.0	4.0	3.6	3.6	70	4.6	4.6	4.0	0.0
21	3.3	4.3	3.3	5.0	71	4.3	3.0	4.6	3.6
22	0.0	4.3	5.0	5.0	72	3.3	2.0	3.6	4.3
23	5.0	4.0	2.6	5.0	73	5.0	4.6	4.6	3.0
24	4.6	5.0	5.0	4.6	74	5.0	3.0	4.6	5.0
25	4.0	4.0	4.3	4.0	75	5.0	5.0	4.6	5.0
26	3.6	4.3	2.0	4.3	76	5.0	4.6	4.6	4.3
27	4.0	4.6	5.0	3.6	77	5.0	4.3	2.6	4.3
28	5.0	4.0	4.3	4.0	78	3.0	5.0	4.3	4.3
29	5.0	4.0	4.3	4.0	79	0.0	4.0	0.3	2.6
30	4.3	4.0	4.6	4.3	80	0.3	5.0	2.6	5.0
31	4.6	0.6	4.3	4.6	81	4.3	4.0	4.3	5.0
32	5.0	3.3	4.0	4.3	82	4.6	4.3	3.6	4.6
33	3.3	0.0	4.0	5.0	83	5.0	4.6	3.6	5.0
34	3.0	0.0	0.0	4.6	84	4.0	3.3	2.6	3.3
35	3.6	0.0	0.0	3.6	85	5.0	4.6	4.3	0.0
36	5.0	4.0	5.0	3.0	86	4.6	3.6	4.3	3.3
37	3.6	0.3	3.0	0.0	87	4.0	5.0	3.3	0.0
38	5.0	4.6	4.3	4.0	88	3.6	2.6	4.3	5.0
39	4.6	4.3	4.0	2.6	89	3.0	3.6	4.3	2.6
40	5.0	0.0	4.3	5.0	90	3.6	4.6	4.3	4.6
41	3.3	4.0	5.0	3.3	91	5.0	4.6	2.6	4.0
42	4.6	5.0	4.6	0.0	92	4.6	5.0	4.0	4.6
43	3.0	4.0	0.3	3.3	93	3.0	4.0	3.6	4.0
44	4.6	4.3	4.3	5.0	94	4.0	4.0	5.0	3.0
45	4.3	4.0	4.3	5.0	95	5.0	3.6	3.6	4.6
46	3.3	0.3	4.3	4.6	96	4.0	2.6	4.0	5.0
47	4.6	5.0	3.0	4.3	97	4.3	4.3	4.0	4.6
48	3.0	0.0	5.0	5.0	98	5.0	4.3	3.0	2.6
49	0.3	5.0	2.3	4.3	99	4.6	5.0	4.3	5.0
50	4.0	0.6	5.0	4.0	100	4.0	3.6	0.0	5.0

Table 4.4 Cross 2, (continued).

Plant	Isolate				Plant	Isolate			
no.	M2	M3	M4	M5	no.	M2	M3	M4	M5
101	4.6	0.6	2.3	4.6	105	5.0	3.6	3.0	4.3
102	4.3	4.0	5.0	4.0	106	2.6	4.3	4.0	4.6
103	5.0	4.0	4.0	4.6	107	4.3	4.6	4.3	3.6
104	3.6	4.0	4.6	3.6					

Table 4.5 Cross 3. Mean infection score of 107 plants tested against 4 isolates.

Plant	Isolate				Plant	Isolate			
no.	M1	M2	M5	M11	no.	M1	M2	M5	M11
1	0.0	0.3	0.3	0.3	46	0.3	0.0	0.3	0.3
2	4.7	3.3	2.3	0.0	47	0.3	0.0	3.7	0.3
3	0.0	0.0	0.3	0.0	48	0.0	0.0	4.3	0.7
4	0.7	4.7	0.3	0.3	49	0.3	4.0	0.0	0.0
5	0.3	0.3	3.3	0.0	50	0.0	0.3	4.7	0.3
6	0.3	0.3	0.3	4.3	51	0.0	0.3	4.7	0.3
7	0.7	0.0	0.0	0.0	52	0.0	0.0	0.0	0.0
8	4.3	0.0	0.0	0.0	53	3.0	0.7	4.7	0.0
9	0.0	1.0	0.0	0.0	54	0.0	3.7	0.3	0.0
10	0.0	4.0	4.3	0.0	55	0.0	3.7	0.0	4.3
11	3.3	0.3	4.0	0.0	56	3.0	3.7	4.3	0.3
12	0.7	0.0	0.3	3.3	57	0.3	0.0	0.0	0.0
13	0.3	0.0	0.0	0.0	58	0.3	4.0	0.0	0.0
14	0.0	3.7	0.0	0.0	59	0.0	0.3	0.0	3.7
15	0.3	0.0	0.0	0.3	60	0.0	0.0	3.3	0.0
16	0.0	0.0	2.7	1.0	61	0.0	0.0	0.0	0.0
17	3.3	0.0	0.0	0.0	62	0.3	0.3	0.3	4.0
18	4.0	0.0	0.0	4.3	63	3.3	0.0	3.7	0.7
19	0.3	0.3	0.3	0.3	64	0.0	0.0	0.3	0.0
20	0.0	0.0	0.0	0.0	65	0.3	0.0	0.3	0.0
21	0.0	0.0	0.0	0.0	66	0.0	0.7	4.7	0.7
22	0.0	3.7	3.3	3.3	67	0.0	0.0	4.3	0.0
23	0.7	0.0	0.3	0.0	68	0.3	0.0	0.7	4.3
24	1.0	0.3	0.0	0.0	69	0.0	3.3	0.0	0.0
25	0.7	0.3	3.7	3.7	70	0.0	0.0	0.3	0.0
26	0.3	4.3	0.0	0.0	71	0.0	0.3	0.3	0.0
27	0.3	0.7	0.0	0.3	72	3.7	0.0	3.0	0.7
28	0.0	0.3	0.0	0.3	73	0.0	0.7	3.7	0.0
29	0.0	0.3	4.0	0.0	74	0.7	0.0	0.0	4.3
30	4.0	3.7	0.0	0.0	75	0.3	3.7	1.0	0.0
31	0.0	0.0	0.0	0.0	76	0.0	0.0	0.0	0.0
32	0.7	3.3	0.0	3.0	77	0.0	0.7	0.7	3.7
33	0.0	0.3	0.0	0.0	78	0.3	0.0	0.0	0.0
34	3.7	3.7	4.0	0.0	79	0.0	0.0	3.7	0.0
35	0.3	0.0	0.3	3.3	80	0.0	0.0	0.3	3.7
36	0.3	0.0	0.3	3.3	81	3.3	0.0	0.0	0.7
37	0.0	0.0	3.3	3.0	82	0.3	0.7	0.0	0.0
38	5.0	3.7	0.0	0.0	83	0.0	0.7	0.0	5.0
39	0.3	0.0	0.0	0.0	84	0.0	0.3	0.0	0.3
40	0.0	3.5	0.3	0.0	85	0.0	2.7	0.3	0.0
41	0.3	3.0	3.3	3.7	86	0.7	0.7	3.7	0.0
42	0.3	0.7	0.0	0.7	87	3.3	0.0	0.0	4.3
43	3.7	0.0	4.0	0.0	88	0.3	0.7	0.0	0.0
44	0.0	0.3	0.3	0.0	89	0.0	0.3	0.0	0.0
45	3.3	3.3	0.7	0.0	90	2.3	0.0	0.3	0.0

Table 4.5 (cont.) Cross 3, Mean infection score of 107 plants tested against 4 isolates.

Plant	Isolate			
no.	M1	M2	M5	M11
91	0.7	0.3	0.7	0.7
92	0.0	0.0	3.7	0.0
93	0.0	3.3	0.0	0.0
94	0.0	0.0	0.0	0.0
95	0.0	0.0	0.0	0.0
96	0.7	3.7	0.3	0.0
97	0.0	0.0	4.3	0.3
98	0.7	0.0	0.3	0.0
99	0.0	4.3	3.3	0.0
100	0.0	0.0	3.7	0.0
101	0.0	0.3	2.7	0.0
102	0.0	0.0	3.7	0.0
103	4.0	0.0	0.0	0.0
104	0.3	0.0	0.3	0.3
105	0.0	0.0	4.3	0.0
106	0.0	3.3	0.3	0.7
107	0.3	0.0	0.0	3.7

Table 4.6 Cross 3, Mean Infection scores of 214 F2 plants tested against 8 isolates.

Plant Line	Isolate							
	M2	M3	M4	M5	M13	M14	M15	M16
1	0.0	3.0	0.6	0.3	4.0	2.7	4.0	4.3
2	0.3	3.7	3.0	0.0	2.7	3.3	3.7	4.0
3	3.7	3.0	2.7	1.0	2.7	0.3	0.7	3.3
4	0.3	4.7	3.0	4.0	4.0	0.0	3.0	5.0
5	0.7	0.0	0.0	0.3	4.7	3.0	3.7	0.7
6	5.0	3.7	2.7	0.0	4.7	3.7	3.3	3.7
7	3.7	0.3	3.3	0.3	4.3	4.7	3.7	4.0
8	0.3	0.7	0.3	0.0	3.7	1.0	3.7	3.7
9	5.0	0.0	0.3	0.0	2.7	3.7	0.3	3.3
10	0.0	3.7	0.3	0.0	1.7	0.7	1.0	0.7
11	0.0	3.0	0.0	0.7	0.7	0.0	0.3	0.7
12	0.0	3.3	1.0	0.0	1.7	0.0	0.3	0.7
13	3.7	4.0	0.3	0.0	2.7	0.3	0.7	4.7
14	5.0	3.3	3.3	3.3	2.7	0.7	2.7	3.3
15	0.0	0.3	0.7	0.3	2.0	0.0	0.7	3.0
16	0.0	3.7	3.3	0.0	3.3	1.0	2.3	3.3
17	0.0	2.7	3.7	0.7	3.7	3.3	4.3	4.0
18	2.7	3.7	3.3	0.3	4.0	0.3	2.7	2.7
19	0.3	0.3	2.3	0.0	3.3	3.3	3.0	1.0
20	0.3	0.3	2.3	0.0	3.3	3.7	3.0	1.0
21	1.7	3.3	0.0	0.3	2.0	4.7	1.0	3.3
22	4.7	4.0	0.7	0.7	2.3	3.7	3.3	3.0
23	0.3	2.7	0.7	0.0	2.3	0.7	0.0	1.0
24	0.0	3.7	4.0	0.7	3.0	3.0	0.3	1.0
25	4.7	2.7	3.3	0.0	2.0	0.7	5.0	3.7
26	0.0	3.0	0.7	0.7	0.7	0.3	0.3	3.0
27	2.0	4.0	0.7	0.7	2.7	0.3	0.3	3.0
28	0.7	3.7	3.7	0.3	2.3	0.3	3.0	0.7
29	0.7	3.7	0.0	0.0	1.3	0.0	0.3	4.0
30	4.7	3.0	4.3	5.0	3.3	0.3	4.7	2.3
31	1.0	4.0	3.7	0.0	4.0	0.7	0.0	3.3
32	1.0	4.7	3.3	0.3	3.3	1.0	0.7	3.3
33	0.7	3.7	4.0	0.0	3.7	1.0	2.0	4.3
34	4.0	3.3	3.3	0.3	4.0	3.3	0.0	0.3
35	0.7	3.3	0.0	0.7	2.7	3.0	0.3	0.7
36	3.0	3.7	1.0	3.3	3.3	3.7	0.7	3.3
37	3.7	2.7	0.7	0.3	4.0	0.7	0.1	0.0
38	1.0	3.0	2.0	1.0	4.7	0.7	3.0	3.3
39	3.7	2.7	0.7	1.0	2.7	2.7	3.7	0.0
40	2.3	0.7	1.0	3.3	3.0	0.7	3.3	2.5
41	3.7	5.0	2.7	0.3	4.7	4.3	3.7	4.0
42	1.0	4.3	3.7	3.0	3.7	4.0	3.3	3.7
43	2.3	1.7	2.7	3.3	5.0	0.7	0.7	2.7
44	0.0	2.7	0.0	0.3	2.0	0.0	3.7	3.3
45	0.0	3.3	0.7	0.7	2.3	0.0	0.0	3.7

Table 4.6 Cross 3, Mean Infection scores of 214 F2
(cont.) plants tested against 8 isolates.

Plant	Isolate							
Line	M2	M3	M4	M5	M13	M14	M15	M16
46	3.3	4.0	1.0	0.7	4.3	0.7	0.0	0.7
47	5.0	0.7	0.7	3.7	2.3	0.3	3.3	3.7
48	0.3	3.7	0.7	0.7	2.7	0.3	3.3	3.7
49	3.0	4.0	4.7	0.0	3.7	5.0	0.0	0.7
50	4.0	4.7	3.0	4.3	4.0	3.3	0.0	0.7
51	0.0	2.7	1.0	0.0	3.7	3.3	0.7	2.7
52	1.0	3.0	4.3	0.0	3.0	0.7	1.0	3.7
53	0.3	3.3	1.0	0.7	4.0	3.0	0.3	4.3
54	3.0	3.7	0.7	2.7	4.0	0.7	2.3	0.7
55	2.7	4.3	0.7	4.0	2.0	4.7	2.3	0.3
56	1.0	4.0	2.3	4.0	2.7	3.3	0.3	4.0
57	1.0	0.7	0.7	3.3	3.3	3.3	0.0	0.0
58	1.0	3.3	4.7	0.3	4.0	0.7	2.3	3.7
59	3.7	4.3	3.0	3.3	3.3	0.7	0.7	4.0
60	2.0	3.3	3.3	1.0	3.7	4.0	3.7	3.3
61	3.7	4.3	2.7	0.7	4.7	3.7	0.0	3.3
62	3.0	0.7	0.0	0.7	3.3	4.0	2.3	0.7
63	1.0	1.7	0.3	4.3	3.7	4.3	4.3	3.7
64	3.0	4.3	0.3	1.0	2.7	3.3	3.3	4.7
65	0.0	4.7	4.7	0.7	4.7	0.7	0.3	3.3
66	1.0	0.7	0.0	0.3	2.7	4.0	4.0	4.3
67	4.3	2.0	0.7	3.3	4.0	3.0	4.7	5.0
68	5.0	3.3	0.7	3.0	3.7	0.0	4.0	5.0
69	4.7	3.3	3.3	3.7	3.7	0.7	3.3	0.3
70	5.0	1.0	3.3	0.7	1.3	0.7	3.0	0.3
71	5.0	1.3	3.0	0.7	3.7	0.3	3.7	3.7
72	3.7	2.7	0.7	3.0	2.0	0.3	1.0	3.0
73	3.3	3.3	2.7	1.0	3.3	0.7	0.3	3.3
74	3.3	3.0	0.7	4.0	4.7	3.7	4.0	3.0
75	4.7	3.3	0.0	3.7	3.7	3.3	3.7	2.7
76	1.0	3.0	2.7	1.0	4.0	0.7	0.3	0.3
77	0.7	2.7	0.3	0.7	2.7	0.3	0.0	0.7
78	0.0	2.7	3.0	0.7	3.7	4.0	0.0	0.7
79	5.0	0.7	0.7	1.0	3.0	3.0	4.3	4.0
80	0.7	5.0	3.0	3.7	2.3	2.7	0.7	0.7
81	0.0	2.0	0.3	3.3	3.0	3.3	0.3	3.3
82	0.0	0.3	3.0	3.0	4.0	3.0	0.0	3.0
83	0.0	2.3	0.0	0.7	3.7	0.7	3.3	0.0
84	0.0	2.7	4.0	0.0	4.7	3.3	2.0	0.7
85	1.0	4.0	2.7	3.7	4.0	3.3	3.0	2.7
86	0.3	3.0	0.7	5.0	3.3	3.0	3.3	0.7
87	0.0	0.7	0.0	1.0	3.7	2.7	2.7	1.0
88	0.0	3.3	3.3	3.0	2.7	4.0	1.0	0.3
89	0.0	3.0	0.7	3.4	4.0	3.7	3.3	0.0
90	0.3	2.7	0.7	4.0	3.3	3.0	2.7	1.0

Table 4.6 Cross 3, Mean Infection scores of 214 F2
(cont.) plants tested against 8 isolates.

Plant Line	Isolate							
	M2	M3	M4	M5	M13	M14	M15	M16
91	4.3	3.0	0.7	5.0	2.7	3.3	2.0	3.7
92	0.3	3.3	0.7	4.0	4.3	4.3	1.0	3.3
93	0.0	4.0	0.3	3.3	4.3	4.3	3.3	0.7
94	0.0	3.7	0.0	1.0	3.7	4.7	0.3	0.3
95	0.0	3.7	0.0	1.0	3.7	4.7	0.3	0.3
96	0.3	0.7	0.3	0.0	2.7	3.3	2.7	3.0
97	0.0	2.7	0.7	0.3	2.7	3.0	0.7	0.0
98	0.0	5.0	0.0	4.0	3.3	4.0	3.3	0.7
99	0.0	1.0	0.7	2.0	3.7	2.7	5.0	3.3
100	0.0	3.3	0.3	4.7	2.3	3.7	2.7	0.7
101	0.0	3.0	0.0	2.7	2.7	3.3	2.7	4.0
102	0.7	3.7	0.0	4.3	2.3	4.0	2.3	0.7
103	0.0	0.7	0.0	0.3	3.0	0.7	0.3	4.0
104	0.0	2.7	0.3	0.0	3.3	0.7	3.3	2.7
105	5.0	0.7	0.0	4.3	4.0	4.0	5.0	3.3
106	3.0	0.0	0.0	0.3	4.3	1.0	3.3	0.7
107	0.0	3.0	3.0	4.0	4.0	5.0	0.0	0.7
108	2.7	0.7	0.0	3.3	3.3	1.0	0.0	0.3
109	0.3	3.3	0.7	3.3	2.7	0.3	0.3	0.0
110	3.0	3.3	3.0	3.7	2.7	0.3	0.7	0.3
111	0.3	3.3	0.0	0.7	3.3	0.3	0.7	0.3
112	0.0	2.7	0.3	3.0	1.7	1.0	4.7	0.0
113	0.3	3.3	0.3	4.0	2.3	0.3	3.3	0.3
114	1.0	4.3	0.0	1.0	3.3	1.0	4.0	0.0
115	4.0	3.0	2.7	3.3	2.7	0.3	0.0	0.3
116	0.3	2.7	0.0	0.0	0.7	0.3	0.7	0.3
117	0.7	4.0	1.0	3.7	2.3	4.0	2.7	0.7
118	0.3	3.3	0.0	3.7	3.7	4.3	1.0	0.3
119	2.7	1.0	0.0	0.0	2.3	0.7	1.0	0.7
120	0.0	3.0	3.0	0.7	3.0	0.0	0.3	0.3
121	0.0	3.3	2.7	5.0	1.7	1.0	0.3	0.3
122	4.0	3.3	3.3	3.7	2.7	3.3	3.0	0.0
123	0.3	0.7	1.0	3.3	2.7	5.0	0.0	0.0
124	0.0	5.0	3.3	1.0	2.3	0.7	0.3	0.3
125	3.3	3.7	2.3	3.3	3.7	0.7	0.7	0.0
126	2.7	3.3	0.3	3.0	3.3	3.3	3.0	0.7
127	0.0	1.0	0.3	1.0	2.3	2.7	2.0	2.7
128	0.0	1.7	0.7	0.3	2.0	0.0	0.0	0.7
129	0.0	0.3	0.0	1.0	1.3	0.0	0.0	0.3
130	0.0	1.0	0.7	1.0	2.3	0.7	2.3	0.7
131	3.0	3.3	3.7	3.7	3.3	4.0	3.3	3.3
132	0.7	3.7	3.0	3.3	4.0	3.7	4.0	3.0
133	2.7	3.3	0.7	4.7	1.3	4.3	3.3	3.3
134	3.7	3.3	0.3	3.0	1.7	3.3	3.7	0.3
135	2.7	4.3	3.3	3.3	3.7	3.3	0.7	3.7

Table 4.6 Cross 3, Mean Infection scores of 214 F2
(cont.) plants tested against 8 isolates.

Plant	Isolate							
Line	M2	M3	M4	M5	M13	M14	M15	M16
136	0.7	0.3	3.7	0.7	1.7	1.0	0.7	0.7
137	3.7	4.0	3.3	3.3	3.3	3.7	4.7	3.3
138	3.7	3.7	0.0	0.7	3.0	1.0	0.7	0.7
139	3.0	1.0	0.7	0.0	3.0	1.0	0.7	0.7
140	2.7	3.7	3.3	3.7	4.3	3.7	3.3	3.3
141	0.7	3.7	4.0	5.0	2.3	3.0	1.0	1.0
142	5.0	5.0	0.3	3.0	3.0	3.0	0.7	3.0
143	1.3	3.7	2.3	4.7	3.7	1.0	0.0	3.7
144	2.7	3.7	0.7	3.0	2.7	0.7	0.7	4.0
145	4.0	3.3	2.7	1.0	2.7	3.7	1.0	3.0
146	0.3	2.3	0.0	1.0	4.0	0.7	3.0	1.0
147	0.0	1.7	0.7	4.0	3.7	3.3	3.3	3.3
148	0.3	3.3	0.7	4.3	3.7	3.3	0.3	3.3
149	0.0	3.0	0.3	0.3	4.7	3.7	4.7	0.7
150	0.3	4.0	4.0	4.0	3.0	2.7	0.0	0.7
151	0.0	3.7	3.0	2.3	2.3	0.7	0.7	0.3
152	0.7	3.0	2.7	4.0	3.3	1.0	1.0	0.3
153	0.0	3.3	1.0	0.7	3.3	4.0	0.0	0.3
154	0.7	3.0	1.0	0.0	3.0	4.0	0.0	1.0
155	1.0	3.0	2.7	1.0	2.7	0.7	0.3	0.7
156	5.0	0.3	3.7	0.7	2.0	3.7	1.0	1.0
157	5.0	3.7	2.7	3.0	1.7	4.0	4.3	0.7
158	0.0	2.7	0.7	2.7	2.7	4.7	3.7	2.7
159	0.3	0.7	0.0	0.3	2.7	2.3	1.0	0.3
160	3.7	3.7	3.0	0.7	1.3	3.7	1.0	0.3
161	0.3	0.7	1.0	0.7	2.3	2.7	0.3	0.0
162	0.0	3.0	3.0	0.3	4.0	0.7	0.3	2.7
163	0.0	3.7	2.7	3.3	3.3	0.7	2.7	1.0
164	0.0	2.3	2.7	4.0	3.7	2.7	4.3	2.7
165	0.0	3.0	0.0	0.3	3.0	0.3	1.0	0.3
166	3.7	3.3	0.7	0.7	3.3	3.0	3.7	3.0
167	0.3	3.7	0.7	3.0	3.7	2.7	2.3	3.3
168	0.0	0.3	0.3	0.7	3.3	0.3	1.0	3.7
169	0.3	4.0	3.0	2.7	3.7	1.0	0.7	4.3
170	0.3	3.7	0.0	3.7	4.0	2.7	0.7	3.3
171	1.0	2.3	0.3	0.7	3.3	0.0	1.0	3.0
172	3.0	3.3	0.5	0.3	3.3	4.3	5.0	3.3
173	0.7	3.0	0.0	0.3	4.0	1.0	0.0	3.0
174	0.0	0.7	0.7	0.7	3.7	0.3	0.7	3.0
175	4.7	3.7	3.3	3.0	4.0	3.3	4.3	3.3
176	0.0	1.0	2.7	0.0	3.7	3.0	1.0	2.7
177	4.0	4.0	0.3	0.0	3.7	0.7	3.7	3.3
178	0.0	0.7	0.3	0.0	3.3	2.3	3.3	2.7
179	0.0	0.3	0.3	0.0	2.7	0.0	0.3	0.7
180	5.0	3.0	2.7	3.0	4.0	0.7	0.7	2.7

Table 4.6 (cont.) Cross 3, Mean Infection scores of 214 F2 plants tested against 8 isolates.

Plant	Isolate							
Line	M2	M3	M4	M5	M13	M14	M15	M16
181	3.0	3.3	0.3	3.7	2.7	0.7	3.3	3.0
182	2.3	3.3	0.3	0.7	2.7	3.7	2.7	2.3
183	3.0	3.7	0.7	1.0	3.3	2.7	0.3	4.0
184	3.0	2.7	4.0	2.3	4.7	3.3	3.3	4.0
185	2.7	4.0	0.7	0.3	3.3	3.7	0.3	3.3
186	2.3	3.3	0.3	0.7	3.3	1.0	0.3	3.7
187	0.0	0.3	3.5	0.7	3.3	0.7	1.0	2.7
188	4.0	4.0	0.7	5.0	3.3	3.3	0.0	0.7
189	1.3	4.7	0.3	5.0	3.7	3.3	0.7	3.3
190	3.0	3.0	0.3	2.7	2.0	3.7	0.7	3.0
191	3.0	4.0	1.0	1.0	2.7	3.7	1.0	3.0
192	3.7	3.7	0.7	1.0	3.7	5.0	1.0	3.0
193	2.7	2.3	3.0	2.7	2.7	3.0	0.0	2.5
194	1.0	3.3	0.7	3.0	2.0	3.7	0.7	4.0
195	3.3	3.3	3.7	3.7	3.7	3.0	0.5	3.0
196	3.7	3.7	3.3	3.0	3.7	2.7	2.7	3.3
197	4.3	3.7	0.3	2.7	3.0	3.7	0.7	4.0
198	5.0	3.7	0.0	2.7	3.7	0.7	0.7	3.3
199	4.7	3.3	0.3	4.0	3.3	3.0	2.7	3.3
200	4.7	1.0	0.0	0.7	3.7	1.0	0.3	3.0
201	5.0	4.7	2.7	1.0	3.3	1.0	0.3	3.0
202	3.0	3.3	0.0	2.7	2.3	0.3	1.0	0.5
203	2.7	2.7	0.0	0.3	4.0	0.7	0.7	2.7
204	3.0	4.0	1.0	0.7	2.7	3.7	0.7	3.7
205	4.0	3.7	0.0	3.7	3.7	3.7	2.3	3.3
206	0.0	3.0	0.0	0.7	3.3	3.7	2.7	3.3
208	0.0	4.7	0.3	0.0	3.3	3.0	0.7	0.0
209	0.0	5.0	0.3	0.0	3.3	3.0	0.7	0.0
210	2.0	3.3	0.7	0.7	2.7	2.3	2.7	2.3
211	3.3	4.0	3.7	3.7	2.7	2.7	3.7	3.0
212	3.0	3.7	3.0	0.3	3.3	4.0	0.3	4.0
213	2.0	3.3	3.0	0.7	2.7	2.7	2.7	3.0
214	2.7	3.7	1.0	0.0	3.3	0.3	1.0	2.7