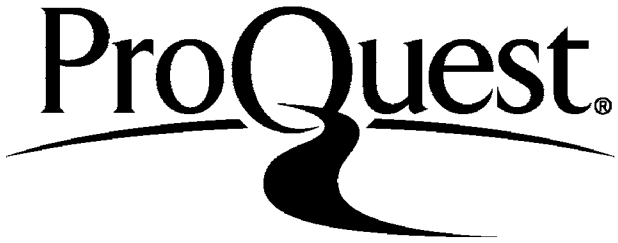
Breeding for rust-resistance in Antirrhinum

Thesis submitted for the
Degree of Ph.D.
in the University of London

Thomas Richard Butler-Stoney
Royal Holloway and Bedford
New College

1988



ABSTRACT

Recent trials of Antirrhinum majus cultivars have revealed a wide range of susceptibility to rust (Puccinia antirrhini). The best plants were used as parents in a breeding programme to produce a useful level of durable field resistance.

The F1 generation was self-pollinated but the F2 to F4 were selectively cross-pollinated within lines. The segregating generations were grown outdoors at two sites in Surrey, where they were subject to natural rust epidemics encouraged by the use of spreader rows. Individual plants were selected for rust-resistance and horticultural quality. By the F4 generation, lines showed greater resistance to rust than existing varieties and were becoming uniform in flower colour, growth habit and horticultural quality.

Many floral abnormalities arose, especially in the F3. Their expression was usually highly variable and was enhanced by environmental stress.

Tests of rust isolates against individual antirrhinum plants showed that there is considerable genetic variation within the rust population. Some monitoring of resistant varieties is desirable to identify future changes in the rust population.

The spread of spores between widely spaced patches of susceptible host was simulated using a computer, which indicated that small, isolated patches may escape infection. The effect of rate-reducing resistance is increased when plants are widely separated. This is in agreement with practical experience.

The urediniospores of Puccinia antirrhini cannot survive the winter in Britain and, though teliospores are regularly produced, no alternate host is known. However, overwintered antirrhinum plants produce viable urediniospores which start epidemics in early summer: there is no need to postulate an alternate host in the British Isles. Treating antirrhinum as a summer annual might be an important hygienic measure, reducing the local build up of inoculum early in the season. Acceptable control of rust should be possible, provided only the more resistant varieties are grown.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1
INTRODUCTION

The garden snapdragon, Antirrhinum majus L. is well known as a garden flower. It is normally treated as a hardy annual, flowering from mid-summer onwards. It has been one of the most popular of bedding plants and is widely distributed around the world.

Antirrhinum rust, Puccinia antirrhini Dietel and Holway, was first reported from California where it quickly became a serious disease of the garden antirrhinum. It subsequently spread around the world. The damage is caused by the uredinia which occur on leaves, stems, calyces and capsules. The urediniospores produced reinfect antirrhinum. Epidemics of the rust quickly kill susceptible plants. The epidemics commonly become apparent at the beginning of flowering. The lesions of severely infected plants disfigure the leaves, and the plant soon dies.

From California the rust has spread around the world. It was first reported in England in 1933. The antirrhinum has been reduced from a reliable hardy and reasonably drought tolerant plant to one that is quite unreliable.

Breeding for resistance to the antirrhinum rust began in America. The first "rust-resistant" varieties developed were immune to rust, and for a time these controlled the ravages of the disease and allowed the antirrhinum to continue as a successful bedding plant. In 1936 rust appeared on the previously immune varieties in California. In Britain they remained free of rust until 1962. Trials of the available antirrhinum varieties conducted by the Royal Horticultural Society at Wisley in 1969 found some rust on plants of every variety entered.

Trials conducted in 1978 and 1979 by the R.H.S. showed that cultivated varieties of antirrhinum had a wide range of response to rust epidemics. Some varieties were destroyed early in the season, but others were able to survive until well after flowering began, or even to the end of the season, although they were growing with the susceptible varieties. There is evidence (see Chapter 6 below) to show that there is variation

within the P. antirrhini population to match that in the host.

The breeding programme described below started with crosses between some of the best varieties and continued with selection in the field over several generations. It was hoped that a broadly based resistance could be built up in this way. Different breeding lines were kept separate so that resistance could be developed independently in the different lines. It is hoped that this broadly based resistance might prove more durable than single-gene resistance.

Teliospores are produced by Puccinia antirrhini, and these are able to germinate and produce basidiospores. Neither teliospores nor basidiospores have been known to infect Antirrhinum or any other species. If the basidiospores can infect some host and complete the heteroecious Puccinia life cycle this would have implications for the development of new varieties of antirrhinum as the sexual cycle of the rust would allow the more rapid development of races with multiple virulence genes.

The breeding programme is described in Chapter 3. The design and statistical analysis of the trials is discussed separately in Chapter 4. Although rust-resistance was the primary objective, other horticultural qualities of the plants were considered in making the selections. Many different aberrant forms were observed, especially of the flowers (Chapter 5). The lifecycle and variability of the antirrhinum rust are considered in Chapter 6. Chapter 7 considers the epidemiology of a disease affecting ornamentals such as Antirrhinum and other crops which are grown in many small and often isolated plots. The prospects for control of Puccinia antirrhini by further breeding are considered in Chapter 8.

Plate 1 shows a fine display of bedding plants that was spoilt by the ravages of Puccinia antirrhini. Plate 2 gives a view of the trial at Wisley in 1982 described in Chapter 3.

Plate 1. A fine display of bedding plants spoilt by antirrhinum rust.

Plate 2. Antirrhinum trial at Wisley in 1982.

CHAPTER 2

ANTIRRHINUMS AND RUST-RESISTANCE

2.1 THE CULTIVATED ANTIRRHINUM *Antirrhinum majus*

Antirrhinum majus L. has been cultivated since the sixteenth century or earlier, first as a medicinal herb and later as an ornamental plant. The eighteenth century nurseryman, John Kingston Galpine lists *A. majus* as the "Greater Snapdragon" and *A. foliis variegatis* as the "Striped leav'd Snapdragon" (Galpine, 1782). Before the nineteenth century it was only known with white, purple or yellow flowers. During the nineteenth and twentieth centuries, the range of colours increased and the snapdragon became one of the most popular bedding plants. It is tolerant of a wide range of conditions and is cultivated in many parts of the world. Antirrhinums are also important as a glasshouse crop for the cut-flower trade. Although it is normally grown as a hardy annual, *Antirrhinum majus* is a perennial in its native habitat. Plants can survive through one or more winters in Britain.

The genus *Antirrhinum* is divided into two morphologically distinct sections. *A. majus* is a member of the section Antirrhinum Rothm. This section is indigenous to Mediterranean Europe, and especially Spain. *A. siculum* Miller has also been widely cultivated and is naturalized in many places in the Mediterranean region. The section Saerorhinum Gray is indigenous to California.

The popularity of antirrhinum as a bedding plant has declined because of the destruction caused by the antirrhinum rust. The rust of glasshouse antirrhinums was a serious problem for a short time in the United States. It was controlled by hygienic measures, and especially by a change from propagating by cuttings to growing of plants from seed, which prevented carry-over of rust between consecutive antirrhinum crops (Mains, 1935).

2.2 THE ANTIRRHINUM RUST *Puccinia antirrhini*

The first report of the rust was published by Blasdale (1903). He had found it on cultivated *A. majus* in his garden at San Leandro near San Francisco, California in 1895. He reported that the rust was common in the region around San Francisco Bay. Specimens were sent by him to Dietel and to Holway, who published the name *Puccinia antirrhini* Dietel and Holway (Dietel, 1897). There are earlier records of the rust in California according to Barbe (1964 & 1967). The first was on cultivated *A. majus* near Santa Cruz in 1879. It was also collected in 1886. The first record of the rust on the indigenous Californian species is from 1892 on *A. virga* Gray.

Blasdale concluded that the rust must be a native Californian species that had adapted to the introduced *A. majus*. The rust is now known to attack four of the *Antirrhinum* species native to California.

From California the rust has spread around the world. The spread across North America was reviewed by Peltier (1919) and by Doran (1921). The first report from outside California was from Oregon in 1909. By 1921 most of the United States and parts of Canada were affected. The disease was reported in France in 1931 and England in 1933 (Green, 1933 & 1934). It spread rapidly throughout Europe in that decade. It was reported from South Africa in 1939 and Australia (Sydney, New South Wales) in 1952 (Walker, 1954). Within two years it had also been found in Tasmania and New Zealand. First reports of the rust from each country have been collated by Gawthrop (1980).

The complete life cycle of rusts involves five spore stages and is well documented for *P. graminis* and some other rusts. In most species the sexual phase and two associated spore stages occur on an alternate host. The *P. graminis* life cycle is illustrated in Fig. 2.1.

No alternate host is known for the antirrhinum rust, and only two spore stages, urediniospores and teliospores are normally seen. The known life cycle of *P. antirrhini* (Fig. 2.2) is only part of the complete rust life cycle. The urediniospores are the most common spore type. They infect *Antirrhinum* and give rise to new uredinia. The

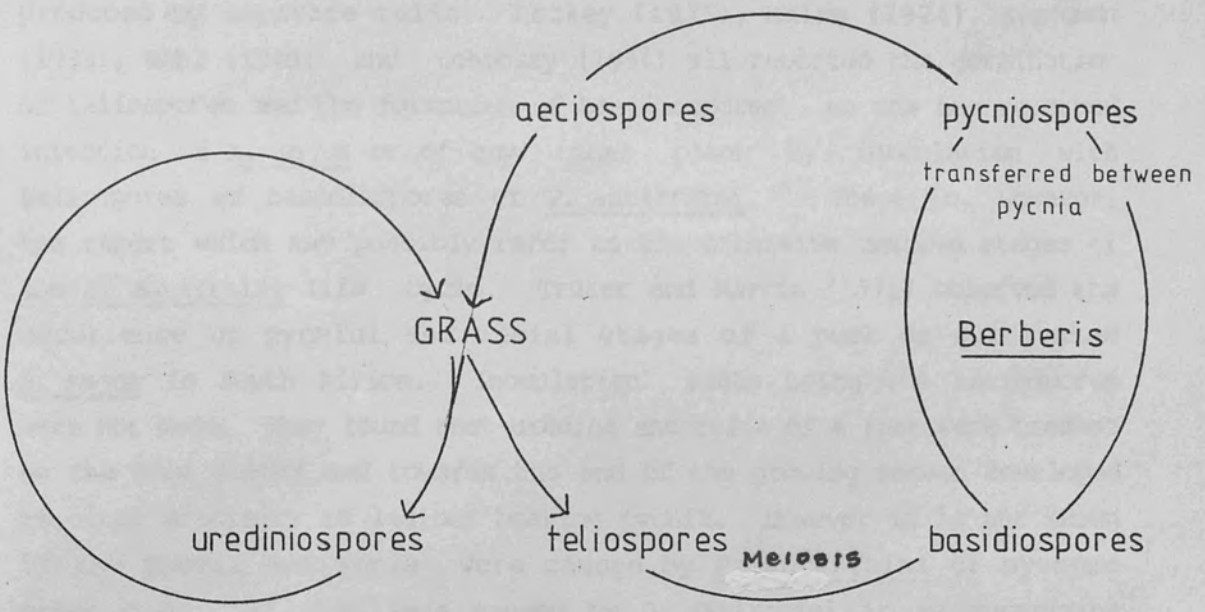


Fig. 2.1 Life cycle of Puccinia graminis, a typical heteroecious rust.

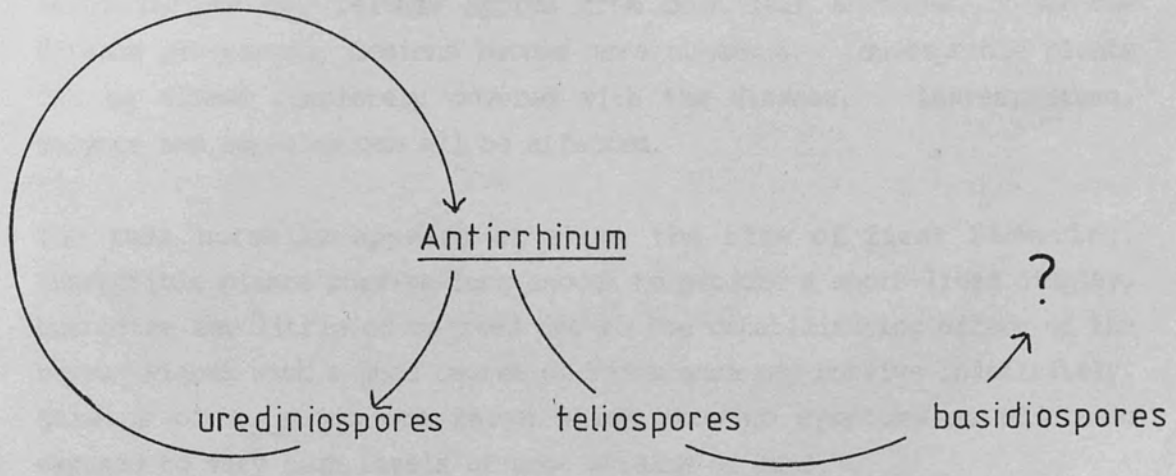


Fig. 2.2 Life cycle of Puccinia antirrhini. It is not known if the teliospore and basidiospore stages have any function in this species.

teliospores are produced mainly at the end of the growing season. They may be found mixed among the urediniospores, but are also produced by separate telia. Hockey (1921), Mains (1924), Kochman (1938), Wahl (1949) and Lehoczky (1954) all reported the germination of teliospores and the formation of basidiospores. No one has reported infection of A. majus or of any other plant by inoculation with teliospores or basidiospores of P. antirrhini. There is, however, one report which may possibly refer to the otherwise unknown stages of the P. antirrhini life cycle. Truter and Martin (1971) observed the occurrence of pycnial and aecial stages of a rust on cultivated A. majus in South Africa. Inoculation tests using the aeciospores were not made. They found that uredina and telia of a rust were present on the same leaves and towards the end of the growing season developed in close proximity to lesions bearing pycnia. However it is not known if the pycnia and aecia were caused by P. antirrhini or by some other rust. If they were caused by P. antirrhini it is surprising that there have been no other reports of such lesions.

P. antirrhini first appears as yellowing spots on the leaves. Within a few days these erupt into uredinia, usually on the underside of the leaf. Occasionally spores are produced on the upper surface, and large lesions may release spores from both leaf surfaces. As the disease progresses, lesions become more numerous. Susceptible plants can be almost completely covered with the disease. Leaves, stems, calyces and capsules can all be affected.

The rust normally appears at about the time of first flowering. Susceptible plants survive long enough to produce a short-lived display, but often set little or no seed due to the dehabilitating effect of the rust. Plants with a good degree of resistance may survive indefinitely. Strains of A. majus are known which show no symptoms at all when exposed to very high levels of some strains of rust.

2.3 BREEDING FOR RUST-RESISTANCE IN ANTIRRHINUMS

The antirrhinum rust was first observed in California. This is also the main area of antirrhinum seed production in the United States of

America. By 1921 the rust was having severe consequences for the seed industry. Emsweller and Jones (1934) searched the flower-seed multiplication ranches of California for rust-resistant plants. They found rust in every field visited, but failed to find any individual plants showing resistance.

The production of the first rust-resistant lines of A. majus was described by E.B. Mains (1935). In 1922, two plants survived with only moderate infection from a group of snapdragons of mixed colours severely infected with rust in the garden of Dr. Mains in Indiana. These two plants were transplanted and self-pollinated. The progeny were grown outside during the following summer. A few plants were killed by rust, most were moderately rusted, but a few were much less rusted and the uredinia on them were small. Further cycles of self-pollination and selection resulted in the production, by 1928, of two inbred lines on which little rust developed and the uredinia were small. However, these plants were of very poor horticultural quality. Slightly resistant plants were found in a few of the commercial varieties of the time. Selection and inbreeding produced lines derived from these plants which were more resistant than the original varieties, while retaining acceptable flowers. Only one of these lines, derived from the variety "Giant White", approached the resistance of the two resistant lines derived from the garden plants. From 1928 onwards these and later lines were distributed to other breeders.

Elmsweller and Jones (1934) produced plants derived from the "Giant White" line that showed no symptoms of rust. Test crosses to commercial varieties indicated the presence of a single gene controlling rust-resistance. They also found evidence of modifying factors. The development of the immune lines from partially resistant lines was presumably the result of segregation and selection of the modifying genes. Resistance from these lines was introduced into commercial varieties by backcrossing. Both Mains (1935) and White (1933) demonstrated a major gene in lines descended from a cross between the Giant White line and A. glutinosum. Mains produced another independently derived resistant line from a cross between seed supplied from Europe by E. Baur under the name Antirrhinum ibanjezii and a susceptible commercial pink snapdragon. F2 segregation of approximately

3 : 1 indicated another dominant gene. Although Mains apparently found three independent sources of resistance (the two plants in his garden, the commercial variety "Giant White" and the European material supplied as "A. ibanjezii") it seems that only the second source was used in the rust-resistant commercial varieties before 1937. Mains selected only for rust-resistance, and gave no attention to other qualities. His aim was to develop resistance that other breeders could incorporate in their breeding of commercial varieties. As a result his plants were of poor habit, and had small magenta (wild type) or mottled flowers.

In 1936 all the resistant lines in America were attacked by rust and this new virulent form was designated "Physiologic Race 2" (Yarwood, 1937).

Following the first reports of rust in England in 1933, British breeders began to take an interest in resistant varieties. There is little published on the development of many of the British rust-resistant varieties as much of the work was done within the seed companies.

W.H. Simpson & Sons Ltd. offered resistant American varieties in their catalogue from 1936 onwards. Their seed came from the Ferry Morse Seed Co., and from the Waller Franklin Seed Co. These companies had both cooperated in the work of Elmsweller and Jones and so the resistance of their varieties was probably derived from Mains's "Giant White" line. These varieties were tested in trials at Wisley by D.E. Green, the Royal Horticultural Society mycologist.

The origins of the Wisley breeding programme are described by Green (1937a, 1937b & 1941). From 1935 onwards Green tested new stocks received from America and his own selected inbred lines which were derived from the American stocks. Green considered plants as resistant only if they were completely free of rust. Where he needed a qualitative measure of resistance he used "percentage resistance", the percentage of all the plants showing no rust at all. From 1935 until 1940 rust-free plants were self-pollinated. Selection between rust-free plants was made on the grounds of colour and horticultural quality. At the end of the 1939 trial five stocks remained completely

free of rust. Continual selection was resulting in a gradual improvement of the flowers of these lines. These five lines were grown again in 1940 and crosses were made between them. The five are of importance as the source of all the Wisley varieties. They were (Green, 1941):

1. "Wisley No. 3": derived from the first rust-resistant lines received from America, a magenta coloured self-pollinated line that had been tested and rust-free for six seasons.

2. "Orange Pink": an American stock; apricot yellow with white tube; tested and rust-free for three seasons.

3. "Terra Cotta Pink": an American stock; apricot yellow with faint purple tube; tested and rust-free for three seasons.

4. "Yellow Sport": a yellow variant of "Terra Cotta Pink"; tested and rust-free for two seasons.

5. "Brightness": received from America in 1939; magenta with yellow-orange lip; tested and rust-free for two seasons.

Gawthrop (1980) studied records retained at Wisley and worked out pedigrees for the later Wisley varieties developed by Green. Unfortunately breeding records for the period 1941 to 1950 are lost. However, among the crosses made in 1940 must be the origins of the varieties "Wisley Golden Fleece", "Wisley Cheerful" and "Wisley Bridesmaid", which received awards of merit in the 1949 Trial of Rust-Resistant Antirrhinums held by the R.H.S. at Wisley.

Simpson's "Rust Resistant Pink" (later known as "Pink Freedom") was also given an Award of Merit in 1949. This variety was probably bred from American material and its rust-resistance would share the same genetic origins as Green's lines and the Wisley varieties.

The fifth variety to receive an award in 1949 was "Rustproof Orange Glow". This was developed by R. Gould from a single plant of the susceptible variety "Orange Glow" and may represent a different source

of resistance (Gawthrop, 1980). "Rustproof Orange Glow" was later known as "Orange Monarch".

These five antirrhinum varieties were the basis of further breeding work by Green. They were crossed among themselves and with rust-susceptible varieties (including "Malmaison"). Crosses were followed by generations of repeated selfing and selection. Crosses between breeding lines, or less often to varieties, were made only occasionally. Fewer than 10 plants of a breeding line were grown in each generation. Gawthrop (1980) gives pedigrees of the varieties "Toreador", "Polaris", "Titan" and "Aurora" (synonym "Juno"). "Toreador" and "Titan" appear to derive their resistance from "Wisley Golden Fleece". "Aurora" ("Juno") is descended from "Pink Freedom". "Polaris" was derived from a cross between "Pink Freedom" and "Orange Glow" and could therefore contain genes for either or both types of resistance.

In the winter of 1937, Green received news from America of the new virulent race of the rust. He wrote (Green, 1941):

> "Our reaction was that as the second strain was not yet in Great Britain, we should continue to develop our resistant varieties in the hope that we should escape "Physiologic Race 2" of the Rust, at least for many years to come."

Thus from 1937 onwards, Green knew that he was relying on geographical isolation to keep Race 2 out of Great Britain. The Wisley breeding programme was abandoned after the disastrous trial of 1969 in which all varieties were infected with rust.

The R.H.S. conducted trials of Rust Resistant Antirrhinums in 1949, 1958, 1962 and 1969. Two rows of "Malmaison" a rust-susceptible variety were grown between trial entries. The trials were sprayed with suspensions of urediniospores several times in the season. These trials were therefore a severe test of rust resistance. Only stocks which remained completely free throughout the season were considered for an award. In 1969 no varieties satisfied this condition, although a number of varieties exhibited only slight infection when exposed to this very high level of inoculum. No awards were given in that year.

Since 1969 there has been some confusion over the application of the

term "rust-resistant". No varieties have been available which show complete immunity to the rust, but most of the recent commercial varieties develop much less rust than highly susceptible varieties such as "Malmaison". The cultivars with most resistance have been described by the seed trade as "rust-resistant", a term which should not now be taken to indicate immunity.

2.4 GENETIC CHANGE IN *Puccinia antirrhini*

When the first rust-resistant antirrhinum varieties were produced, Mains (1935) warned of the possibility that new physiologic forms of rust might appear on the resistant varieties.

The "breakdown" of the rust resistance derived from Mains's material was first observed in 1936 (Yarwood, 1937). By inoculating detached leaves floating on sucrose solution with spores collected from susceptible and from previously resistant varieties, Yarwood demonstrated that the change was due to one or more new forms of *P. antirrhini*. He designated the virulent rust as "Physiologic Race 2". The early epidemics of the Race 2 rust were especially severe in the Salinas Valley in California (Blodgett & Mehlquist, 1941). It is interesting that Race 2 should first be reported within 200 miles of the site of the first reports of rust on *A. majus*. Could it be that the origin of Race 2 was influenced in some way by strains of *P. antirrhini* growing on the indigenous *Antirrhinum* species ? This possibility was not considered by Gawthrop and Jones (1980), but if *P. antirrhini* can ever complete a sexual life cycle, it is likely to do so here in its natural habitat. Gawthrop (1980, Appendix 7.1) visited a number of sites in the Salinas Valley during her search for wild *Antirrhinum* species. Sites visited were carefully selected as the locations of recent specimens in a number of herbaria and after consultation with Californian botanists. It is therefore likely that she had some evidence of wild *Antirrhinum* growing in the Salinas Valley even though she failed to find any.

Blodgett and Mehlquist (1941) considered that the Race 2 rust was less destructive than the Race 1. An alternative explanation might be that

the varieties immune to Race 1 had some residual resistance effective against Race 2.

Gawthrop and Jones (1980) compared the published results of five rust resistance trials conducted between 1921 and 1954. They interpreted differences in the ranking of the 37 varieties common to two or more trials as due to changes in the rust genotypes. They considered that the rust was changed between trials conducted in 1921 (Doran, 1921) and 1935 (Mains, 1935). It would seem that this change is not the appearance of "Race 2" or Mains would not have considered his breeding lines resistant. Rust fungi are all highly specialized in their host specificity. These trials were conducted only 30 to 40 years after P. antirrhini had moved from the indigenous Californian Antirrhinum species to A. majus. It is perhaps not surprising that the rust should still be changing to adapt to its new host. Gawthrop and Jones also conclude that the race of rust in Australia in the 1950s was different both from those in California (Race 2) and in Britain (still Race 1) at that time. They recorded a change in the rust in Britain between 1958 and 1962, the dates of two trials organised by the R.H.S. However, five varieties received awards of merit in the 1962 trial. It was not until the 1969 trial that all varieties were infected with rust.

The breakdown of resistance in the rust immune varieties in Britain must have involved more complicated changes than just the appearance (introduction) of a "Physiologic Race 2" genotype of rust. There must have been at least three races or genotypes of rust active in the world during the 1950s and in England during the 1960s. Experiments reported by Barbe (1967) and Gawthrop (1980) indicate that rust strains multiplied from different sources respond differently when inoculated on to test plants or leaves of various genotypes. This is discussed in more detail in Chapter 6 below. P. antirrhini must be more variable than is generally reported. The presence of a varied mix of strains in the existing rust populations must be of great importance to the plant breeder. If there is a large number of strains with local variations in their relative abundance, then the interpretation of resistance trials is very difficult, and to test varieties against all strains might require the use of many trial sites.

Blasdale (1903) found that the rust affecting cultivated A. majus attacked the native A. vagans with almost the same intensity. Linaria reticulata and L. amethystina were attacked to a much lower degree. However, Barbe (1967) could not get rust collected from native Californian species to infect cultivated A. majus varieties believed susceptible to all races of rust, nor could he infect the Californian species with rust collected from cultivated A. majus. Some difference must therefore have developed between the rust of the cultivated and wild species in California. This process has involved the loss as well as the gain of virulence. The loss of virulence to the old host may be expected after a highly specialised and host-specific pathogen has adapted to a new host species. The difference between the results of Blasdale in the first years of the rust of cultivated antirrhinums and Barbe half a century later may indicate that the gain of the new virulence preceded the loss of the old. If this is so, then the genetic changes within P. antirrhini populations have been more gradual and involved more gene loci than have been recognised in the literature.

2.5 GENETIC RESOURCES IN THE GENUS Antirrhinum

The work of Emsweller and Jones was continued by Blodgett and Mehlquist (1941). They could not find any strains of A. majus that were immune to rust of Race 2, although some showed considerable resistance. They found other Antirrhinum species that were not infected, but considered most of these too far removed taxonomically to be of value in a breeding programme. Their results with other species were similar but not identical to the results obtained by Mains.

Mehlquist and Rahmani (1948) report experiments on the genetics of resistance to Race 2. They found one plant which was better than other plants resistant to Race 2. The resistance to Race 2 appeared to be controlled by a single gene, unlinked to the Race 1 resistance. Genotypes heterozygous for the Race 2 resistance were only moderately resistant to Race 2.

Sampson (1960) mapped a rust-resistance gene between the Eos and Inc

loci controlling flower colour. It is not clear to which race this gene confers resistance.

There is a very wide range of genetic variation available in the wild Antirrhinum species. The European species (section antirrhinum) have been crossed experimentally in many combinations. These interspecific hybrids are listed by Gawthrop (1980). These species are often only distinguished by one or two characters. The latest monograph on the genus is that of Rothmaler (1956). He divided the European section, Antirrhinum, into three subsections, nine series and 24 species. Webb (1971) considered that this did not allow for the variants that provide bridges between all the species. In Flora Europaea, he recognised only 17 species, with two subspecies of A. hispanicum. There appear to be negligible barriers between species. The situation has probably been confused by hybridization between locally endemic species and the cultivated species, A. majus L. and A. siculum Miller. Changes in the distribution of locally endemic species could also lead to hybridization which would obscure species differences. Many of the species may never have had a very distinct genetic identity. The taxonomy within the European species is therefore complicated, with many synonyms published.

The exact relationships between the wild populations is not of importance to the plant breeder. It is certain that there are a wide range of genotypes that could be incorporated into breeding programmes. The wild species mostly have small and narrow flowers which would not be acceptable in cultivated varieties. Although the rust will not grow on many of these species, they are not likely to contain specific adaptations for rust-resistance as Puccinia antirrhini was originally a parasite of the Californian Antirrhinum species. It is here that Antirrhinum and Puccinia populations have coexisted, and balanced populations of host and parasite can still be found on the wild species (Barbe, 1967).

Crosses have not been made between the two sections of the genus. Unless this can be done the gene pools of the original host species of P. antirrhini are not available to antirrhinum breeders. At present this is probably not a serious problem, as the range of variation within

A. majus has not been fully exploited.

For all wild material of the section Antirrhinum, chromosome counts have been reported as 2n = 16, although autotetraploid cultivars of A. majus have been developed and are commercially available. For the section Saerorhinum counts have been reported of 2n = 32 from two species: A. multiflorum Penn. (Munz, 1968) and A. nuttallianum; and of 2n = 30 for two species: A. coulterianum Benth. and A. elmeri Rothm. (Günther & Rothmaler, 1963). Gawthrop (1980) examined plants of A. multiflorum and A. virga and found 2n = 32 for both species.

2.6 DISEASE RESISTANCE IN OTHER CROPS

Early work on breeding for resistance to disease of all types on many crops was based on the use of single genes giving resistance to all pathotypes of the disease that were abundant at the time and place of breeding. Resistant material was widely distributed amongst breeders. Transfer of single effective genes between varieties was easy and many varieties were produced sharing a common resistance. The work on antirrhinum rust-resistance from 1930 onwards is typical of the approaches made at the time.

The gene-for-gene hypothesis of Flor (1955), now widely accepted for many plant diseases, provided a basis for understanding the boom-and-bust cycles observed in many crops. This with the concepts of vertical (race specific) and horizontal (general) resistance introduced by Van der Plank (1963) have produced a change in the general tactics of breeders for many crops. The use of successive resistance genes conferring immunity is seen as achieving only temporary success. Attempts are being made to use known resistance genes together in a more controlled and more effective way, or to find forms of horizontal resistance. There has been a tendency to equate vertical resistance with single gene (oligogenic) resistance and horizontal resistance with polygenic resistance.

2.7 SCREENING FOR RUST-RESISTANCE

During 1978 and 1979, Gawthrop (1980) ran trials of 131 different accessions of Antirrhinum majus to assess rust-resistance. The trials were spread over two years and two sites, which were at the Botanic Supply Unit of London University at Egham in Surrey and on land belonging to the Royal Horticultural Society at Wisley. In each year the same trial was repeated at both sites. 67 accessions were grown in the trials of 1978 and 11 of these were included as controls in the trials of 1979. In both years, the very susceptible variety "Malmaison" was used to form rust spreader rows. This is the same susceptible variety that was in the R.H.S. rust-resistant antirrhinum trials. A complete row of Malmaison was planted as every third row in the trial. In addition, two rows of Malmaison were planted round the edge of each trial.

The accessions included in the trials came from a wide variety of sources. Most were commercial varieties. It was also possible to include various lines from confidential sources. In addition, a few wild or near wild populations were included. The commercial varieties included a number of old varieties which were not in current seed lists. Many of these varieties had been considered rust-resistant until the R.H.S. antirrhinum trial of 1969.

As might be expected, this wide range of A. majus genotypes showed a wide range of rust-resistance. The general pattern was that a severe epidemic swept through the trials at around flowering time. Plants of the more susceptible varieties were killed. Plants of the more resistant varieties survived until the autumn. None of the varieties showed complete immunity to rust. Some of the old varieties incorporating the genes for immunity to Race 1 performed very well in these trials.

It was on the basis of the trials in 1978 and 1979 that the R.H.S. and Royal Holloway College decided to pursue a breeding programme aiming to achieve a useful improvement in horizontal resistance to antirrhinum rust.

The breeding programme proposed to improve resistance by the accumulation of many genes, each on its own of small effect, but which would together give a good level of rust-resistance. These genes would have to be selected from a wide range of sources. The final varieties must include a range of colours. The horticultural qualities including habit and flower size and shape need to be maintained or improved during the breeding programme.

Three ways were proposed in which new varieties might be created to meet these requirements (Gawthrop, 1980). These are summarized in Fig. 2.3 (after Gawthrop, 1980). All three programmes use a pedigree method and require the repeated use of a quantitative assessment of rust-resistance. The three programmes would support each other and movement of material between the three programmes might be necessary.

The programmes were based on crosses between the best available varieties chosen on the basis of the 1978 and 1979 trials. Progenies from these crosses would be selected for rust-resistance and horticultural qualities from the F2 onwards.

Programme I was designed for the quickest possible production of new rust-resistant varieties. The initial crosses for this programme would be made between varieties of the same or similar colour. It was hoped that this would largely avoid the need to select for colour. This would reduce the number of plants needed for selection in each generation, or allow a tighter selection for rust-resistance and horticultural qualities from any given number of plants. In later generations crosses would only be made between related lines. The plants selected in later generations would be as uniform as possible in such qualities as height, flowering time and shade of colour. It was hoped that in this way lines of uniform colour and habit could be produced in four or five generations. The method was dependent on the availability of suitable parent varieties of similar colour and therefore limited in the range of colours to be produced.

Programme II was a modification of programme I. Where matching pairs of parent varieties were not available for use in programme I, it was proposed that coloured varieties should be crossed with white or ivory

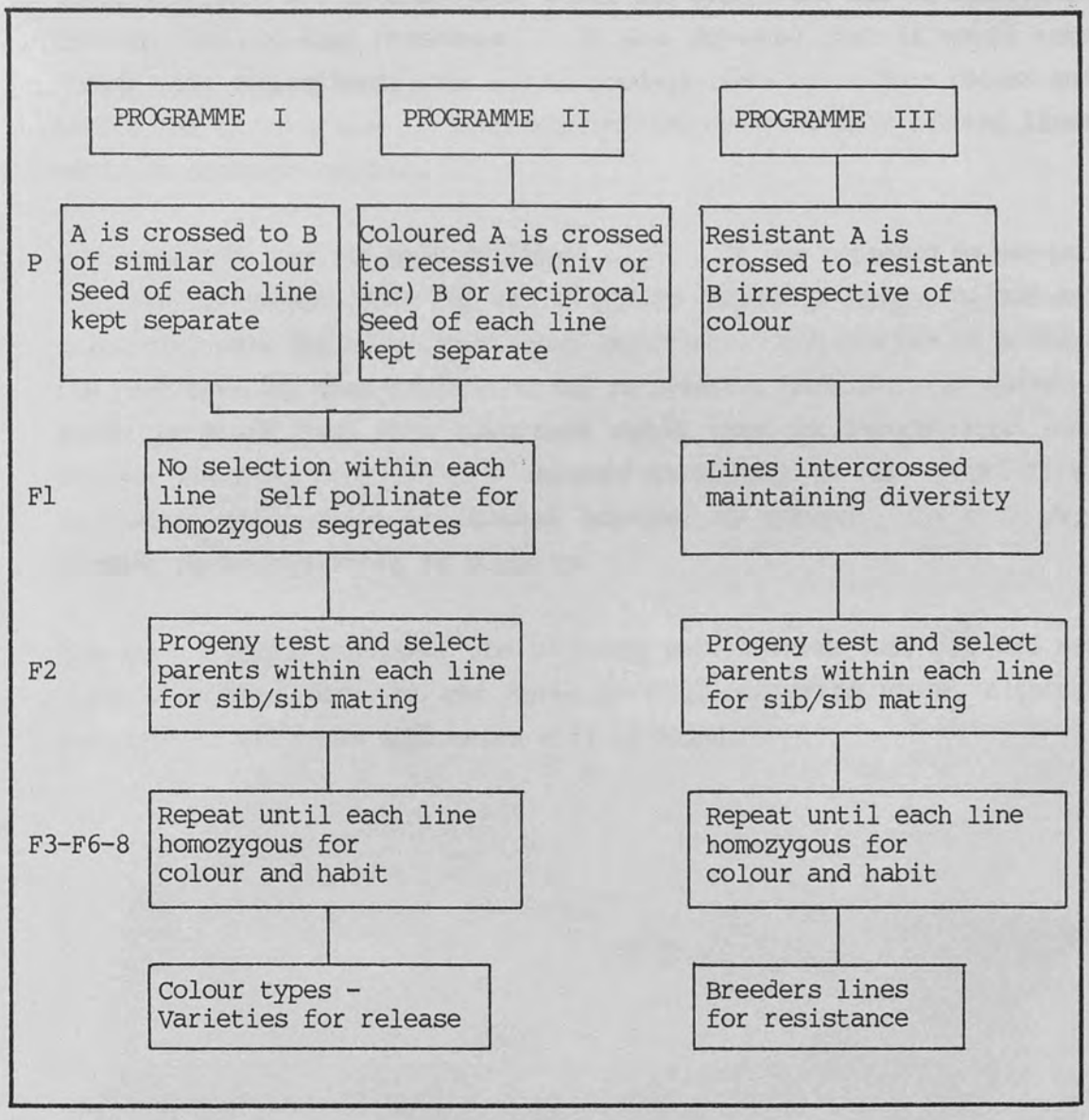


Fig. 2.3 Summary of breeding programmes (after Gawthrop, 1980).

varieties. The white and ivory colours are determined by genes controlling enzymes early in the pigment synthesis pathway. Other corolla colours are dominant over white and ivory, and can be maintained through the breeding programme. It was expected that it would take longer (six generations) this way to produce lines of uniform colour and habit. As in programme I, after the initial crosses, only related lines would be crossed together.

Programme III had slightly different aims. It was intended to produce good rust-resistant lines for use in future breeding work. Colour and uniformity were not to be considered important. The aim was to produce the best possible rust-resistance and to preserve horticultural quality. Lines produced from this programme could then be incorporated into further breeding programmes. Because uniformity is not of priority, unrelated lines could be crossed together as needed. In this way, further resistance might be built up.

The next chapter describes the breeding work carried out. It did not exactly follow any of the three outlines suggested above, although elements of all three approaches will be found.

CHAPTER 3
THE BREEDING PROGRAMME

3.1 OBJECTIVES

The aims of the breeding programme were:

1. To produce a level of resistance sufficient for the plants to survive, without disfigurement, until the end of the flowering season. Complete immunity to rust was not the aim.

2. That resistance should have a wide genetic base. To achieve this end, the different lines should not be crossed together during development, but each line produced should be the result of an independent selection process.

3. To achieve plants with good horticultural quality: good habit, typical flower shape and large flower size, early and persistant flowering, and free from visible abnormalities. (This is discussed in more detail in section 3.2 below).

4. The different lines should include a wide range of flower colours. Each colour should be bold and uniform.

5. The different lines should be as similar as possible in all characteristics other than colour of the flowers. For example, height and habit should be similar.

3.2 THE IDEAL PLANT

The ideal form of the plant was considered to be as follows:

Habit

Plants should be of medium height, with strong erect branches giving the whole plant a pyramidal shape. Flowering should begin on the main shoot

(which was not pinched out in these trials) and continue on the branches.

Flower spike
Flowers should be carried well above the foliage. The flowers should be evenly spaced along the spike, and the internodes should be neither too long nor too short.

Flowering time
Deliberate selection was not made for time of flowering, but there must have been strong selection pressures on flowering time arising from the implementation of the breeding programme. Plants could not be used that were not flowering at the time of selection and crossing.

Flower size and shape
Individual flowers should be large. Their width should be approximately equal to their height.

Plants were selected primarily for low levels of rust infection. However, as indicated above, other factors were taken into consideration. The parents of all the lines in the breeding programme were all commercial varieties, and therefore most plants within the programme were of acceptable horticultural quality. The selection for horticultural quality consisted mostly of eliminating unsuitable plants. The most common reasons for eliminating plants were associated with the flowers. Many of the more extreme morphological forms that were observed are described in Chapter 5 below.

3.3 TERMINOLOGY

"Variety" is used of the established named varieties grown in the trials of 1978 and 1979, and by extension, of the various unnamed accessions which were grown in those trials. It is never used of any existing progeny of the breeding programme although it is used of the hoped for end product of this breeding programme.

To avoid confusion, the term "line" is used to describe all plants

descended from the same parent varieties in 1980. "Progeny" refers to the F1 of a cross between two individual plants.

Each line can be followed through the breeding programme by the "accession numbers". Different accession numbers are assigned to the progeny of each pollination. Accession numbers within the breeding programme are of the form:

year - line number - progeny number

An additional number added in brackets at the end is used where it is necessary to identify individual plants.

Accession numbers of seed obtained from other sources are of the form:

year - number

In describing the trials, the term "site" is used to describe the ground covered by the trial. "Block" is used only in the statistical sense as in the term "randomised complete blocks", in which a block of the experiment contains one replicate of each "treatment". Not every trial was divided into blocks. "Row" is used to describe plants in a straight line across the site. A "spreader row" is a row of rust-susceptible plants used to ensure the rapid development of a rust epidemic. A "test row" is a row of plants to be assessed for rust-resistance. All test rows consisted of up to six "plots". "Plot" is used in the statistical sense, of an experimental unit receiving uniform "treatments". The only "treatment" in these trials was the different accessions of seed. Each plot contained plants of the same accession number. This usage differs from that of Gawthrop (1980), who used "plot" to mean site, and "block" to mean both block and plot.

3.4 THE PARENT VARIETIES

The varieties from which the breeding programme was based were selected on the basis of the trial results on the screening programme. Good rust-resistance was the major criterion. The chosen varieties are listed in Table 3.1, and rank positions are given for these varieties in the 1978 and 1979 trials. These ranks are inferred from tables given by Gawthrop (1980). Consideration was also given to the horticultural

Accession number	Variety name	Colour	Rank in rust resistance trials 1978(a)	1979(b)	Horticultural quality (c) Uniformity	Habit	Flowering spike
78-34	Coronette White	white	19				
78-36	Coronette Rose	red	30				
78-39	Coronette Cherry	red	14				
78-66	Carioca White	white	13				
78-83	White Monarch	white	46				
78-88	Amber Monarch	yellow	5	4	3	3	3
78-90	Scarlet Monarch	red	16				
78-164	Sutton's Rust Resistant Leonard Sutton	red		6	2	3	3
78-178	Wisley Golden Fleece	yellow		1	3	3	3
78-180	Titan	red		10	2	3	3
78-183	Yellow Freedom	yellow		3	3	3	3
78-185	White Freedom	white		17	3	3	2
78-187	Bonfire	red		15	2	3	3
78-195	Frontier White	white		43	2	3	3
78-240	Sutton's Rust Resistant Pale Sulphur	yellow		11	2	3	2
78-246	Sutton's Triumph White	white		41	3	3	3

Table 3.1 Parent varieties used in the breeding programme, and their performance in the trials in 1978 and 1979. (Original table prepared from data given in Gawthrop, 1980)

a) From data of the second scoring at Wisley, and ranked within the 67 varieties grown. Low numbers indicate resistant varieties.

b) Based on combined data from both sites, and ranked within the 79 varieties grown. Low numbers indicate resistant varieties.

c) Horticultural quality was only assessed in the 1979 trial. 1 = poor, 2 = moderate, 3 = good.

qualities, and especially to height. Uniformity in height between the parent varieties was considered an advantage. It was hoped that this would reduce the variation in height within the segregating progenies, and so greatly reduce the need to consider height in the selection process. Nearly all the varieties used were of medium height.

The chosen varieties were grown together in a small trial at Egham in 1980. The initial crosses were made on selected plants in this trial.

3.5 THE BREEDING PROGRAMME

The parent varieties were crossed together in the summer of 1980 and these crosses are listed in Table 3.2. Between one and five pairs of plants were used for each of these crosses. Individual plants used were selected for rust-resistance and for horticultural quality. In some cases crosses were made taking advantage of individual off-type plants within rust-resistant varieties. Where this was done, the number of pollinations made was necessarily limited.

Crosses were also made between resistant and susceptible varieties to investigate the inheritance of rust-resistance (Table 3.3). The resistant varieties used included old ones which were presumed to have the gene for immunity to Race 1 of the rust, and recent varieties for which nothing was known about the inheritance of their resistance.

A generalized scheme of the breeding programme is given in Fig. 3.1. The F1 generation was grown in the glasshouse in the winter of 1980-1981 and self-pollinated. All later generations were grown in field trials and selected plants were cross-pollinated. It was originally hoped that two generations could be grown in the year, by growing one generation in a glasshouse during the winter. Experience with the F1 generation in the winter of 1980-1981 showed that this was not feasible with the available resources. Selection for rust-resistance of the winter generation would have been a problem, and most selection would have had to be done in the summer generation, when larger numbers of plants were possible and natural rust epidemics could occur. The use of winter

Accession number of F1	[n]	Expected colour of cross	Parent varieties Female		Male	
80-31	0	yellow	78-183	Yellow Freedom	78-34	Coronette White
80-32	5	white	78-246	Sutton´s Triumph White	78-83	White Monarch
80-33	4	white	78-195	Frontier White	78-83	White Monarch
80-34	3	pink	78-36	Coronette Rose	78-164	Sutton´s RR Leonard Sutton
80-35	1	orange	78-187	Bonfire (off type)	78-88	Amber Monarch
80-36	5	deep pink	78-39	Coronette Cherry	78-164	Sutton´s RR Leonard Sutton
80-37	3	scarlet	78-90	Scarlet Monarch	78-180	Titan
80-38	4	flame	78-187	Bonfire	78-88	Amber Monarch
80-39	5	pale yellow	78-240	Sutton´s RR Pale Sulphur	78-246	Sutton´s Triumph White
80-40	4	yellow	78-178	Wisley Golden Fleece	78-183	Yellow Freedom
80-41	3	-	78-88	Amber Monarch	78-183	Yellow Freedom
80-42	4	-	78-178	Wisley Golden Fleece	78-240	Sutton´s RR Pale Sulphur
80-43	5	-	78-187	Bonfire	78-164	Sutton´s RR Leonard Sutton
80-44	5	orange	78-66	Carioca White	78-88	Amber Monarch
80-45	3	flame	78-83	White Monarch	78-187	Bonfire
80-46	3	pale orange	78-83	White Monarch	78-187	Bonfire (off type)
80-47	3	deep pink	78-185	White Freedom	78-39	Coronette Cherry
80-48	4	white	78-185	White Freedom	78-34	Coronette White
80-49	3	pale pink	78-164	Sutton´s RR Leonard Sutton	78-195	Frontier White
80-50	4	red	78-34	Coronette White	78-90	Scarlet Monarch
80-51	2	pale orange	78-195	Frontier White	78-88	Amber Monarch
80-52	3	yellow	78-183	Yellow Freedom	78-195	Frontier White
80-53	5	red or orange	78-180	Titan	78-183	Yellow Freedom
80-54	4	-	78-88	Amber Monarch	unknown	

Table 3.2 F1 crosses between the parent varieties.

[n] is the number of progenies of each cross.

Accession number of F1	Parent varieties			
	Female		Male	
80-55	78-32	Malmaison	78-183	Yellow Freedom
80-56	78-32	Malmaison	78-195	Frontier White
80-58	78-32	Malmaison	78-178	Wisley Golden Fleece
80-78	78-164	Leonard Sutton	78-32	Malmaison
80-80	78-88	Amber Monarch	78-32	Malmaison

Table 3.3 F1 crosses between resistant and susceptible varieties made to investigate the inheritance of rust-resistance.

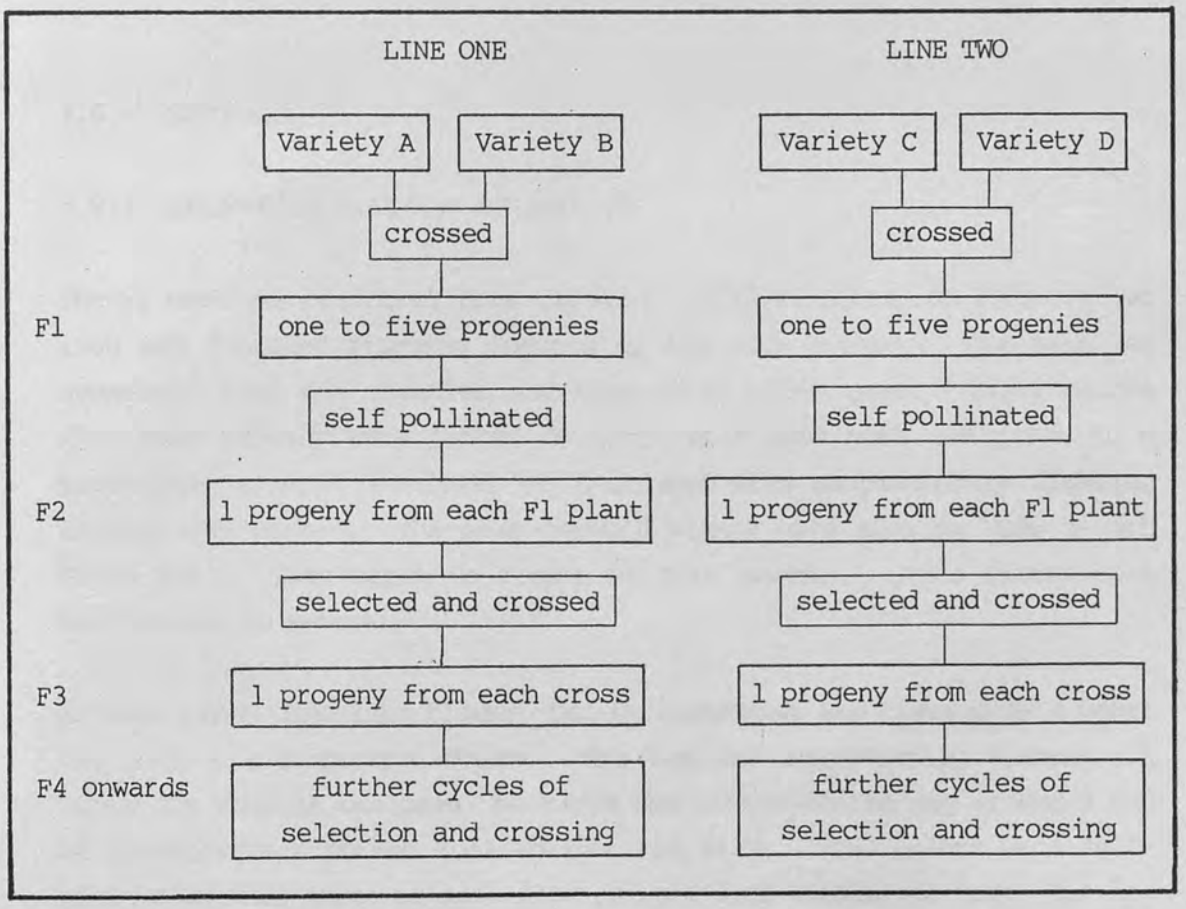


Fig. 3.1 A generalized scheme of the breeding programme.

N.B. No crosses were made between the separate lines.

generations would have allowed quicker development of a uniform population.

All generations from the F2 onwards were grown in field trials. Individual plants were selected and crossed together within lines. Crosses were made both within progenies (between siblings) and between progenies. Plants were usually crossed to plants of the same colour and habit in order to achieve uniformity as quickly as possible, although in some cases the number of suitable plants was limited. Usually a mixture of crosses between and within progenies were made. Where the number of suitable plants was limited, only one type of cross might be possible. Occasionally plants were self-pollinated. Some whole lines or progenies with severe faults were abandoned.

3.6 METHODS

3.6.1 SELF-POLLINATION OF THE F1

The F1 seed was collected from the small trial at Egham on 13th October 1980 and finished ripening indoors by the 24th October. The seed was separated from the capsules and germinated under mist. Three plants from each progeny were potted on into three inch pots and grown in a glasshouse with a low level of heat and with supplementary lighting through the winter. The most forward plants were showing buds by 4th March 1981. They began to flower on 25th March. Other plants were much slower to mature.

As each plant came into flower, the inflorescence was covered by a paper bag with a transparent window. The bag was supported by a cane. A miniature stapler was used to close the bottom of the bag around a pad of non-absorbent cotton wool against the stem. The plants were self-pollinated when three or more flowers were open within the bag. The bag was removed and a cotton stick used to transfer pollen between all the open flowers starting from the top and working downwards. A new stick was used for each plant. The bag was replaced after pollination.

The corollas would be shed about a week after pollination.

Flowers which had not been pollinated would last longer. The bags were removed as soon as the flowers showed signs of having set seed. The main stem was cut just above the highest seed containing capsule and any flowering side shoots were also removed. The seed was left to ripen on the plant until the capsule pores were about to open. At this stage the seed capsules and a length of the main stem were harvested, and allowed to finish ripening in paper bags in the laboratory. These seed packets were kept in a wire cage to allow air circulation around them.

3.6.2 CROSS-POLLINATION

Cross-pollination was carried out on selected plants from the breeding trials. In some cases pollination was done in the field. When crosses had to be made late in the season the plants were dug up and brought into a glasshouse in pots. This had the additional advantage of bringing all the plants together in a small area, and allowing the work to be done at a more convenient height.

Flowers to be used as female parents were emasculated using fine forceps. Usually this was done just before the buds opened. In a few cases newly opened flowers which had not reached anthesis were used. All flowers (or seed capsules) on the stem below the flowers emasculated were removed. It was usually possible to emasculate two flowers together on the same spike. Most progenies consisted of the seed set in two such flowers which were emasculated and pollinated together. After emasculation female parents were protected from pollinating insects with paper bags as described above. The spikes of plants selected to act as males were bagged at the same time and all open flowers were removed. Thus any flowers open in the bags were known not to have been visited by pollinating insects. Many plants were used as both male and female parents in different crosses. In these cases, flowers above the emasculated flowers were available for use as males.

Crosses were made when the emasculated flowers were fully open. The male flowers were brought to the females. In 1981, and when pollen was scarce, individual anthers were used. They were removed using forceps and rubbed against the stigma of the emasculated flowers. The forceps were sterilized between each pollination done in this way. When plenty

of flowers were available for use as males, the corolla was pulled back from the anthers of the male flower, and the anthers and stigma of the male flower rubbed against the stigma of the female. In this way, the reproductive parts touched nothing but each other, and there were no instruments to sterilize.

When flowers of the same spike were used as both male and female, emasculated flowers were marked by removing one of the upper corolla lobes at the time of emasculation. The other could be removed to indicate which flowers had been pollinated.

3.6.3 THE TRIALS

Two sites were available for the trials. They were at the Botanic Supply Unit of London University at Egham (henceforth the Egham site) and on land belonging to the Royal Horticultural Society at Wisley (henceforth the Wisley site).

The Egham site was the same land that had been used for the trials in 1978, 1979 and 1980. By the end of the programme described here it had been used for Antirrhinum trials for six consecutive years. However, it should be noted that none of the trials occupied the whole site (which was not rectangular) and that the trials varied in size. There was therefore a large but varying overlap between trials of different years.

The Wisley site was adjacent to the site of the 1978 and 1979 trials. It was ploughed up from permanent pasture before the 1981 trial. Both 1981 and 1982 trials occupied most of the available land.

Seeds were germinated and pricked out by the glasshouse staff at each institution. At Wisley, 45 plants were pricked out into a standard seed tray. At Egham 35 plants were pricked out into the each seed tray. This follows the standard practices of the two institutions. Plants were grown in the seed trays until the majority were 7 to 12cm tall when they were all planted out together. In dry conditions they were watered in using a hose pipe or sprinkler. Throughout the season they were kept watered using sprinklers. Planting of a trial never took

longer than two days.

The statistical design and analysis of the trials is discussed in the next chapter. Two different approaches to trial design were used. In trials designed to compare all the available material, a randomised complete block design was used. This was the case in both 1982 trials. Where the main aim was selection within lines, the related plants were not spread over the whole trial site as in a randomised block design, but were kept together. This was the case in the trials in 1981 and 1983.

THE F2 TRIALS IN 1981

The trials of 1981 were to select good plants from the breeding lines represented by 173 F2 progenies. Approximately 30 plants of each entry were grown. For some entries the number of plants was limited by the number of seedlings available. When there were many progenies descended from the same parent varieties the size of some of the plots was reduced to give more uniform allocation of resources between the different lines.

The sowing of the seed for the trials of 1981 was delayed by the later than expected maturation of the F2 seed on the glasshouse grown F1 plants. The first seed to be available was grown at Wisley. Later seed was grown in two groups at Egham.

THE TRIAL AT WISLEY IN 1982

65 lines of control and parent varieties, and F1, F2 and F3 generations were compared in the trial at Wisley in 1982. Parent varieties of all the lines grown were included in this trial. A few additional varieties were included to give a wide selection of the different rust-susceptibilies encountered in the 1978 and 1979 trials, and so allow a greater confidence in comparisons between the trials. Seed of five crosses between resistant varieties and the susceptible variety Malmaison was also grown. Only two of these five accessions proved viable.

THE TRIAL AT EGHAM IN 1982

89 entries were selected to be grown in the the trial at Egham in 1982. They included all the F3 material, some F2 entries and 8 of the parent varieties as controls. These control varieties were all chosen from among the control varieties grown at Wisley in 1982. Of the 89 entries, 78 germinated and grew to be available at planting out time.

THE TRIAL AT EGHAM IN 1983

23 progenies and five control varieties were grown in a smaller trial at Egham in 1983. Of the 23 progenies, 17 were from crosses made in 1982, and 6 were F2 crosses made in 1981 and had been grown in the 1981 trial.

3.7 RESULTS

The F1 plants growing in the glasshouse began to flower in early April 1981, and were self-pollinated as soon as the flowers were open. The seed capsules reached their full size about two weeks after pollination, but were slow to ripen, seed not being ready until early June. The slower later plants were only beginning to flower at the end of May, although ripening was quicker in the hotter summer weather. Some plants took six months from sowing in November to coming into flower in May. It may have been that temperature and photoperiod requirements for flowering were not met, or that a higher temperature in the glasshouse during the winter would have produced faster growth. The differences between varieties were not simply the result of different growth rates, so it is probable that flowering requirements were not met.

The colours of the F1 plants are listed in Table 3.4. A few flower abnormalities were observed at this stage, the most common being the split corolla (described in Chapter 5 below).

The later than expected maturity of the F1 plants in the glasshouse resulted in F2 seed not being available until rather late in the 1981 season. As the date of maturity differed between the crosses the lines were therefore divided into four groups according to the time of

Accession number	Colour	Accession number	Colour
32-1	white	45-1	pink
32-3	white	45-2	
32-4	white	45-3	pale pink
32-5	white	46-1	pink
33-1	white	46-2	pink
33-2	white	46-3	pink
33-3	white	47-2	pink
33-4	white	48-1	white
34-1	pink	48-2	white
34-2	pink	48-2	white
34-3	pink	48-3	white
35-1	pink-orange	48-4	white
36-1	pink	49-1-1	pink
36-2	pink	49-1-2	magenta
36-3	pink	49-1-3	magenta
36-4	pink	49-2	magenta
36-5	pink	49-3-1	pale pink
37-1	red	49-3-2	purple
37-2	red	49-3-3	purple
37-3	red	50-2	pink
38-1	pink, orange	50-3-1	dark purple
38-2	pink, orange front	50-3-2	dark purple
38-3	pink, orange front	50-3-3	pink
39-1	white	50-4-1	pink
39-2	white	50-4-2	dark purple, paler tube
39-3	white	50-4-3	dark purple, paler tube
39-4	white	51-1-1	pale pink
39-5	white	51-1-2	magenta
40-2	pale pink, orange-yellow front	51-1-3	magenta tube, red front
40-2	pale pink, orange-yellow front	51-2-1	magenta tube, red front
40-3	very pale pink	51-2-2	pale pink
40-4	pale pink tube, orange-yellow front	51-2-3	magenta tube, red front
40-5	pale pink tube, orange-yellow front	52-1-1	magenta
41-2	pale pink tube, orange-yellow front	52-1-2	pale pink tube, white front
41-3	pale pink tube, orange front	52-1-3	pale pink tube, yellow front
41-6	pale pink tube, orange-yellow front	52-2-1	pale pink, yellow front
42-1	pale pink, yellow front	52-2-2	magenta
42-2	pale pink, yellow front	52-2-3	magenta
42-3	pale pink, yellow front	52-3	pale pink
42-4	pale pink, yellow front	53-1	pink, orange front
42-4	pink	53-3	pink tube, red front
43-2	pink	53-4	pink, orange front
43-3	pink	53-5	pink tube, orange front
43-3	pink	54-1	pink tube, orange front
43-4	pink	54-2	pink orange
43-5	pink	54-3	pink tube, orange front
44-1	magenta	54-4	pink tube, orange-yellow front
44-2	magenta		
44-3	magenta		
44-4	magenta		
44-5	magenta		

Table 3.4 Flower colour of F1 plants grown in the glasshouse in 1981.

ripening of the seed. The first three of these were used in the trials of 1981:

1. The earliest seed set was grown and planted at Wisley.
2. First sowing of seed at Egham.
3. Second sowing of seed at Egham.
4. Seed set late or not at all was not used.

The number of progenies from each of the breeding lines that were grown in the trials in 1981 are given in Table 3.5. No lines were represented in trials at both Wisley and Egham, but there were progenies of the lines 32 and 48 in both plantings at Egham.

The late planting of the trials in 1981 resulted in only a slight epidemic, and therefore selection of suitable plants for breeding could not begin until the end of September. Even the spreader rows at the Wisley trial which had been planted earlier were slow to take the rust and so did not provide the higher levels of inoculum that might have been expected. When plants were selected, it was too late for crosses to be made in the field. The selected plants from both trials were dug up and brought into a glasshouse at the Botanical Supply Unit. Crosses were made in the glasshouse in the autumn. Further crosses were made on the same plants in the spring of 1982. These crosses provided the F3 seed for the trials of 1982.

The trial at Wisley in 1982 was conducted to compare the different generations and the different lines. Pedigrees and mean rust scores of all the entries in this trial are given in Table 3.6. Statistical analysis (see Chapter 4 below) showed that there were significant differences between these accessions in their rust scores (F test for variety effect significant at 0.1% level) although care has to be taken in ascribing significance to differences between individual pairs of trial entries (see Chapter 4 below). Figs. 4.13 and 4.14 summarize the t tests between all possible pairs of entries.

The control varieties at Wisley were chosen to span the range of rust-resistance found by Gawthrop (1980) and included some of the old varieties which had been immune to Race 1 antirrhinum rust. The old varieties developed little rust. This probably reflects a decrease in the prevalence of rust strains virulent on these varieties since they

Cross number	Wisley	Egham 1st planting	Egham 2nd planting	Total
32		3	6	9
33			6	6
34			7	7
35		3		3
36			8	8
37			4	4
38	9			9
39	10			10
40			10	10
41			7	7
42		11		11
43	9			9
44		9		9
45	6			6
46	9			9
47			6	6
48		6	4	10
49			7	7
50			8	8
51			6	6
52			7	7
54	12			12
Total	55	32	86	173

Table 3.5 Number of F2 progenies of each initial cross grown in the trials in 1981.

Trial entry number	Accession number	Generation	Variety name		(1) Mean rust score 12th Aug.	30th Sept.
1	78-180	CONTROL	Titan		1.56	4.68
2	78-92	CONTROL	"B"		dead	
3	78-95	CONTROL	"E"		dead	
4	78-88	CONTROL	Amber Monarch		1.48	3.86
5	78-32-III	CONTROL	Malmaison		2.78	4.69
6	MALMAISON	CONTROL	Malmaison		2.66	5.79
7	78-246	CONTROL	Triumph White		1.72	4.80
8	78-240	CONTROL	Pale Sulphur		1.90	5.52
9	78-164	CONTROL	Leonard Sutton		2.47	4.76
10	78-195	CONTROL	Frontier White		2.30	5.46
11	78-178	CONTROL	Golden Fleece		1.54	3.58
12	78-183	CONTROL	Yellow Freedom		1.49	3.92
13	78-187	CONTROL	Bonfire		2.53	5.15
14	82-1	CONTROL	Yellow Freedom		2.68	4.22
15	82-3	CONTROL	Scarlet Monarch		2.20	4.95
16	82-2	CONTROL	Victory		2.01	3.08
			Parents of progenies			
			female	male		
17	80-38-1	F1	Bonfire	Amber Monarch	1.61	4.57
18	80-38-3	F1	Bonfire	Amber Monarch	1.83	4.82
19	81-38-1	F2	80-38-1	self	1.75	4.97
20	81-38-3	F2	80-38-1	self	1.90	4.86
21	81-38-7	F2	80-38-3	self	1.96	4.06
22	82-38-1	F3	81-38-1	81-38-5	1.53	4.09
23	82-38-6	F3	81-38-3	81-38-7	dead	
24	82-38-7	F3	81-38-7	81-38-3	2.07	3.54
25	80-39-1	F1	Pale Sulphur	Triumph White	1.71	5.59
26	80-39-4	F1	Pale Sulphur	Triumph White	1.65	4.79
27	81-39-1	F2	80-39-1	self	2.52	5.87
28	81-39-4	F2	80-39-2	self	1.78	4.68
29	81-39-7	F2	80-39-4	self	1.77	4.96
30	82-39-1	F3	81-39-1	81-39-1	1.94	5.42
31	82-39-3	F3	81-39-4	81-39-7	1.63	4.84
32	82-39-4	F3	81-39-7	81-39-7	dead	

Table 3.6 Entries in the trial at Wisley in 1982 (continued on next page)

(1) See Section 4.5 (below)

Trial entry number	Accession number	Generation	Parents of progenies		Mean rust score 12th Aug.	30th Sept.
			female	male		
33	80-43-3	F1	Bonfire	Leonard Sutton	1.96	4.48
34	80-43-5	F1	Bonfire	Leonard Sutton	2.24	3.82
35	81-43-1	F2	80-43-2	self	1.67	4.96
36	81-43-3	F2	80-43-3	self	1.64	4.21
37	81-43-9	F2	80-43-5	self	1.90	4.60
38	82-43-9	F3	81-43-9	81-43-9	1.81	5.35
39	82-43-2	F3	81-43-3	81-43-3	2.25	4.86
40	82-43-10	F3	81-43-9	81-43-1	2.02	4.40
41	80-40-3	F1	Golden Fleece	Yellow Freedom	1.93	4.00
42	81-40-9	F2	81-40-2	self	2.22	5.13
43	81-40-10	F2	81-40-3	self	1.19	3.30
44	80-41-2	F1	Amber Monarch	Yellow Freedom	1.73	4.33
45	81-41-3	F2	81-41-2	self	1.56	4.17
46	81-41-5	F2	81-41-3	self	1.29	4.55
47	80-42-1	F1	Golden Fleece	Pale Sulphur	2.37	4.41
48	81-42-1	F2	80-42-1	self	2.74	5.03
49	81-42-5	F2	80-42-2	self	1.73	4.87
50	80-49-3	F1	Leonard Sutton	Frontier White	2.61	5.18
51	81-49-2	F2	81-49-1	self	2.70	5.53
52	81-49-6	F2	81-49-3	self	1.92	4.96
53	80-52-2	F1	Yellow Freedom	Frontier White	2.04	5.20
54	81-52-1	F2	81-52-1	self	2.19	4.42
55					1.69	4.06
56	80-55-1	F1	Malmaison	Yellow Freedom	2.19	5.54
57	80-56-1	F1	Malmaison	Frontier White	2.87	5.81
58	80-58-1	F1	Malmaison	Golden Fleece	dead	
59	80-78-1	F1	Leonard Sutton	Malmaison	dead	
60	80-80-1	F1	Amber Monarch	Malmaison	dead	
			Variety name			
61	78-38	CONTROL	Coronette Bronze		1.74	4.46
62	78-35	CONTROL	Coronette Scarlet		2.79	5.03
63	78-63	CONTROL	Carioca Yellow		1.97	4.68
64	78-64	CONTROL	Carioca Cherry Red		1.92	4.35
65	82-6	CONTROL	Amber Monarch		1.85	4.53

Table 3.6 List of trial entry numbers, accession numbers, parents of progenies and mean rust scores in the trial at Wisley in 1982.

have not been widely grown.

Three cultivated varieties, Yellow Freedom, Amber Monarch and Malmaison were entered into the trial twice. One entry of each of these used seed from the accession used by Gawthrop in 1978 and 1979. The other entry (1982 accession numbers) was new commercial seed. The entry listed as Malmaison (without an accession number) was commercial seed obtained by the R.H.S. staff at Wisley and used for the spreader and guard rows in the Wisley trials of 1981 and 1982. This seed was not given a separate accession number. The accession 78-32-III was seed of the original Malmaison accession multiplied by Gawthrop at Egham. Any shift in rust-resistance of these three varieties should be revealed by comparison of the two accessions of each. The two accessions of Yellow Freedom differed greatly at the first scoring (t test significant at 99% level). The 1982 accession was relatively heavily rusted at that time, but the rust did not continue to develop, and both accessions were among the less rusted entries by the time of the second scoring. This change might represent different and uneven distribution of two or more pathotypes of rust within the trial. The two accessions of each of the varieties Malmaison and Amber Monarch were not significantly different from each other.

Most of the entries in the trial at Wisley in 1982 were F1 and F2 progenies from some of the crosses. It was hoped that this trial would enable useful comparisons to be made between these accessions and their parent varieties. Five crosses with the susceptible variety Malmaison were included, though three of these failed to germinate. Precise estimates of rust-resistance in the progenies of these crosses would have revealed information about the genetic background to the rust-resistance. However, with the precision obtained it is not possible to draw many conclusions from the data of these accessions. Most of them had rust scores intermediate between their parent varieties. Of those that fell outside the range of their parent varieties, only one, the F2 progeny 81-40-9, is significantly different from both of its parents in the t test (second scoring). This is probably a chance effect, and illustrates the risks of making large numbers of comparions with the t test. Both parents, Wisley Golden Fleece and Yellow Freedom were among the more rust-resistant accessions.

The F1 progenies of the crosses Malmaison X Yellow Freedom and Malmaison X Frontier White have rust scores nearer to Malmaison than to their rust-resistant parent. Even if this is taken as an indication of dominance by the rust-susceptible alleles, the effect would be changed by transformation of the data or by the use of a different scale for scoring rust infection.

Selection of plants for breeding was made on the trial at Egham in 1982. The entries are listed with their mean rust scores in Table 3.7. Mean rust scores for the second scoring are compared by Tukey's test in Fig. 4.12. Mean rust scores for both scorings are compared by t test in Figs. 4.15 & 4.16. Flower colours of the individual plants are summarized in Table 3.8.

Selection was made in the field for individual healthy plants. Some of the progenies of cross 54 were noted at that time as having a low level of rust. They also produced strong plants of good horticultural quality. Three of the cross 54 progenies, 81-54-13, 82-54-5 and 82-54-4, had the lowest rust scores at the second scoring at Egham in 1983. These also were among the better accessions at the first scoring. The two related progenies, 81-54-1 and 82-54-9, were more heavily infected with rust. All progenies of lines 43 and 46 were among the more heavily infected accessions. Within other lines there was a wide range of rust scores between progenies. For example, 81-36-6 and 81-48-2 had little rust at the second scoring while 81-36-10 and 81-48-10 were much more severely rusted.

Line 37 had F2 progenies of two types in the trial at Egham in 1981. Some progenies were segregating for flower colour, while others were uniformly red. Plants of the red progenies were selected for breeding, but in the trial at Egham in 1982 progenies of this line were identical with Scarlet Monarch, the female parent of the line. The red F2 progenies must have resulted from self-pollination on the breeding plot in 1980.

The most promising material from the breeding programme was grown in a smaller trial at Egham in 1983. Accessions in this trial and their mean rust scores are listed in Table 3.9. Four of the control varieties of

Trial entry number	Accession number	Generation	Variety name		Mean rust score 10th Aug.	28th Sept.
2	78-92	CONTROL	"B"		2.96	5.96
8	78-240	PARENT	Pale Sulphur		1.99	4.93
9	78-164	PARENT	Leonard Sutton		2.48	4.71
14	82-1	PARENT	Yellow Freedom		3.06	6.10
15	82-3	PARENT	Scarlet Monarch		2.30	5.27
16	82-2	PARENT	Victory		2.15	4.24
			Parents of progenies			
			female	male		
22	82-38-1	F3	81-38-1	81-38-5	2.38	4.76
30	82-39-1	F3	81-39-1(1)	81-39-1	2.42	5.13
31	82-39-3	F3	81-39-4	81-39-7(2)	1.98	4.89
66	82-39-7	F3	81-39-8	81-39-10(1)	2.11	5.41
67	82-39-8	F3	81-39-10(1)	81-39-10(2)	2.15	4.79
38	82-43-9	F3	81-43-9(2)	81-43-9(4)	2.40	5.36
39	82-43-2	F3	81-43-3(1)	81-43-3(3)	2.93	5.82
69	82-43-6	F3	81-43-6(2)	81-43-6(3)	2.68	5.16
70	82-45-2	F3	81-45-6(3)	81-45-1(1)	2.60	5.81
71	82-45-3	F3	81-45-1(2)	81-45-1(1)	2.30	5.37
72	82-45-4	F3	81-45-3	81-45-1(1)	2.56	5.41
73	82-45-5	F3	81-45-5	81-45-6(2)	2.27	5.30
74	82-45-7	F3	81-45-6(1)	self	2.02	5.32
75	82-46-1	F3	81-46-1(1)	81-46-1(2)	2.01	5.10
76	82-46-2	F3	81-46-1(2)	81-46-4(1)	2.06	5.42
77	82-46-3	F3	81-46-1(3)	81-46-1(4)	2.13	5.72
78	82-46-4	F3	81-46-1(4)	81-46-7(2)	2.04	5.54
79	82-46-6	F3	81-46-4(1)	81-46-4(2)	1.79	5.14
80	82-46-7	F3	81-46-4(2)	81-46-7(1)	2.42	5.15
81	81-54-1	F3	81-54-4	81-54-7	2.67	5.33
86	82-54-4	F3	81-54-4(1)	81-54-4(2)	1.93	3.97
87	82-54-5	F3	81-54-4(2)	81-54-8(1)	2.16	3.87
91	82-54-9	F3	81-54-8(1)	81-54-8(3)	2.21	5.31
94	81-54-13	F3	81-54-9(3)	81-54-9(2)	1.80	3.82
96	81-32-1	F2	80-32-5	self	2.22	5.45
97	81-32-3	F2	80-32-5	self	2.16	5.50
98	81-32-6	F2	80-32-1	self	2.53	5.62
99	81-32-7	F2	80-32-3	self	2.19	5.06
100	81-32-9	F2	80-32-4	self	1.93	4.81
101	81-34-4	F2	80-34-2	self	2.26	4.41
102	81-34-6	F2	80-34-3	self	2.54	5.49

Table 3.7 Entries in the trial at Egham in 1982 (continued on next page)

Trial entry number	Accession number	Generation	Parents of progenies		Mean rust score	
			female	male	10th Aug.	28th Sept.
103	81-36-1	F2	80-36-1	self	2.67	4.56
104	81-36-3	F2	80-36-1	self	2.77	5.31
105	81-36-6	F2	80-36-2	self	2.03	4.11
106	81-36-10	F2	80-36-4	self	2.82	5.47
107	81-36-12	F2	80-36-4	self	2.87	5.02
108	81-37-1	F2	80-37-1	self	2.51	5.67
109	81-37-3	F2	80-37-2	self	2.32	5.24
110	81-37-4	F2	80-37-3	self	2.58	5.19
111	81-37-5	F2	80-37-1	self	3.00	5.28
112	81-37-6	F2	80-37-2	self	2.38	5.05
113	81-41-5	F2	80-41-3	self	1.88	4.76
114	81-41-7	F2	80-41-6	self	2.12	4.60
115	81-42-8	F2	80-42-4	self	2.38	4.62
116	81-42-9	F2	80-42-4	self	2.96	5.56
117	81-42-10	F2	80-42-4	self	1.98	5.09
118	81-42-12	F2	80-42-2	self	2.20	4.68
119	81-42-13	F2	80-42-4	self	2.33	4.70
120	81-42-14	F2	80-42-4	self	2.27	4.44
121	81-48-2	F2	80-48-1	self	2.02	4.39
122	81-48-5	F2	80-48-3	self	3.30	4.83
123	81-48-6	F2	80-48-3	self	2.31	5.00
124	81-48-7	F2	80-48-2	self	2.03	4.82
125	81-48-10	F2	80-48-4	self	2.71	5.64
126	81-49-5	F2	80-49-3	self	2.76	4.96
127	81-49-6	F2	80-49-3	self	2.11	4.76
128	81-49-1	F2	80-49-1	self	2.30	4.38
129	81-49-2	F2	80-49-1	self	3.08	5.24
130	81-50-3	F2	80-50-3	self	1.87	4.43
131	81-50-6	F2	80-50-4	self	2.08	5.02
132	81-50-7	F2	80-50-4	self	2.37	4.84
133	81-52-1	F2	80-52-1	self	2.09	4.72
134	81-52-4	F2	80-52-2	self	1.91	5.10
135	81-52-7	F2	80-52-3	self	2.11	5.10
136	81-53-1	F2	80-53-1	self	2.18	4.75
137	81-53-3	F2	80-53-3	self	1.97	4.52
138	81-53-5	F2	80-53-5	self	2.10	5.17

Table 3.7 List of trial entry numbers, accession numbers, parents of progenies and mean rust scores in the trial at Egham in 1982.

Entry number	Accession number	Number of plants with flowers of each colour								
		White	Yellow	Orange	Flame	Pink	Red	Magenta	Black	Total
2	78-92	0	0	9	6	1	2	0	0	18
3	78-95	0	0	0	0	0	0	0	0	0
7	78-246	18	0	0	0	0	0	0	0	18
8	78-240	1	26	0	0	0	0	0	0	27
9	78-164	0	0	0	0	27	0	0	0	27
14	82-1	0	26	0	0	0	1	0	0	27
15	82-3	0	0	0	0	0	27	0	0	27
16	82-2	0	0	13	9	3	0	0	0	25
22	82-38-1	0	9	18	0	0	0	0	0	27
23	82-38-6	0	0	0	17	0	0	0	0	17
30	82-39-1	4	23	0	0	0	0	0	0	27
31	82-39-3	0	27	0	0	0	0	0	0	27
32	82-39-4	0	0	0	0	0	0	0	0	0
66	82-39-7	0	26	0	0	0	0	0	0	26
67	82-39-8	0	27	0	0	0	0	0	0	27
68	82-39-11	0	0	0	0	0	0	0	0	0
38	82-43-9	0	0	0	27	0	0	0	0	27
39	82-43-2	2	0	8	0	17	0	0	0	27
40	82-43-10	0	0	0	0	18	0	0	0	18
69	82-43-6	0	0	9	1	8	0	0	0	18
70	82-45-2	0	15	0	12	0	0	0	0	27
71	82-45-3	0	11	10	5	0	0	0	0	26
72	82-45-4	0	2	1	15	0	0	0	0	18
73	82-45-5	0	7	7	7	2	0	0	0	23
74	82-45-7	0	4	3	11	0	0	0	0	18
75	82-46-1	4	0	0	0	23	0	0	0	27
76	82-46-2	4	0	0	0	22	0	0	0	26
77	82-46-3	4	2	0	2	8	0	0	0	16
78	82-46-4	6	1	0	1	10	0	0	0	18
79	82-46-6	4	0	1	0	10	0	0	0	15
80	82-46-7	5	0	0	0	13	0	0	0	18
81	81-54-1	0	0	18	9	0	0	0	0	27
82	82-54-1	0	0	0	0	0	0	0	0	0
83	82-54-2	0	0	0	0	0	0	0	0	0
84	81-54-3	0	0	0	0	0	0	0	0	0
85	82-54-3	0	0	0	0	0	0	0	0	0
86	82-54-4	0	0	18	9	0	0	0	0	27
87	82-54-5	0	0	27	0	0	0	0	0	27
88	82-54-6	0	0	0	0	0	0	0	0	0
89	82-54-8	0	0	18	0	0	0	0	0	18
90	81-54-9	0	0	0	0	0	0	0	0	0
91	82-54-9	0	25	0	0	0	0	0	0	25
92	82-54-10	0	0	0	0	0	0	0	0	0
93	82-54-11	0	0	0	0	0	0	0	0	0
94	81-54-13	0	0	27	0	0	0	0	0	27
95	82-54-13	0	0	0	0	0	0	0	0	0

Table 3.8 Number of plants of each colour at Egham in 1982 (continued on next page).

Entry number	Accession number	Number of plants with flowers of each colour								
		White	Yellow	Orange	Flame	Pink	Red	Magenta	Black	Total
96	81-32-1	27	0	0	0	0	0	0	0	27
97	81-32-3	27	0	0	0	0	0	0	0	27
98	81-32-6	23	0	0	0	0	0	0	0	23
99	81-32-7	26	1	0	0	0	0	0	0	27
100	81-32-9	25	0	0	0	0	0	0	0	25
101	81-34-4	0	0	0	3	20	0	0	0	23
102	81-34-6	0	0	0	2	25	0	0	0	27
103	81-36-1	0	0	2	3	22	0	0	0	27
104	81-36-3	0	0	0	1	14	1	0	0	16
105	81-36-6	0	0	1	0	34	0	0	0	35
106	81-36-10	0	0	2	2	23	0	0	0	27
107	81-36-12	0	0	0	0	27	0	0	0	27
108	81-37-1	0	0	0	0	0	27	0	0	27
109	81-37-3	0	0	0	0	0	27	0	0	27
110	81-37-4	0	0	0	0	0	27	0	0	27
111	81-37-5	0	0	0	0	0	26	0	0	26
112	81-37-6	0	0	0	0	0	27	0	0	27
113	81-41-5	0	4	12	2	0	0	0	0	18
114	81-41-7	0	1	13	4	0	0	0	0	18
115	81-42-8	0	0	4	0	14	0	0	0	18
116	81-42-9	0	0	0	6	11	0	0	0	17
117	81-42-10	0	0	9	3	14	0	0	0	26
118	81-42-12	0	9	14	4	0	0	0	0	27
119	81-42-13	0	4	9	5	0	0	0	0	18
120	81-42-14	0	12	9	4	0	0	0	0	25
121	81-48-2	27	0	0	0	0	0	0	0	27
122	81-48-5	27	0	0	0	0	0	0	0	27
123	81-48-6	27	0	0	0	0	0	0	0	27
124	81-48-7	27	0	0	0	0	0	0	0	27
125	81-48-10	27	0	0	0	0	0	0	0	27
126	81-49-5	2	1	1	1	12	0	0	0	17
127	81-49-6	5	0	0	0	4	0	18	0	27
128	81-49-1	3	0	0	2	13	0	0	0	18
129	81-49-2	4	1	0	1	2	0	19	0	27
130	81-50-3	8	1	0	0	3	1	1	2	16
131	81-50-6	5	0	0	1	8	1	2	0	17
132	81-50-7	5	3	0	0	7	2	0	4	21
133	81-52-1	12	4	1	0	3	1	3	0	24
134	81-52-4	0	9	5	4	0	0	0	0	18
135	81-52-7	9	1	4	2	11	0	0	0	27
136	81-53-1	0	2	9	7	0	0	0	0	18
137	81-53-3	0	7	9	10	0	1	0	0	27
138	81-53-5	0	6	7	3	2	0	0	0	18
Total:		368	297	298	201	431	171	43	6	1815

Table 3.8 Number of plants of each colour at Egham in 1982.
(continued from previous page)

Trial entry number	Accession number	Generation	Variety name		Mean rust score
8	78-240	CONTROL	Pale Sulphur		1.89
9	78-164	CONTROL	Leonard Sutton		2.24
15	82-3	CONTROL	Scarlet Monarch		2.60
16	82-2	CONTROL	Victory		1.22
166	83-1	CONTROL	Golden Queen		3.93
			Parents of progenies		
			female	male	
100	81-32-9	F2	80-32-4	self	1.87
141	82-32-2	F3	81-32-1(1)	81-32-1(2)	2.48
142	82-32-3	F3	81-32-1(2)	81-32-3	1.92
105	81-36-6	F2	80-36-2	self	1.22
144	82-36-1	F3	81-36-12(1)	81-36-12(2)	1.89
120	81-42-14	F2	80-42-4	self	2.04
147	82-42-1	F3	81-43-9(1)	81-42-9(2)	1.30
149	82-42-3	F3	81-42-10(2)	81-42-10(1)	1.61
121	81-48-2	F2	80-48-1	self	1.40
150	82-48-1	F3	81-48-2(1)	81-48-2(3)	1.39
151	82-48-3	F3	81-48-10(1)	81-48-10(2)	1.56
152	82-48-4	F3	81-48-7	self	1.24
153	82-48-5	F3	81-48-10(3)	81-48-2(1)	1.44
154	82-48-6	F3	81-48-2(2)	81-48-10(3)	1.59
128	81-49-1	F2	80-49-1	self	2.22
155	82-49-1	F3	81-49-6(1)	81-49-6(2)	1.58
157	82-49-4	F3	81-49-6(2)	81-49-1(2)	1.44
137	81-53-3	F2	80-53-3	self	1.95
159	82-53-3	F3	81-53-1(2)	81-53-3	2.00
160	82-53-5	F3	81-53-3	81-53-1(3)	1.60
86	82-54-4	F3	81-54-4(1)	81-54-4(2)	1.34
163	82-54-5	F4	82-54-8	82-54-13(1)	1.44
165	82-54-8	F4	82-54-1	82-54-4	1.66

Table 3.9 List of trial entry numbers, accession numbers, parents of progenies and mean rust scores in the trial at Egham in 1983.

Trial entry number	Accession number	Mean rust score	Number of plants	Range of rust score best	worst	S.E.M.	Colour	Maturity
8	78-240	1.89		1.0	4.2	0.22	CONTROL	2
9	78-164	2.24		1.4	3.8	0.10	CONTROL	3
15	82-3	2.60		1.0	5.0	0.16	CONTROL	3
16	82-2	1.22		1.0	1.8	0.09	CONTROL	0
166	83-1	3.93		2.4	4.8	0.43	CONTROL	2
100	81-32-9	1.87	30	1.2	2.8	0.07	white	3
141	82-32-2	2.48	24	1.0	4.6	0.21	white	3
142	82-32-3	1.92	29	1.2	3.8	0.01	white	3
105	81-36-6	1.22	23	1.0	1.8	0.06	pink+	2,3
144	82-36-1	1.89	25	1.0	3.2	0.11	pink	2,3
120	81-42-14	2.04	28	1.0	3.0	0.09	yellow+	2
147	82-42-1	1.30	29	1.0	2.4	0.05	pink+	1,2
149	82-42-3	1.61	20	1.0	3.4	0.13	pink+	2,3
121	81-48-2	1.40	24	1.0	2.2	0.07	white	3
150	82-48-1	1.39	29	1.0	2.0	0.54	white	1,2
151	82-48-3	1.56	25	1.0	3.2	0.11	white	3
152	82-48-4	1.24	28	1.0	2.2	0.06	white	2
153	82-48-5	1.44	26	1.0	2.0	0.06	white	1,2
154	82-48-6	1.59	26	1.0	3.8	0.12	white	3
128	81-49-1	2.22	12	1.0	3.8	0.25	pink+	1,2
155	82-49-1	1.58	12	1.0	2.6	0.17	white	2
157	82-49-4	1.44	15	1.0	2.4	0.10	white	1,2
137	81-53-3	1.95	11	1.0	2.6	0.14	red+	2
159	82-53-3	2.00	19	1.2	4.4	0.16	red	1,2
160	82-53-5	1.60	23	1.0	3.0	0.11	red+	1,2
86	82-54-4	1.34	10	1.0	1.6	0.07	orange	0,2
163	82-54-5	1.44	27	1.0	3.0	0.09	orange	2
165	82-54-8	1.66	32	1.0	3.2	0.10	orange+	3

Table 3.10 Rust scores and horticultural qualities in the trial at Egham in 1983.

1982 were grown and one variety, "Golden Queen", which had not been grown in any of the previous trials. Mean rust scores, the range of scores of the plants of each accession, the predominant colour and a maturity score are given in Table 3.10. Comparisons between accessions must be treated with caution because the trial was designed for a further cycle of selection within accessions (Chapter 4 below). Golden Queen had the highest mean rust score of all the entries in the trial. The other control varieties and the breeding programme progenies had low rust scores and were not badly affected by rust. The lowest mean rust scores were achieved by the control variety Victory and one of the progenies of line 36. The progenies of line 54 were acceptably free of rust.

Maturity at 9 July 1983 was scored on a five point scale:

0. no sign of flowering
1. flower buds developing on the main stem
2. flowers open on the main stem but no seed set
3. flowers and seed capsules on the main stem
4. flowers on more than one stem

Some accessions contained plants at differing stages of maturity, and this range is indicated by giving two numbers in Table 3.10.

Selected plants were again crossed together within the breeding lines and seed of these crosses was available at the end of the breeding programme.

The pedigrees of all the progenies grown in all the trials are given in Table 3.11. Accessions in the trials are listed in Appendix 1.

3.8 DISCUSSION

The logistic problems in the summer of 1981 caused the loss of the slowest maturing lines and progenies. It is uncertain how much effect this had on the breeding programme. The absence of these lines allowed resources of time and space to be devoted to the other lines. Early maturity is a desirable feature. Due to the effect of the seasons, there were many stages in the programme when plants were selected for

having flowers available for crossing. Crosses made in the warmer and drier weather were generally more successful than those made in the less favourable conditions in the autumn. While this selection pressure was not intentional, it was strong at some stages in the breeding programme. Its effect will be beneficial if the result is the production of quicker maturing varieties, and as long as this is not achieved at the expense of persistence.

The slow ripening of the seed caused delay at all stages of the breeding programme. Even when time was not critical, slow ripening left the capsules exposed to the elements and to disease for longer than might have been necessary. Some progenies were lost because although the seeds had formed in the capsule, they succumbed to moulds before the capsules were ripe for harvesting. It may be that the hormone balance of the spike is different when many capsules are ripening seed. Ripening inducing hormones from seed containing capsules may normally promote the ripening process in other capsules and change the physiology of the whole spike. In stems with only one or two capsules setting seed, the hormone balance could be very different from that of a normal spike with many seed capsules. This may have slowed the ripening process and have caused the plants, or at least the flower spikes, to remain in a more juvenile physiological condition following hand pollination. The seeds were fully formed long before the capsules ripened. The seeds may have been sufficiently mature to have germinated successfully if spikes had been harvested earlier and dried slowly in the laboratory.

The stems were originally kept to one or two capsules to avoid contamination, and in the hope that the better nutritional status would hasten ripening. Simple experiments would show whether or not ripening is faster when there are many capsules on a spike. If it is, then it might be better not to cut off all the top of the spike, but to allow these flowers to be open pollinated or hand self-pollinated. The capsules containing the pedigree seed would be identified as the one or two capsules remaining nearest the base of the spike. A region of flowers removed would serve to separate them from the open pollinated capsules at the top of the spike. Extra care would be needed to avoid mixing of seed or harvesting of the wrong capsules.

The trials were less conclusive than had been hoped. The reasons for this are discussed in Chapter 4 (below). However, the overall level of rust on the trials was less than in the earlier trials and this may reflect a generally higher level of rust-resistance. Some of the accessions had very little rust. Those which performed best in 1982 were also good in 1983. The 1983 trial was designed for selection of individual plants and comparisons between accessions should be treated with caution. Material was still in early generations and not genetically uniform but the general level of rust infection was low. These lines are not immune to rust, but could form the basis for the production of a range of varieties that develop little rust, or develop rust only slowly. Such varieties would provide a good display of flowers without succumbing to disease. It is not possible to test the durability of disease resistance except by the prolonged use of cultivars on a wide scale. Lines in the breeding programme were independently derived from different parents, and the aim has been to accumulate polygenic resistance. It is therefore less likely that all the lines should all succumb at once to a single new genotype of the rust.

P1		P2	F1		F2			F3
78-246	X	78-83	80-32-5 (1)	X self	81-32-1 (1)	X	81-32-1(2)	82-32-2
					(2)	X	81-32-3	82-32-3
			(2)	X self	81-32-2			
			(3)	X self	81-32-3			
			80-32-1 (1)	X self	81-32-4			
			(2)	X self	81-32-5			
			(3)	X self	81-32-6			
			80-32-3 (1)	X self	81-32-7			
			(2)	X self	81-32-8			
			80-32-4 (1)	X self	81-32-9			
			(2)	X self	81-32-10			
78-36	X	78-164	80-36-1 (1)	X self	81-36-1			
			(2)	X self	81-36-2			
			(3)	X self	81-36-3			
			80-36-2 (1)	X self	81-36-4			
			(2)	X self	81-36-5			
			(3)	X self	81-36-6			
			80-36-3 (1)	X self	81-36-7			
			(2)	X self	81-36-8			
			(3)	X self	81-36-9			
			80-36-4	X self	81-36-10			
78-187	X	78-88	80-38-1 (1)	X self	81-38-1	X	81-38-5	82-38-1
			(2)	X self	81-38-2			
			(3)	X self	81-38-3	X	81-38-7	82-38-6
			80-38-2 (1)	X self	81-38-4			
			(2)	X self	81-38-5			
			(3)	X self	81-38-6			
			80-38-3 (1)	X self	81-38-7	X	81-38-3	82-38-7
			(2)	X self	81-38-8			
78-240	X	78-246	80-39-1 (1)	X self	81-39-1 (1)	X	81-39-1(2)	82-39-1
					(2)	X	81-39-10(3)	82-39-11
			(2)	X self	81-39-2			
			(3)	X self	81-39-3			
			80-39-2 (1)	X self	81-39-4	X	81-39-7(2)	82-39-3
			(2)	X self	81-39-5			
			(3)	X self	81-39-6			
			80-39-4 (1)	X self	81-39-7 (1)	X	81-39-7(2)	82-39-4
			80-39-5 (1)	X self	81-39-8	X	81-39-10(1)	82-39-7
			(2)	X self	81-39-9			
			(3)	X self	81-39-10(1)	X	81-39-10(2)	82-39-8

Table 3.11 Pedigrees of progenies grown in trials (continued on next page)

P1		P2	F1		F2			F3
78-178	X	78-240	80-42-1 (1)	X self	81-42-1			
			(2)	X self	81-42-2			
			(3)	X self	81-42-3			
			80-42-2 (1)	X self	81-42-4			
			(2)	X self	81-42-5			
			80-42-3 (1)	X self	81-42-6			
			(2)	X self	81-42-7			
			80-42-4 (1)	X self	81-42-8			
			(2)	X self	81-42-9 (1)	X	81-42-9(2)	82-42-1
			(3)	X self	81-42-10(1)	X	81-42-9(1)	82-42-2
					(2)	X	81-42-10(1)	
			(4)	X self	81-42-11			
78-187	X	78-164	80-43-2 (1)	X self	81-43-1			
			(2)	X self	81-43-2			
			80-43-3 (1)	X self	81-43-3 (1)	X	81-43-3(3)	82-43-2
			(2)	X self	81-43-4			
			(3)	X self	81-43-5			
			80-43-4 (1)	X self	81-43-6 (2)	X	81-43-6(3)	82-43-6
			(2)	X self	81-43-7			
			(3)	X self	81-43-8			
			80-43-5 (1)	X self	81-43-9 (2)	X	81-43-9(4)	82-43-9
					(3)	X	81-43-3(1)	82-43-10
			(2)	X self	81-43-11			
			(2)	X self	81-43-12			
78-83	X	78-187	80-45-1	X self	81-45-1 (2)	X	81-45-1(1)	82-45-3
			80-45-2 (1)	X self	81-45-2			
			(2)	X self	81-45-3	X	81-45-1(1)	82-45-4
			(3)	X self	81-45-4			
			80-45-3 (1)	X self	81-45-5	X	81-45-6(2)	82-45-5
			(2)	X self	81-45-6 (1)	X	self	82-45-7
					(3)	X	81-54-1(1)	82-45-2
78-83	X	78-187	80-46-1 (1)	X self	81-46-1 (1)	X	81-46-1(2)	82-46-1
					(2)	X	81-46-4(1)	82-46-2
					(3)	X	81-46-1(4)	82-46-3
					(4)	X	81-46-7(2)	82-46-4
			(2)	X self	81-46-2			
			(3)	X self	81-46-3			
			80-46-2 (1)	X self	81-46-4 (1)	X	81-46-4(2)	82-46-6
					(2)	X	81-46-7(1)	82-46-7
			(2)	X self	81-46-5			
			(3)	X self	81-46-6			
			80-46-3 (1)	X self	81-46-7			
			(2)	X self	81-46-8			
			(3)	X self	81-46-9			

Table 3.11 Pedigrees of progenies grown in trials (continued on next page)

P1		P2	F1		F2			F3
78-185	X	78-34	80-48-1 (1)	X self	81-48-1			
			(2)	X self	81-48-2 (1)	X	81-48-2(3)	82-48-1
			(3)	X self	81-48-3			
			80-48-2 (1)	X self	81-48-4			
			(2)	X self	81-48-7	X	self	82-48-4
			(3)	X self	81-48-8			
			80-48-3 (1)	X self	81-48-5			
			(2)	X self	81-48-6			
			(3)	X self	81-48-9			
			80-48-4	X self	81-48-10(1)	X	81-48-(2)	82-48-3
78-164	X	78-195	80-49-1 (1)	X self	81-49-1 (1)	X	81-49-1(2)	82-49-3
			(2)	X self	81-49-2			
			80-49-2 (1)	X self	81-49-3			
			(2)	X self	81-49-4			
			80-49-3 (1)	X self	81-49-5			
			(2)	X self	81-49-6 (1)	X	81-49-6(2)	82-49-1
					(2)	X	81-49-1(2)	82-49-4
			(3)	X self	81-49-7			
78-180	X	78-183	80-53-1	X self	81-53-1 (1)	X	81-53-1(1)	82-53-1
					(2)	X	81-53-3	82-53-3
			80-53-3	X self	81-53-3	X	81-53-1(3)	82-53-5
			80-53-5	X self	81-53-5			
78-88	X	?	80-54-1 (1)	X self	81-54-1 (2)	X	81-54-1(1)	82-54-1
					(3)	X	81-54-4(2)	82-54-2
			(2)	X self	81-54-2	X	81-54-9(4)	82-54-3
			(3)	X self	81-54-3			
			80-54-2 (1)	X self	81-54-4	X	81-54-7	F3-81-54-1
					(1)	X	81-54-4(2)	82-54-4
					(2)	X	81-54-8(1)	82-54-5
			(2)	X self	81-54-5			
			(3)	X self	81-54-6	X	81-54-4(1)	82-54-6
			80-54-3 (1)	X self	81-54-7 (2)	X	81-54-8(1)	82-54-8
			(2)	X self	81-54-8 (1)	X	81-54-8(3)	82-54-9
					(2)	X	81-54-1	82-54-10
			(3)	X self	81-54-9	X	81-54-10(2)	F3-81-54-3
					(1)	X	81-54-4(2)	82-54-11
					(3)	X	81-54-9(2)	F3-81-54-13
			80-54-4 (1)	X self	81-54-10			
			(2)	X self	81-54-11			
			(3)	X self	81-54-12			

F4 of cross 54:

F3 82-54-5	X	F3 82-54-13(1)	F4 82-54-5
F3 82-54-8	X	F3 82-54-4	F4 82-54-8

Table 3.11 Pedigrees of progenies grown in trials.
(Continued from previous two pages)

CHAPTER 4
DESIGN AND ANALYSIS OF DISEASE RESISTANCE TRIALS

4.1 INTRODUCTION

During the antirrhinum rust research at Royal Holloway College since 1978, 401 different accessions of Antirrhinum majus have been grown in trials to compare their rust resistance. In the initial survey, during 1978 and 1979, 131 accessions from various sources (mostly established varieties) were compared (Gawthrop, 1980). In the breeding programme 249 progenies descended from crosses between the best of these were grown in one or more trials between 1981 and 1983. These trials also included 21 accessions of control varieties and one commercial variety not grown previously. It would not have been practical (or useful) to grow and compare all these accessions at the same time and in the same place. Even the numbers of lines that have been grown at the same time and place are too large for an observer to make all the useful comparisons by eye alone. The discipline and methodology of statistics allow many comparisons to be made and provide an estimate of the reliability of these comparisons. The reader is referred to R.A. Fisher's discussion on the (comparatively simple) investigation of a lady's claim concerning cups of tea (Fisher, 1935) for an illustration of the value of statistical methodology for interpreting numerical data.

In a plant breeding programme the practical constraints of growing and observing large numbers of plants are a major limitation on the rate of progress. Consider, for example, a cross between two plants involving the segregation of 10 genes, each with two alleles. Plants of each of the 10 homozygous genotypes will each be produced at a frequency of one in 4^{10}; ie one plant in 1048576. It is therefore important that experiments are designed to facilitate the comparisons that are most needed. In any research there is a limit to the available resources of manpower and materials. Thus there is a practical limit on the number of plants that can be grown in trials. Also the design must not be overcomplicated or there will be logistic problems at planting and scoring times. For example, it will be argued below that neighbouring plants and plots affect each other within the trial. This effect might

have been reduced if the plants had been arranged in an hexagonal manner so as to be equidistant from six neighbours, all of randomised varieties. The planting of such an arrangement would have been very time consuming and error prone. Selection of plants for hand crossing within related lines would also have been very difficult. In practice the plants were grown in plots consisting of nine or more plants of the same variety in a straight line. The whole plot could then be planted taking the plants out of one seed tray and working in complete rows across the site.

The data should be as easy to collect as possible. Any subjective scoring is hard to interpret later. Different people may give different scores to the same material. If possible the whole experiment should be scored by one person. At some times in the season the material may be changing fast. Scores associated with maturity or disease severity can change very quickly. Thus at certain times it is physically impossible for one person to score the whole of a large experiment.

If the data is to be entered into a computer, the scores should be arranged in such a way that they can be entered with the minimum of error-prone hand reordering and reformatting.

The design of an experiment must reflect the most important comparisons to be made. In this breeding programme, the most important comparisons were sometimes considered to be between accessions (in 1978, 1979 and 1982) and sometimes between sibling plants and within the breeding lines (in 1981 and 1983).

A distinction must be made between trials from which expert judges are to pick "the best" and qualitative trials to be analysed statistically. Agricultural trials have used replicated designs and have encouraged the development of advanced statistical techniques to extract the information. Horticultural trials have often been based on growing a single small patch to achieve the best (rather than typical) quality. Assessment has been by the eye of specialist judges. This approach is obviously necessary for some of the aesthetic qualities of ornamental plants. It is much less suitable for quantitative qualities that can be given a numerical score. This is equally true for ornamental and other

plants. Selection for the production of prize individuals under ideal conditions is not necessarily suited to the production of varieties for general use. For example, the "corn shows" in the United States of America were intended to encourage the production of high quality and high yielding maize. However, judging plants by the quality of a single ear led to the production of land races producing only one ear on each plant. These "show types" yielded less than the less spectacular many-eared races. (Goodman, 1976)

While antirrhinum rust could be controlled by monogenic resistance, it was possible to judge varieties as rust-resistant or rust-susceptible by the presence or complete absence of rust on the leaves of a small plot exposed to the disease. This was the practice at the trials organised by the Royal Horticultural Society in 1949, 1958, 1962 and 1969. (Gawthrop, 1980; Gawthrop & Jones, 1980) In the 1969 trial no varieties satisfied this condition for resistance, although some had very little rust.

Since that time, the aims of plant breeders have changed in favour of increasing levels of partial resistance. (see Chapter 2 above) In a breeding programme such as that reported here it is necessary to have criteria for comparing "little rust" with "very little rust". This is more open to chance effects, and more difficult to achieve than the all or nothing approach.

4.2 SOURCES OF ENVIRONMENTAL VARIATION

In field experiments involving infectious disease, nonuniform levels of inoculum across the site will contribute to the environmental variation. With soil borne diseases this may reflect previous cropping. With aerially dispersed pathogens, variation is likely to arise from the random nature of the initial inoculum. Epidemics of such diseases often start as discrete foci around the sites of single infection events early in the season.

In field experiments of all kinds, there are often unwanted effects at the edge of plots. If paths or empty rows separate plots, then the

plants at the edge of each plot will suffer less competition than those in the middle. If plots are close together, then between treatment competition at the edge of the plots may produce anomalous effects. With chemical treatments the effect becomes more severe. Spray drift or leaching of fertilizers may cause treatments to directly affect neighbouring plots.

The problem of plots affecting the performance of their neighbours is worst for trials involving readily dispersed infectious disease. Spores are dispersed from highly infected plots to their neighbours (especially on the downwind side).

Inoculum dispersal between plots within the trial will have a systematic effect of reducing differences between neighbouring plots. The effect of treatments controlling the disease will be underestimated because of the influx of spores into treated plots. The effect of treatments leading to high levels of disease may be underestimated because of the net loss of inoculum from highly infected plots. This loss of contrast need not affect the ranking of the treatments, unless there are other effects involved. If vertical resistance is a component of the treatment effects, then the effective inoculum potential may be different for different treatments.

There will also be a tendency for interplot spore dispersal to make the performance of plots more like their immediate neighbours. This effect has been demonstrated in many experimental pathosystems, for example mildew of barley (Jenkyn et al., 1979) and late blight of potato (James et al., 1976).

Plant breeders and other research workers in agriculture and horticulture need experimental and statistical techniques to cope with all the various forms of environmental variation outlined above. The major effects are well understood and controlled. There is as yet no satisfactory answer to the problem of dispersal of inoculum within an experiment.

The control of experimental error by experimental design and by statistical analysis are closely related. Three basic principles can be

recognised:

1. Every precaution should be taken to minimize the physical sources of residual or unexplained variation. (This has always been the aim of good experimental technique).

2. Where random variation cannot be eliminated it should be arranged to contribute to the least useful comparisons. For example, in the randomised complete blocks design, environmental variation is confounded with the comparison of replicates.

3. All unknown effects should be properly randomised. This is in effect part of the null hypothesis of all conventional statistical analysis. (Fisher, 1935)

In order to minimize edge effects, it is common practice not to harvest the outside rows of plots in cereal trials. This is not possible in the early generations of breeding programmes because of the very small amounts of seed available. The problem is reduced at this stage, because the aim is to screen large numbers of genotypes, rather than to obtain precise estimates.

For mobile or aerially dispersed diseases, spreader rows of susceptible plants are usually used to encourage the build up of inoculum. In many experiments these are occasional rows or occasional plants within rows. In other experiments the experimental plots have been separated by borders of a susceptible variety. Border rows should also be used around the whole experiment in order to prevent the effects of lowering inoculum level through spore loss from the edge of the experiment. If spreader rows are too thinly spaced, and the disease develops much more severely on them than on the experimental material, then these spreader rows may act as discrete foci of infection, causing the surrounding plants to have a higher level of infection than plants further from the spreaders. Too much spreader material will give a very high inoculum potential over the whole experiment. Any effect of rate reducing resistance will be masked. For its effect to be fully developed rate reducing resistance needs to be multiplied over many generations of pathogen within the host population. With an artificially high

inoculum potential which is unrelated to disease severity, this cannot occur.

Sometimes spreader rows have been artificially inoculated to start the epidemic. There is then the question of the choice of the inoculum used. If only one race of pathogen is used, race specific resistance active against that race will be given (undue) prominence in the results. (This is likely to be more important in variety trials than in fungicide trials because of the vertical component of much disease resistance.) A mixture of known races has sometimes been used. Norwood, Freeman and Crute (1982) describe trials to test field resistance to downy mildew (Bremia lactucae) in lettuces. They used randomised complete block designs for up to ten varieties and partially balanced incomplete block designs for experiments with 12 or more genotypes. 1.5m wide spreader rows on each side of the experimental area were drilled with a susceptible variety approximately six weeks before the experimenal lines were transplanted. The spreader rows were inoculated by atomizing spores onto plants at intervals along the centre of the spreader beds. Isolates were chosen to reduce the effectiveness of all known race specific resistance, but to avoid the introduction of new races, only isolates of U.K. origin were used.

Clothier (1987) describes methods used for routine evaluation of oat and barley varieties for disease resistance. Spreader drills are used to encourage natural epidemics or are artificially inoculated. Net blotch (Pyrenophora teres) spores can be injected into the leaf sheaf of barley plants, allowing disease to be established even during unfavourable weather conditions.

In experiments involving soil borne pathogens which disperse only slowly, uniformly high inoculum levels are achieved by the "sick plot" technique. High levels of the pathogen are built up by repeated growing of the susceptible host on the same land (in deliberate contravention of normal good husbandry). When this land is subsequently used for field experimentation, the experimenter can be confident that all material is exposed to a uniformly high inoculum. This technique is not normally relevant to aerially dispersed pathogens that spread into the experiment from elsewhere in each growing season.

The inoculum level is likely to be more uniform in a small compact experiment.

Paths or the use of some rows of a control treatment between each plot have been used to reduce interplot interference effects. Such practices mean that all edges have the same properties. Thus there can be no direct (for example competitive) interactions between neighbouring plots. The behaviour of these small plots can still be very different from that of larger plots or pure stands of one treatment.

The effects of general environmental trends have been well understood and controlled by the use of randomised complete block design and analysis (in which each treatment occurs once in each block). When numbers of treatments are large and replication limited the randomised complete block design begins to lose its efficiency. With increasing numbers of treatments the complete blocks must increase in size, leading to an increase of within block environmental variation, contributing to the residual or error sum of squares.

Latin square designs are useful for controlling general environmental trends. The restriction that replication must equal the number of treatments can make them unsuitable for experiments involving many treatments. The partitioning of error variance into row and column effects allows general (additive for diagonal) trends in all directions to be controlled. It is not efficient at controlling error variance due to uneven or irregular effects and therefore would not be very efficient for controlling variation due to irregular dispersal of inoculum over a disease resistance trial.

Designs which allow a block size that is smaller than the number of treatment levels are discussed by Cochran and Cox (1957). The smaller block size allows much more of the environmental variation to be identified, and thus increases the precision of the comparison of the treatment levels. There are a number of these smaller blocks in each complete replicate. The layout, analysis and interpretation of these designs is a little more complex than for randomised complete block or latin square designs. Now that large experiments can be analysed by

computer this should not present any great problem, but some of the designs are not readily analysed by common ANOVA programs. For example, the ANOVA directive of Genstat can analyse all balanced designs, but unbalanced designs have to be rendered balanced by the use of pseudofactors, each with a single degree of freedom. The effects of these pseudofactors have to be combined with the main effects before the analysis can be interpreted.

In balanced lattice designs each treatment occurs once in the same block with every other treatment. If the number of units per block is k, the number of treatment levels (t) is k squared and the number of replications (r) is equal to k. Other balanced designs can be constructed with any number of treatments and any size of blocks, but the minimum number of replicates is fixed by these two variables. The large number of replications required makes these designs unsuitable for use with large numbers of treatments.

The partially balanced designs allow a lower level of replication. Estimates of means are made for all treatments with equal precision, although not all comparisons are made with the same precision. Any one variety occurs in the same block with only a subset of the other varieties. Those varieties which occur in the same block can be compared with greater precision than those which do not occur in the same block. Lattices with two replications are called simple lattices, while those with three replications are the triple lattices. For these designs the number of varieties must be a perfect square, k squared, where k is the number of units in a block. Other more complex designs relax these constraints. Amongst those are listed by Cochran and Cox (1957) are the rectangular lattices of Harshbarger (1949). These have s(s-1) varieties arranged in blocks of s-1. Other designs have been developed. Patterson et al. (1978) describe the alpha designs used in some statutory variety trials in the United Kingdom. Alpha designs are available for any number of treatments between 20 and 100, and with replication of two, three or four. These and other designs can supplement the simple and triple lattice designs and allow a greater choice of number of experimental units.

Fasoulas (1981) has developed "honeycomb selection" as a bulk selection

procedure for yield of cereals, but the method could be adapted to select for other qualitative measures. In this method, no attempt is made to estimate means and variances of the plants in selection trials, but each plant is compared with a variable number of neighbours. Single plants are widely spaced in a hexagonal pattern. Planting in this arrangement is done by marking the field with parallel rows and staggering the planting of one row relative to the next. Each plant has six neighbours all at the same distance. Data is collected for the individual plants and arranged on paper in the same hexagonal arrangement as the plants in the field. Each plant in turn is compared with all the others in the hexagon of which it is the centre. If the central plant has the best score it is then selected. The proportion of plants selected can be varied by changing the size of hexagon used for selection. Thus one plant can be selected from hexagons of 7, 19, 37, 61 or 91 plants. This method could be adapted for breeding for disease resistance. Selection is based entirely on individual plants, and a formal comparison is always made with the nearest neighbouring plants. Bos (1981) compared the effectiveness of honeycomb and other methods for bulk selection to increase yield and decrease culm length of winter rye. He found the response to honeycomb selection disappointing and considered that there was too much environmental variation within groups of seven plants.

Methods of analysis using moving averages or using neighbouring plots as covariates could help improve the accuracy of disease resistance trials.

4.3 MATERIALS AND METHODS

The accessions which were grown in the trials are described in Chapter 3 above.

Statistical analysis was performed using the Genstat statistical package running on the Amdahl computer at the University of London Computer Centre, and on Genstat 5 running on the VAX computer at the Welsh Plant Breeding Station.

4.4 FIELD TRIALS 1981 - 1983

In all the trials in the breeding programme, the main aim was to assess rust-resistance. The trials were generally conducted in a similar way to those of 1978 and 1979 (Gawthrop, 1980). It was considered an advantage that the breeding trials should be comparable with these earlier trials. In practice the earlier procedures were modified to meet the changing aims of the breeding trials. The aims and designs of all the trials from 1978 to 1983 are summarized in Table 4.1.

In most trials the primary purpose was to select individual plants from within progenies, and from within related progenies of the same line. For this purpose the main unit of the experiment was the single plant. At this level a replicated experiment was not practical. Although antirrhinums can be clonally propagated by cuttings, to do so would clearly add considerably to the work involved. The breeding programme would be slowed down and the total number of genotypes that could be tested with the available resources would be reduced. The plants to be compared should be grown in as compact an area as possible. This has advantages for practical reasons of ease in making the comparisons, and for theoretical reasons of making comparisons in the most uniform environment possible. Trials laid out in this manner are not very suitable for statistical analysis. Without replication of plants or even of progenies in different parts of the trial site there is no way of estimating the variation associated with position. In these trials this did not matter as the best plants to be used for further breeding were selected by eye from within small groups (progenies and lines). The trial can be considered as many small trials for each of the breeding lines.

When the comparison of different lines and progenies was part of the aim of a trial, a replicated and randomised experiment was possible. The main unit of the experiment was the plot, a group of individual plants grown close together. Following earlier practice a randomised complete block design with three blocks was used.

Year	Site	Aim	Design	Spreader rows
1978	Wisley) Egham)	variety trial	randomised blocks	Malmaison
1979	Wisley) Egham)	variety trial	randomised blocks	Malmaison
1980	Egham	initial crosses	one plot of each variety	Malmaison
1981	Wisley) Egham)	selection of F2	one plot of each progeny	Malmaison
1982	Wisley	comparison of lines and generations	randomised blocks	Malmaison
1982	Egham	selection and comparison with controls	randomised blocks	Malmaison & Sutton's Triumph Primrose
1983	Egham	selection	one plot of each progeny, randomised blocks of control varieties	Scarlet Monarch

Table 4.1 Summary of the aims and designs of the Antirrhinum trials 1978-1983.

note: Trials from 1978 to 1980 were conducted by F.M. Gawthrop (Gawthrop, 1980).

4.4.1 THE TRIAL AT WISLEY IN 1981

The trial at Wisley was to select good plants from the breeding lines represented by 55 F2 progenies.

Because the aim was to select individual plants for crossing, a randomised complete blocks design was not used although the row spacing and spreader rows of this trial followed the general practice of previous trials (Gawthrop, 1980). Each entry in the trial was grown in one plot of a single line of plants. Every plot was planted 0.4m from a row of the susceptible variety Malmaison. Groups of three rows of plants (a row of Malmaison with a test row each side) were separated by a path 0.7m wide. Plants were spaced 0.2m apart within each row. This is shown diagrammatically in Fig. 4.1. The trial was surrounded by two rows of Malmaison. The rows were aligned to the north - south boundaries of the site, to be across the prevailing westerly wind. Two rows of Malmaison were planted as guard rows around the trial site.

Approximately 30 plants of each entry were grown. For some entries the number of plants was limited by the number of seedlings available. When there were many progenies descended from the same parent varieties the size of some of the plots was reduced to give more uniform allocation of resources between the different lines.

The arrangement of the progenies in their plots is shown in the planting diagram Fig. 4.2.

The sowing of seed for the trials of 1981 was delayed by the later than expected maturation of the F2 seed on the glasshouse grown F1 plants. The first seed available was grown at Wisley. Later seed was grown in two groups at Egham. The Malmaison spreader rows were planted on 13th July and the test rows on 5th August. Plate 3 illustrates the layout of the trial and the difference in maturity between the spreader rows and the experimental plots.

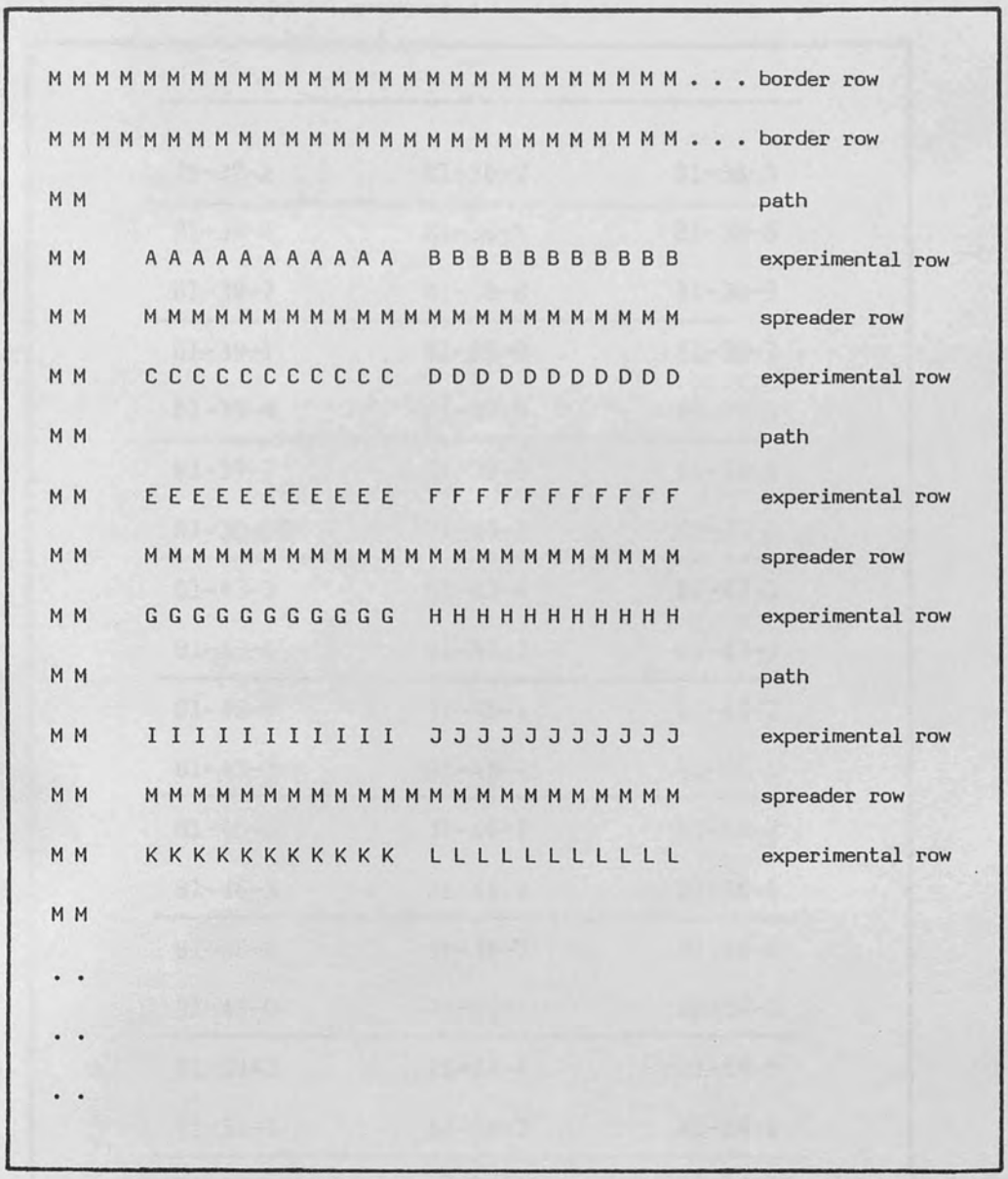


Fig. 4.1 Arrangement of plants in the trials.

Key: M single plant of Malmaison
A,B...L single plant in the experimental rows.
11 plants represent one plot.

Space between plants was 0.2m within rows.
Experimental rows were 0.4m from spreader rows.
Paths between experimental rows were 0.7m wide.
Thus the unit of two experimental rows and one spreader row was repeated every 1.5m.

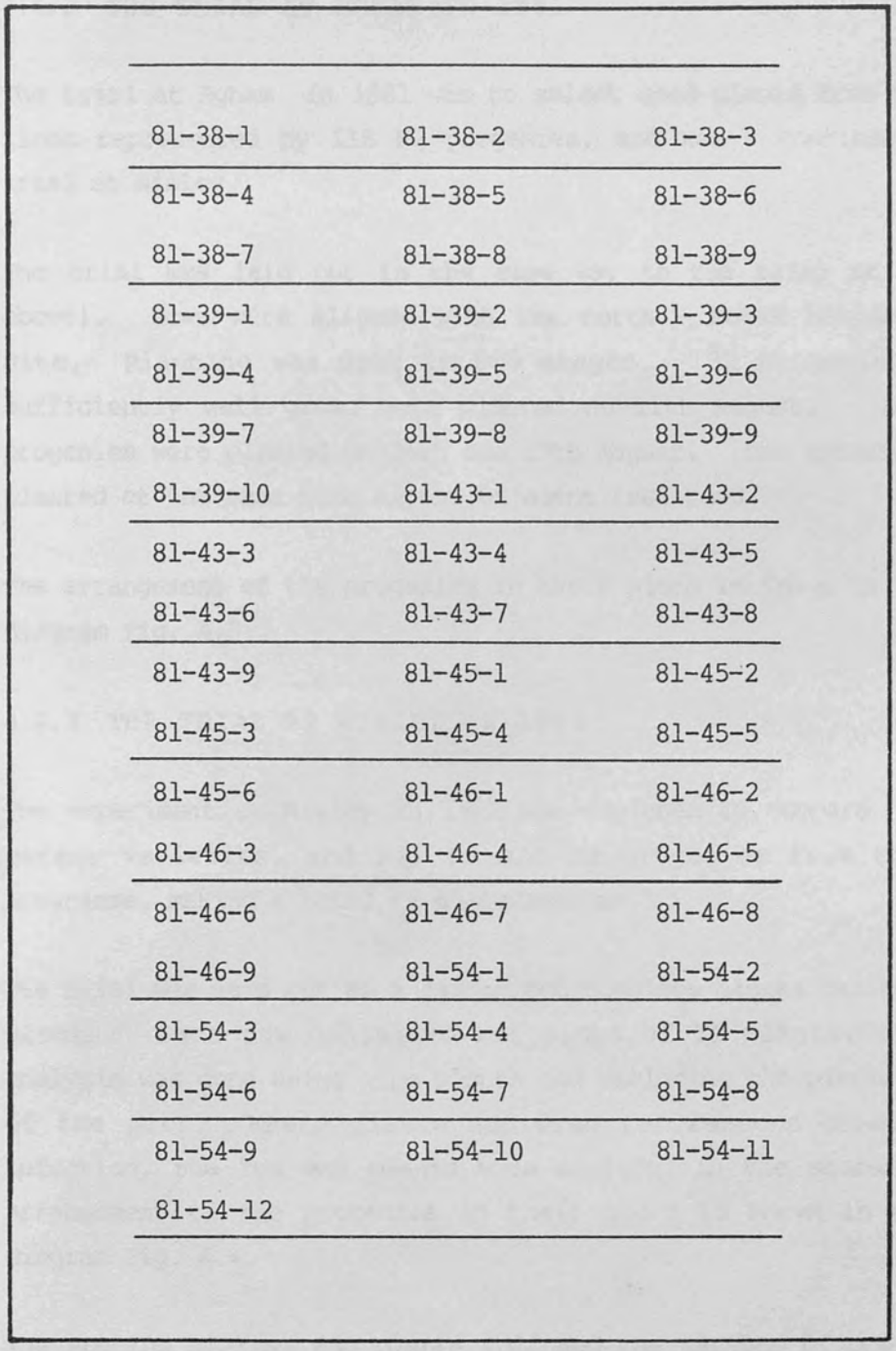


Fig. 4.2 Planting diagram for the trial at Wisley in 1981. Lines represent spreader rows.

4.4.2 THE TRIAL AT EGHAM IN 1981

The trial at Egham in 1981 was to select good plants from the breeding lines represented by 118 F2 progenies, and was a continuation of the trial at Wisley.

The trial was laid out in the same way as the trial at Wisley (see above). Rows were aligned with the north - south boundaries of the site. Planting was done in two stages. 32 progenies that were sufficiently well grown were planted on 12th August. A further 86 progenies were planted on 26th and 27th August. The spreader rows were planted at the same time as the adjacent test rows.

The arrangement of the progenies in their plots is shown in the planting diagram Fig. 4.3.

4.4.3 THE TRIAL AT WISLEY IN 1982

The experiment at Wisley in 1982 was designed to compare control and parent varieties, and F1, F2 and F3 progenies from the breeding programme, making a total of 65 accessions.

The trial was laid out as a randomised complete blocks design with three blocks. Each row contained six plots of 11 plants. Statistical analysis was done using nine plants and excluding the plants at each end of the plot. Where plants had died for reasons other than rust infection, the two end plants were included in the scored plot. The arrangement of the progenies in their plots is shown in the planting diagram Fig. 4.4.

The spacing of rows and plants followed the pattern of previous trials (see above). Rows followed the east - west boundaries of the site for convenience of experimental layout. Two plants of Malmaison were included at the end of each row as guard plants.

The experiment was planted on the 12th and 13th May. Plate 4 shows the site at the end of the season.

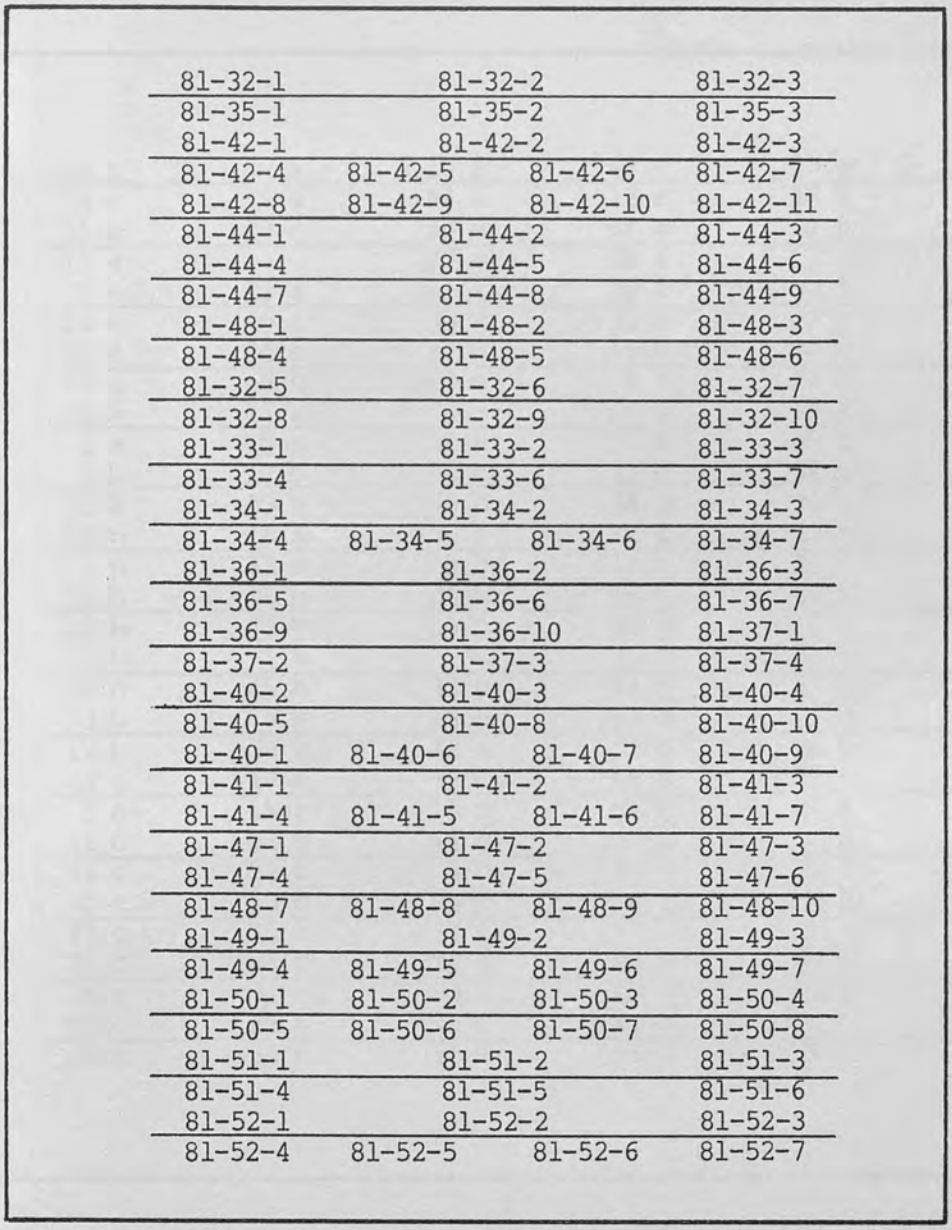


Fig. 4.3 Planting diagram for the trial at Egham in 1981. Lines represent spreader rows.

64 a	62 a	18 a	48 a	53 a	10 a
34 a	19 a	44 a	13 a	43 a	1 a
55 a	17 a	54 a	61 a	5 a	25 a
15 a		27 a	38 a		6 a
36 a	12 a	51 a	39 a	50 a	63 a
14 a	45 a	46 a	31 a	29 a	49 a
52 a	35 a	47 a	16 a	30 a	42 a
41 a	40 a	24 a	4 a	22 a	33 a
20 a	11 a	26 a	56 a	37 a	28 a
8 a	9 a	21 a	57 a	65 a	7 a
7 b	17 b	52 b	41 b	64 b	9 b
20 b	29 b	55 b	28 b	30 b	16 b
61 b	19 b	56 b	11 b	25 b	27 b
4 b	63 b	38 b	14 b	35 b	8 b
18 b	46 b	36 b	49 b	65 b	42 b
39 b	40 b	50 b	34 b	62 b	47 b
5 b	1 b		33 b	51 b	13 b
26 b	45 b	37 b	44 b	31 b	15 b
53 b	6 b	10 b	57 b	21 b	48 b
12 b	54 b	43 b	22 b	24 b	
46 c	17 c	29 c	61 c	37 c	11 c
1 c	18 c	8 c	62 c	20 c	28 c
21 c	53 c	44 c	57 c	6 c	30 c
54 c	52 c	5 c	51 c	35 c	48 c
40 c	13 c	33 c	4 c	36 c	10 c
42 c	50 c	27 c			47 c
56 c	45 c	22 c	49 c	43 c	7 c
26 c	31 c	63 c	25 c	12 c	14 c
64 c	15 c	19 c	41 c		9 c
65 c	34 c	55 c	16 c	38 c	39 c

Fig. 4.4 Planting diagram for the trial at Wisley in 1982.
Lines represent spreader rows.
Numbers are the trial entry numbers. These are given in Table 3.6
Letters a,b and c correspond to the three blocks.

Plate 3. Rust spreader rows in the trial at Wisley in 1981.

Plate 4. Antirrhinum trial at Wisley in 1982.

4.4.4 THE TRIAL AT EGHAM IN 1982

The experiment was designed to compare 89 accessions consisting of 81 progenies from the breeding programme and eight parent varieties as controls. Selection of individual plants for crossing within the breeding programme was also done in this trial.

The experiment was laid out as a randomised complete blocks design with three blocks. Plots consisted of 11 plants as at Wisley. There were six plots in each row across the site. Plants were 0.2m apart as in previous trials, but at planting the rows were all spaced 0.4m apart, leaving no space for paths. Every third row was a spreader row with plants alternating between the rust susceptible varieties Malmaison and Sutton's Triumph Primrose. These spreader rows were removed when the rust epidemic was established. This arrangement allowed a greater number of plants to be tested, while keeping the trial site compact to minimize environmental variation. The spreader rows ensured the presence of inoculum throughout the trial site at an early date. Their subsequent removal ensured that rust multiplication could be assessed on the trial plants. Removal of the spreader rows left paths 0.8m wide, providing more room for crossing work than the 0.7m paths of the previous layout. Two rows of Malmaison and Sutton's Triumph Primrose were planted around the trial site as guard rows. These plants were not removed.

The arrangement of the progenies in their plots is shown in the planting diagram Fig. 4.5.

The trial was planted on 1st June. On 10th August (immediately after the first scoring of rust infection) the spreader rows were removed. They were heavily infected with rust by this time. Plate 5 shows the rust on some of the plants removed. The space occupied by the spreader rows was used to provide access for the second scoring and for the crossing programme.

126 a	74 a	116 a	69 a	138 a	104 a
113 a	102 a	81 a	39 a	125 a	16 a
8 a	80 a	107 a	87 a	2 a	134 a
97 a	124 a	66 a	105 ax	123 a	109 a
78 a	96 a	94 a	122 a	111 a	112 a
119 a	77 a	72 a	115 a	128 a	86 a
40 ax	136 a	110 a	67 a	114 a	108 a
73 a	117 a	133 a	100 a	105 a	98 a
121 a	30 a	91 a	75 a	89 ax	31 a
120 a	127 a	118 a	131 a	70 a	132 a
9 a	135 a	38 a	76 a	129 a	14 a
130 a	99 a	106 a	71 a	137 a	101 a
103 a	79 a	23 ax	22 a	15 a	7 ax
123 b	135 b	116 b	117 b	78 b	106 b
113 b	81 b	38 b	8 b	86 b	132 b
112 b	97 b	31 b	100 b	114 b	126 b
124 b	109 b	76 b	40 bx	77 b	99 b
67 b	74 b	131 b	39 b	136 b	121 b
91 b	22 b	102 b	101 b	9 b	103 b
104 b	137 b	80 b	98 b	71 b	75 b
66 b	72 b	69 b	70 b	16 b	14 b
120 b	108 b	134 b	96 b	105 b	129 b
110 b	94 b	30 b	128 b	115 b	118 b
125 b	2 b	138 b	87 b	89 bx	130 b
133 b	111 b	122 b	73 b	127 b	107 b
114 c	15 b	23 bx	79 b	7 bx	119 b
2 c	117 c	66 c	16 c	97 c	125 c
106 c	30 c	72 c	111 c	131 c	123 c
15 c	94 c	75 c	99 c	110 c	133 c
71 c	136 c	134 c	135 c	80 c	119 c
73 c	67 c	87 c	138 c	14 c	127 c
112 c	70 c	130 c	113 c	78 c	77 c
120 c	126 c	137 c	74 c	38 c	132 c
79 c	124 c	81 c	108 c	105 c	31 c
91 c	109 c	129 c	101 c	118 c	8 c
39 c	115 c	69 c	102 c	86 c	116 c
122 c	76 c	98 c	104 c	100 c	22 c
9 c	107 c	103 c	128 c	96 c	121 c

Fig. 4.5 Planting diagram for the trial at Egham in 1982. See the caption to Fig 4.4 for an explanation.

x - omitted from statistical analysis

Plate 5. Rust on plants of the spreader rows at Egham in 1982.

4.4.5 THE TRIAL AT EGHAM IN 1983

23 progenies and five control varieties were grown at Egham in 1983. The 23 progenies were planted with all progenies from the same breeding line close together for selection of plants from within each line. All the control varieties were grown in each of three rows spread throughout the trial. These control rows were treated as a randomised complete blocks design with the three rows as blocks. No spreader rows were used, but two guard rows of Scarlet Monarch were planted around the outside of the trial area.

The layout of the trial is shown in Fig. 4.6.

4.5 QUANTITATIVE RUST SCORES AND STATISTICAL ANALYSIS

The two trials in 1982 and the trial in 1983 were scored for rust infection. Scores were made on a modified version of the one to five scale used by Gawthrop (1980) to make them comparable with the results of the earlier trials. Five leaves were chosen from each plant, four from around the outside of the plant and one from near the middle. Each leaf was assessed by eye, and the score entered on a score sheet. Rusted leaves were compared with a standard diagram and given the appropriate score.

The Egham 1982 trial was the first scored. At that time many leaves had small white or yellow specks, the first visible signs of rust infection. It was not possible to give a score to these specks in the terms of percentage of the leaf area affected, and yet they were the most common symptom of rust on the site at that time. To allow them to be assessed, the scoring system was modified to include these leaves in a separate category. They were entered in the score sheets with the symbol "W". In subsequent analysis, these were given the value of two. All scores of two or more in the original system were increased by one, to produce a six-point scale. This is shown in Table 4.2 where the new and old scales are compared.

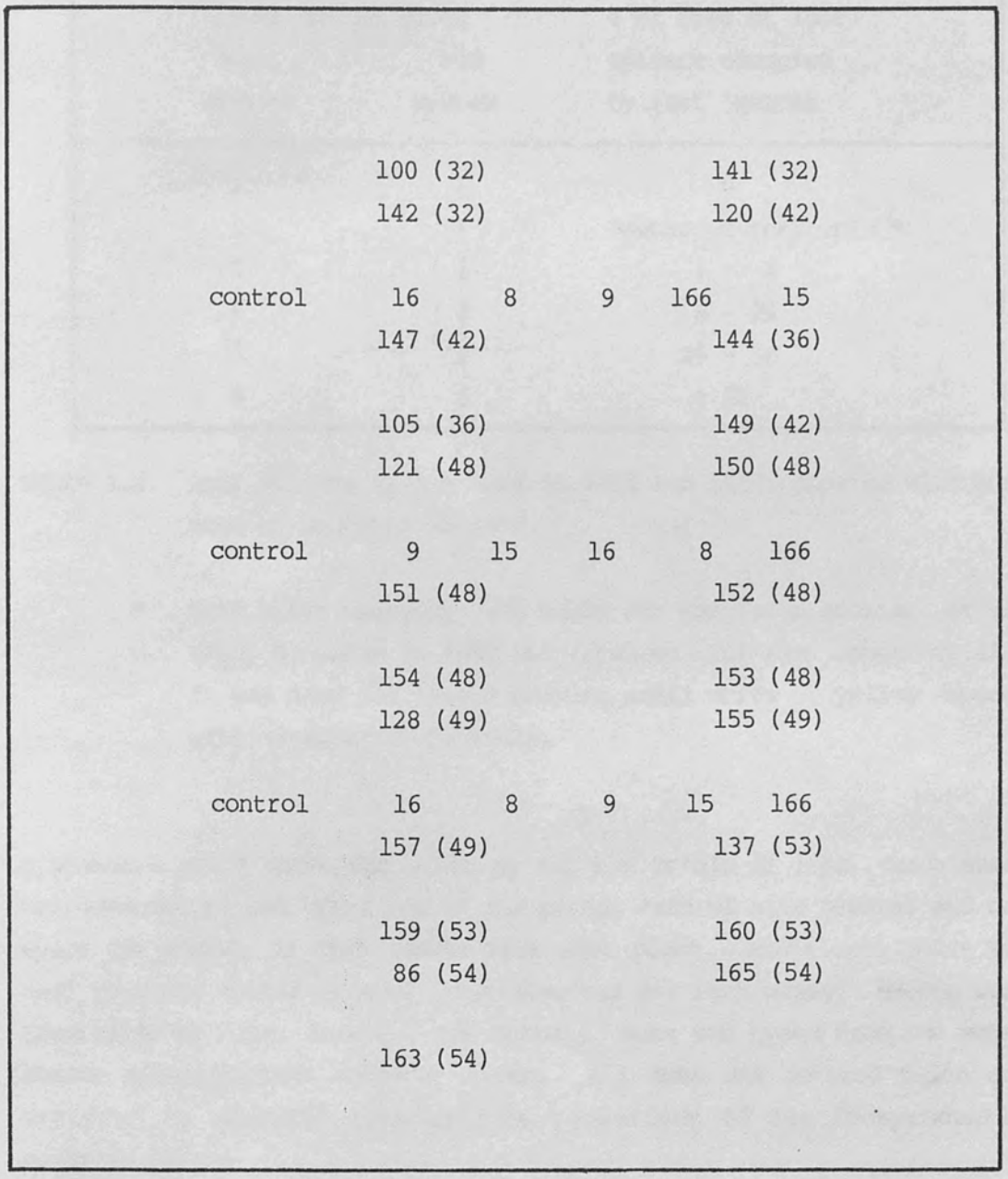


Fig. 4.6 Planting diagram for the trial at Egham in 1983.

Numbers are the trial entry numbers. These are given in Table 3.9.

Rust scores given		% of area of leaf surface occupied by rust lesions
new system	old system	
1	1	0
2	-	specks of rust only *
3	2	1 - 8
4	3	8 - 25
5	4	25 - 50
6	5	> 50

Table 4.2 Rust scoring system used in 1982 and 1983 compared with that used by Gawthrop in 1979.

* This extra category was added for the first scoring of the trial at Egham in 1982 and retained for the other trials. It was used for leaves showing small white or yellow specks with unruptured epidermis.

A standard score sheet was drawn up for the trials of 1982. Each sheet corresponded to one trial row of six plots, each of nine plants; and had space for scores of five leaves from each plant. Additional space was left to score colour or make other comments for each plant. Sheets were identified by site, date and row number. Data was typed from the score sheets directly into computer files. All data was entered twice and verified by computer line-by-line comparison of two independently produced files.

The first scorings in 1982 were made at a time when the amount of rust affecting the plants was increasing rapidly at the start of the epidemic. Four people took part in the scoring in order to score each trial within the shortest possible time. This introduced an extra component into the sources of error within the experiment, because the different people would each interpret the scoring system in their own way. Scoring started at one end of the trial and proceeded through the trial row by row. This had two advantages. Firstly, any effects due to the development of the disease during the time it took to score the

experiment would be classified as a block effect in the analysis of variance. Secondly, the people scoring the trial were working on adjacent rows and could easily compare leaves that were borderline between classes. This was considered important to maintain the uniformity of scoring. However, it might have been better to have assigned one person to score each block. This would have made the effect of disease development during the scoring of the experiment a much more serious problem in the interpretation of the data, but differences between the scorers would have been assigned to the block effect.

Scoring the trials in 1982 for the first time took a total of five days, from the 9th of August to the 11th at Egham and from the 11th to the 13th at Wisley. During this time there was a noticeable change in the level of rust coverage. The second scoring took two days, 28th and 29th September at Egham and one day, 30th September at Wisley. Greater familiarity with the scoring system allowed a steady increase in speed of assessment throughout the exercise. The higher rust scores associated with the second scoring may also have been quicker to determine than the low scores earlier in the season. Some of the plants had died before the second scoring.

Analysis of variance and other related statistical procedures and tests assume that the data has an underlying normal distribution. The rust scores of individual leaves are clearly not normally distributed. There are only six possible values that the data can take, and when plants are very lightly or very heavily infected one of the extreme classes will predominate while the classes near the other extreme will not occur, or will occur only very rarely. The first stage in all analysis was the calculation of plant mean scores as the arithmetic mean of the scores of the five leaves from each plant. These plant means were used as the data for further analysis. Plot mean scores were calculated from the plant mean scores and used as data for analysis of variance. Thus the experimental unit for the variance was the plot mean, which was based on the mean of nine plants. They more nearly follow a normal distribution. The central limit theorem in statistics states that means of a large number of individual data values will be normally distributed. This is true for any distribution within the population

from which the original data was taken. Analysis of variance is considered robust in that it gives useful results even when the data depart from the normal distribution. Using plot means as data efectively ignores any variation between individual plants within plots. This variation, had it been analysed, would not have been of use in making comparisons between accession means.

Analysis of variance is a test to detect the presence of treatment (variety) effects. A number of procedures have been devised to compare the treatment means and indicate which are significantly different from one another. The advantages and disadvantages of the different tests are discussed by Zar (1984). Tukey's test was used to compare all variety means within each of the two trials in 1982. The error rate in Tukey's test indicates the probability of falsely accepting one or more comparisons for each experiment. Thus if the test is made at the 5% level, one experiment in 20 will have one or more differences between treatments wrongly accepted as significant. Differences between variety means are compared with a single critical value calculated as:

$$D = S\bar{X} \quad X \quad Q$$

The value of Q is taken from the Studentized Range, and is dependent on the number of treatments, and the degrees of freedom used to estimate error mean squares.

The randomised complete block design proved ineffective in controlling environmental variation in these trials. Although there were significant overall variety effects in all four scorings (F test) it was not possible to differentiate as many of the accessions as had been hoped by the Tukey and t tests. In order to provide an additional statistical control of variation, the rust scores of the four neighbouring plots were used as covariates in the analysis of variance. This was found to account for some of the environmental variation, and Tukey and t tests were repeated using the new means and residual mean squares. For plots at the edges of the trial there was no neighbouring plot in one direction (two directions for corner plots), and these values were replaced by the plot mean itself. To have left them as missing values would have resulted in an unacceptably large number of missing values, especially as the ANOVA directive within Genstat takes

all variates as missing if any one of the covariates is missing.

Genstat automatically makes estimates for missing values in ANOVA, but in the process the treatment sums of squares are overestimated. However, the total sum of squares and the residuals are estimated correctly in each stratum (Payne et al., 1987). As there was only one treatment factor in these experiments, it follows that where the component sums of squares add to more than the reported total sum of squares, the discrepancy is due to the overestimation of the treatment sum of squares. This was only important for the second scoring of the trial at Wisley. For this one case the treatment sum of squares reported here has been calculated from the other sums of squares. The value reported is less than that calculated by Genstat.

In the covariates analysis it was expected that treatment and covariate sums of squares might be overestimated due to aliasing between these terms in the model. This could have caused the component sums of squares to add to more than the reported total except that the degree of aliasing was low (covariate efficiency factors were reported as above 0.95). For the Wisley trial (with missing values) the reported component sums of squares summed to less than the reported total sum of squares. If the recalculated treatment sum of squares from the analysis without covariates was substituted for that reported in the analysis with covariates, then the sums of squares added to the correct total (the same with or without covariates in the model). Implicit in this substitution is the assumption that the covariate term of the model is taken entirely from the residual term of the model without covariates. Making this substitution has no effect on the residual sums of squares or residual mean square. This residual mean square has been used to calculate new critical values for the Tukey and least significant difference tests. Standard errors of means and critical values are given in Appendix 4. Results of these tests are presented in lower triangular matrix form as Appendix 5. Because part of the original residual sum of squares has been accounted for, the critical values are smaller and more differences are declared significant. Adjustment for the covariates causes the variety means produced with the covariate model to differ from those produced without.

Residual values were plotted against the fitted values for each plot for each of the analyses. This provided a simple check of most of the assumptions of analysis of variance. Residual values should have followed a normal distribution with mean 0 and constant variance across the full range of fitted values. In all cases the plots showed no discernable pattern. Scatter diagrams for the two analyses of the first scoring at Wisley are given as Appendix 6. There is a slight departure from the assumptions when the fitted value was small. It was not possible for the plot to score less than one, and therefore low residuals for low fitted values were not possible. There are no points in the lower left corners of the diagrams in Appendix 6.

The Genstat 5 program written to analyse this data is listed in Appendix 7. This program produced analysis of variance, tables of variety means with and without covariates and plotted scatter diagrams of residual against fitted values. Data was supplied to this program as mean scores for each plant.

Least significant differences were used in a t test to make planned comparisons between related lines in the trial at Wisley in 1982. This trial included parents and F1 and F2 progenies of a number of crosses.

For each trial in 1982 some of the plants of the spreader rows were scored at intervals throughout the season in order to monitor the development of the rust epidemic. Single plants were chosen at one, three and five plot lengths along seven spreader rows in each trial. These plants were scored using the modified scoring system described above. This data was analysed using analysis of variance to test for variation in rust infection of the spreader rows.

4.6 RESULTS

In 1981, the Antirrhinum trials were planted very late in the season. Although there were susceptible Malmaison plants in the spreader rows, epidemic development was slow at both trial sites. The spreader rows of Malmaison at Wisley were planted before any other part of these trials. Although they were well established before the experimental plants were

planted, they too were slow to develop rust and survived until the site was cleared in the winter. Malmaison plants from the trial at Egham were sufficiently healthy to be dug up and survived the winter in pots standing in the open as part of an experiment to investigate the overwintering of rust (Chapter 6 below). This low level of infection is perhaps an indication that by unusually late planting, "disease escape" can allow a healthy display of antirrhinums in the autumn. These trials were not designed for statistical analysis and none was made. The use of the plants in the breeding programme is described in Chapter 3 above.

The trials in 1982 were on the same sites as in 1981. Rust was found on both sites on 13th July. The spreader rows were able to establish a high inoculum level relatively early in the season and were very badly infected at the time of the first scoring, (mid August), when they were removed from the Egham site. Infection was much more severe on the spreader rows than on the trial entries.

The level of rust observed in 1982 is illustrated graphically in Figs. 4.7 to 4.10. The orientation of these figures is the same as that of the planting diagrams Figs. 4.4 and 4.5. Individual plants are shown by a number of vertical lines representing the amount of rust infection. Plants with low rust scores in the range 1.0 to 1.4 are represented by a single vertical line, plants with high rust scores in the range 5.6 to 6.0 by seven vertical lines. One of the plants at Egham on which rust was first found on 13th July is marked in the first row in Fig. 4.9. This plant was noticeably more infected than other plants at the time of first scoring. This was probably the effect of a focus of infection starting from one lesion on this plant but it may have been the result of segregation in this F2 progeny. It is noticeable that some plots were more and some less badly infected than neighbouring plots. Rust was present throughout both sites and there were no obvious trends in rust infection across the sites. The plants that died from wilt diseases at Wisley (represented by X in Fig. 4.8) appear to have been concentrated in the south-east of the site.

Analysis of variance (ANOVA) tables for the 1982 trials are given in Tables 4.3 to 4.6. The variety mean scores for these trials are given in Tables 3.6 and 3.7. These means do not allow for the effect of

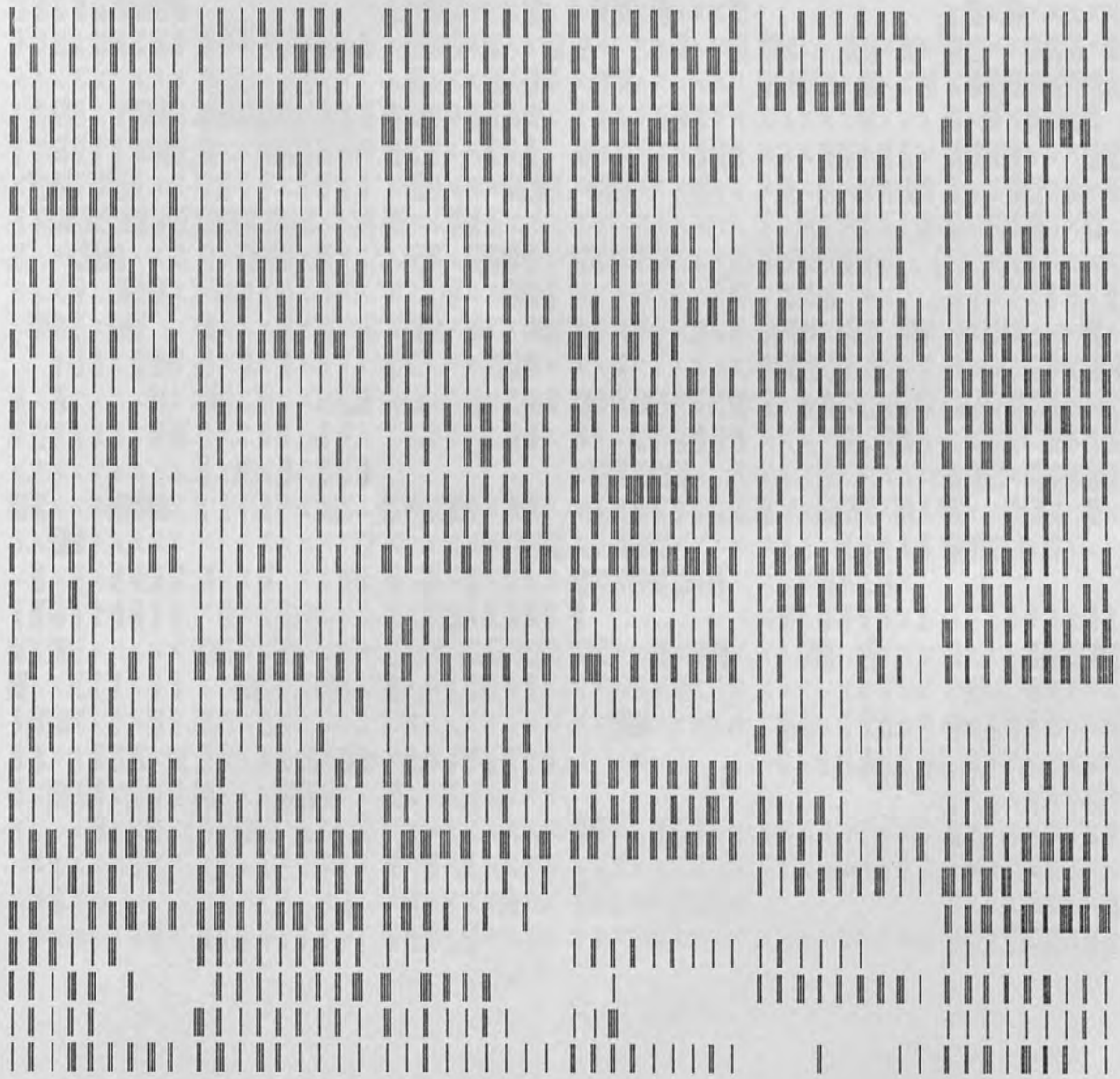


Fig. 4.7 Density of rust infection at the first scoring of the trial at Wisley in 1982.

Groups of vertical lines represent rust scores of individual plants.

1 = score < 1.5
2 = 1.5 to 2.3
3 = 2.3 to 3.1
4 = 3.1 to 3.9
5 = 3.9 to 4.7
6 = 4.7 to 5.5
7 = score > 5.5

Fig. 4.8 Density of rust infection at the second scoring of the trial at Wisley in 1982.

X represents plants that had died from other causes.

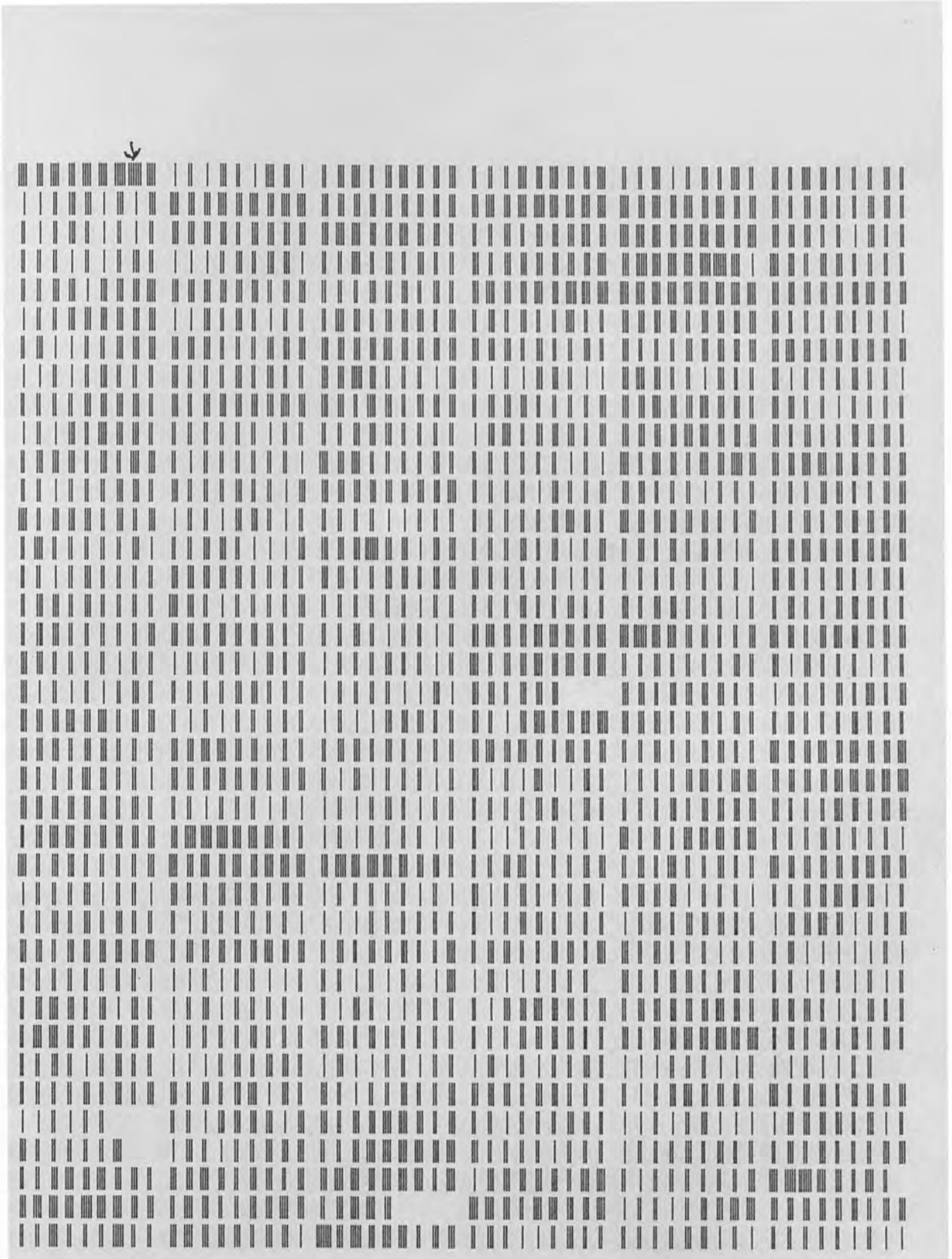


Fig. 4.9 Density of rust infection at the first scoring of the trial at Egham in 1982.

The plant arrowed was the first infected plant observerd.

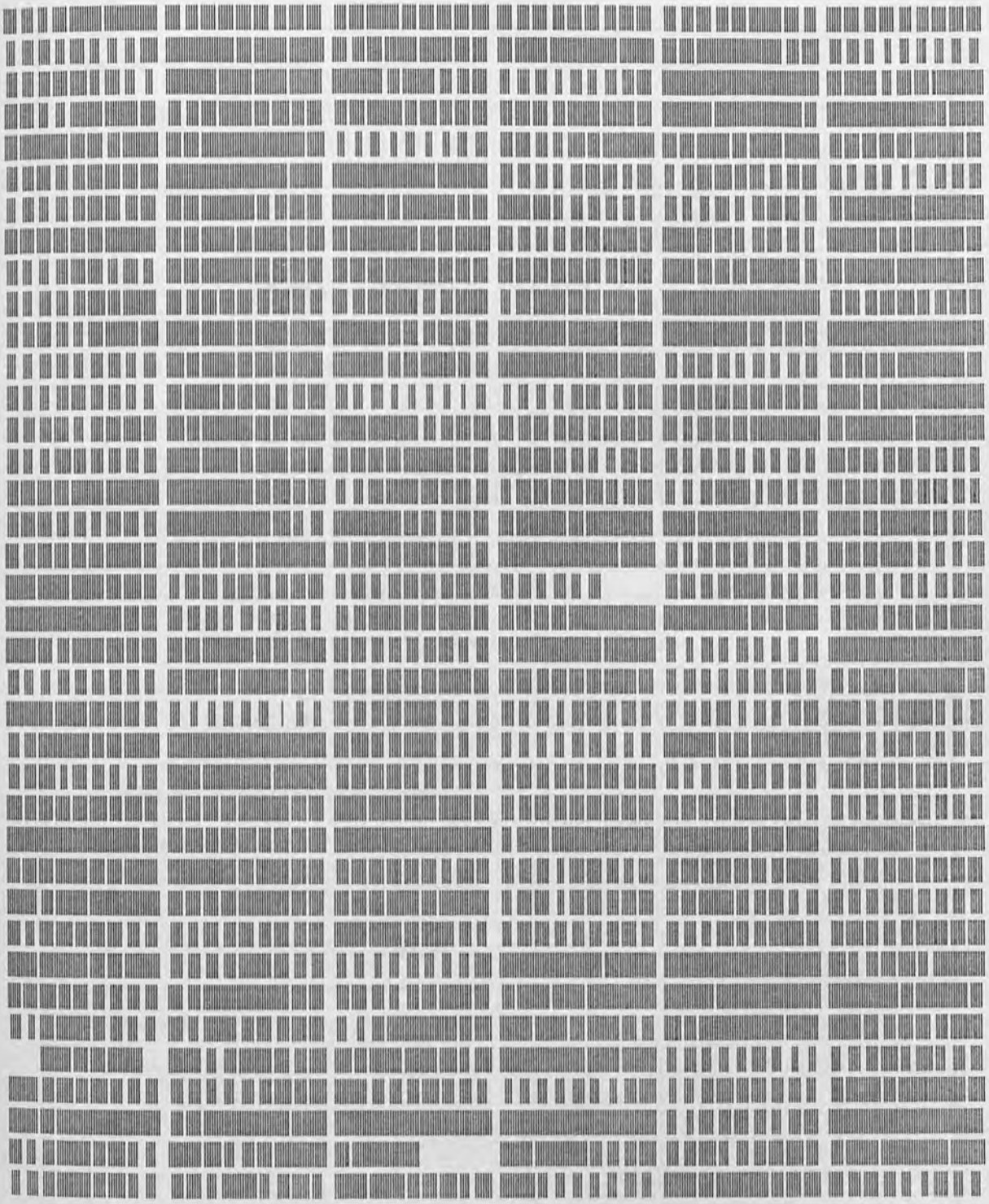


Fig. 4.10 Density of rust infection at the second scoring of the trial at Egham in 1982.

Source of variation:	D.F.	S.S.	M.S.	F.
Randomised complete block design				
Variety effect	57	28.639	0.502	1.74 **
Block effect	2	0.498	0.249	
Residual	112(2)	32.348	0.289	
Total	171(2)	61.482		
Neighbouring plots as covariates				
Variety effect	57	28.639	0.502	3.80 ***
Block effect	2	0.498	0.249	
Covariates	4	18.099	4.525	
Residual	108(2)	14.250	0.132	
Total	171(2)	61.482		

Table 4.3 Analysis of variance table for the first scoring of the trial at Wisley in 1982. Brackets indicate missing degrees of freedom caused by death of some plants.

Source of variation:	D.F.	S.S.	M.S.	F.
Randomised complete block design				
Variety effect	56(1)	50.979	0.910	1.92 ***
Block effect	2	15.059	7.529	
Residual	97(17)	46.507	0.475	
Total	155(18)	112.544		
Neighbouring plots as covariates				
Variety effect	56(1)	50.979	0.910	2.97 ***
Block effect	2	15.059	7.529	
Covariates	4	17.724	4.431	
Residual	93(17)	28.782	0.306	
Total	155(18)	112.544		

Table 4.4 Analysis of variance table for the second scoring of the trial at Wisley in 1982. Brackets indicate missing degrees of freedom caused by death of some plants.

Source of variation:	D.F.	S.S.	M.S.	F.
Randomised complete block design				
Variety effect	72	25.992	0.361	2.52 ***
Block effect	2	2.198	1.099	
Residual	144	20.597	0.143	
Total	218	48.788		
Neighbouring plots as covariates				
Variety effect	72	25.992	0.361	4.01 ***
Block effect	2	2.198	1.099	
Covariates	4	8.016	2.004	
Residual	140	12.581	0.090	
Total	218	48.788		

Table 4.5 Analysis of variance table for the first scoring of the trial at Egham in 1982.

Source of variation:	D.F.	S.S.	M.S.	F.
Randomised complete block design				
Variety effect	72	50.152	0.697	2.95 ***
Block effect	2	2.057	1.028	
Residual	144	33.988	0.236	
Total	218	86.198		
Neighbouring plots as covariates				
Variety effect	72	50.152	0.697	3.32 ***
Block effect	2	2.057	1.028	
Covariates	4	4.607	1.152	
Residual	140	29.381	0.210	
Total	218	86.198		

Table 4.6 Analysis of variance table for the second scoring of the trial at Egham in 1982.

using neighbouring plots as covariates. Means corrected for covariate effects are given in Appendices 2 and 3. The F test without covariates shows that variety effects were significant at the 99% level for the first scoring of the trial at Wisley and at the 99.9% level for the second scoring of the trial at Wisley and both scorings of the trial at Egham. There were thus differences in rust infection between accessions grown in these trials. When covariates were included in the model, the F test was significant at the 99.9% level in all cases.

Critical values for Tukey's test for comparison of all means and for the t test for planned comparison of means are given for each scoring in Table 4.7. The corresponding values using the covariate model are given in Appendix 4. As is to be expected critical values are slightly lower in each case.

Results are described below as analysed with the randomised complete block model without covariates. Differences introduced with the covariate model follow.

For the first scoring of the Wisley trial Tukey's test does not allow any pairs of means to be declared significantly different at the 95% confidence level. Although there are differences between accessions as shown by the F test, it is not possible to declare individual pairs of varieties as significantly different from each other using Tukey's test. For the second scoring, differences between the accession with the lowest rust score (82-2) and the eight most heavily infected accessions are shown by Tukey's test to be significantly different at the 95% confidence level. This difference is shown to be significant at the 99% confidence level in the cases of the three most heavily infected varieties. In addition the second lowest scoring accession (81-40-10) is significantly different from the three most heavily infected varieties. This is shown diagramatically in Fig. 4.11. In this and the following figures (Figs. 4.11 to 4.16) accessions in the trials are listed in order of increasing rust scores down the left hand side. Comparisons between all possible pairs of accessions are shown in lower triangular matrix form. Where the difference is less than the critical value for the test at the 95% confidence level comparisons are represented by a dot. Comparisons for which the difference lies

Error Mean Square	Degrees of Freedom Treatment	Error	Standard Error of Mean	Test	Sig. Level	Tabulated Value	Significant Difference
WISLEY 1st scoring							
0.289	57	112	0.31	Tukey	5%	5.93	1.84
				Tukey	1%	6.59	2.04
				t	5%	1.98	0.86
				t	1%	2.62	1.15
WISLEY 2nd scoring							
0.475	56	97	0.40	Tukey	5%	5.93	2.36
				Tukey	1%	6.59	2.62
				t	5%	1.98	1.11
				t	1%	2.62	1.47
EGHAM 1st scoring							
0.143	72	144	0.22	Tukey	5%	6.04	1.32
				Tukey	1%	6.69	1.46
				t	5%	1.98	0.61
				t	1%	2.62	0.80
EGHAM 2nd scoring							
0.236	72	144	0.28	Tukey	5%	6.04	1.69
				Tukey	1%	6.69	1.88
				t	5%	1.98	0.78
				t	1%	2.62	1.04

Table 4.7 Critical values for Tukey's test and t test of differences between accession mean scores in 1982.

between the 95% and 99% confidence levels are represented by "+" and "*" is used when the difference exceeds the critical value for the 99% confidence level.

Tukey's test for the first scoring of the trial at Egham allows the most severely infected accession (81-48-5) to be declared significantly different at the 95% level from the ten lowest scoring accessions (82-46-6, 81-54-13, 81-50-3, 81-41-5, 81-52-4, 82-54-4, 81-32-9, 81-53-3, 82-39-3, 81-42-10). The two greatest differences are significant at the 99% level.

The results of Tukey's test for the second scoring at Egham are shown in Fig. 4.12.

Comparison of all pairs of means in each experiment using the least significant difference are shown in Figs. 4.13 to 4.16. These comparisons should only be used for planned comparisons, for example comparison of a progeny accession with its parents.

The affect of including the covariate model was to slightly increase the apparent precision of the results. Tukey and least significant difference test results are presented in Appendix 5. In each case a few extra comparisons can be declared significant. The mean scores for each accession are slightly changed between the two models. This is because the covariate model has introduced a correction for each plot representing the rust infection of the neighbouring plots.

The results of monitoring epidemic development on the spreader rows in 1982 are given in Table 4.8. Two way analysis of variance was used to test for differences between the seven rows or between the three positions in the rows for the last scoring for each trial. The analysis of variance tables are given in Table 4.9. No systematic differences between rows or columns were detected.

The five control varieties grown at Egham in 1983 were replicated in each of three rows. The analysis of variance table for these control varieties is given in Table 4.10. The standard error of the mean calculated from the residual mean square is 1.27. Mean scores and

16	82-2	3.08	
43	81-40-10	3.30	.
11	78-178	3.58	..
34	80-43-5	3.82	...
04	78-88	3.86	....
24	82-38-7	3.86	.....
12	78-183	3.92	.....
41	80-40-3	4.00	.....
21	81-38-7	4.06	.....
55		4.06	.....
45	81-41-3	4.17	.....
14	82-1	4.21	.....
36	81-43-3	4.21	.....
22	82-38-1	4.26	.....
44	80-41-2	4.33	.....
64	78-64	4.35	.....
40	82-43-10	4.40	.....
47	80-42-1	4.41	.....
54	81-52-1	4.42	.....
61	78-38	4.46	.....
33	80-43-3	4.48	.....
65	82-6	4.53	.....
46	81-41-5	4.55	.....
17	80-38-1	4.57	.....
37	81-43-9	4.60	.....
01	78-180	4.68	.....
28	81-39-4	4.68	.....
63	78-63	4.68	.....
05	78-32-III	4.69	.....
09	78-164	4.77	.....
26	80-39-4	4.79	.....
07	78-246	4.80	.....
18	80-38-3	4.82	.....
31	82-39-3	4.84	.....
20	81-38-3	4.86	.....
39	82-43-2	4.86	.....
49	81-42-5	4.87	.....
15	82-3	4.95	.....
29	81-39-7	4.96	.....
35	81-43-1	4.96	.....
52	81-49-6	4.96	.....
19	81-38-1	4.97	.....
48	81-42-1	5.03	.....
62	78-35	5.03	.....
42	81-40-9	5.13	.....
13	78-187	5.15	.....
50	80-49-3	5.18	.....
53	80-52-2	5.20	.....
38	82-43-9	5.35	.....
30	82-39-1	5.42	.....
10	78-195	5.46	+.....
08	78-240	5.52	+.....
51	81-49-2	5.53	+.....
56	80-55-1	5.54	+.....
25	80-39-1	5.59	+.....
06	MALMAISON	5.79	*+.....
57	80-56-1	5.80	*+.....
27	81-39-1	5.87	*+.....

Fig. 4.11 Tukey test of differences between variety means at the second scoring of the trial at Wisley in 1982.

+ significant at 5% level, * significant at 1% level.

```
094   81-54-13 3.82
087   82-54-5  3.87  .
086   82-54-4  3.97  ..
105   81-36-6  4.11  ...
016   82-2     4.24  ....
128   81-49-1  4.38  .....
121   81-48-2  4.39  ......
101   81-34-4  4.41  .......
130   81-50-3  4.43  ........
120   81-42-14 4.44  .........
137   81-53-3  4.52  ..........
103   81-36-1  4.56  ...........
114   81-41-7  4.60  ............
115   81-42-8  4.62  .............
118   81-42-12 4.68  ..............
119   81-42-13 4.70  ...............
009   78-164   4.71  ................
133   81-52-1  4.72  .................
136   81-53-1  4.75  ..................
022   82-38-1  4.76  ...................
113   81-41-5  4.76  ...................
127   81-49-6  4.76  ...................
067   82-39-8  4.79  ....................
100   81-32-9  4.81  .....................
124   81-48-7  4.82  ......................
122   81-48-5  4.83  .......................
132   81-50-7  4.84  ........................
031   82-39-3  4.89  ........................
008   78-240   4.93  .........................
126   81-49-5  4.96  ..........................
123   81-48-6  5.00  ...........................
107   81-36-12 5.02  ............................
131   81-50-6  5.02  ............................
112   81-37-6  5.05  .............................
099   81-32-7  5.06  .............................
117   81-42-10 5.09  ..............................
075   82-46-1  5.10  ...............................
134   81-52-4  5.10  ...............................
135   81-52-7  5.10  ...............................
030   82-39-1  5.13  ................................
079   82-46-6  5.14  .................................
080   82-46-7  5.15  ..................................
069   82-43-6  5.16  ...................................
138   81-53-5  5.17  ...................................
110   81-37-4  5.19  ....................................
109   81-37-3  5.24  .....................................
129   81-49-2  5.24  .....................................
015   82-3     5.27  ......................................
111   81-37-5  5.28  ......................................
073   82-45-5  5.30  .......................................
091   82-54-9  5.31  ........................................
104   81-36-3  5.31  ........................................
074   82-45-7  5.32  .........................................
081   81-54-1  5.33  ..........................................
038   82-43-9  5.36  ...........................................
071   82-45-3  5.37  ............................................
066   82-39-7  5.41  ..............................................
072   82-45-4  5.41  ...............................................
076   82-46-2  5.42  ................................................
096   81-32-1  5.45  .................................................
106   81-36-10 5.47  ..................................................
102   81-34-6  5.49  ...................................................
097   81-32-3  5.50  ....................................................
078   82-46-4  5.54  +...................................................
116   81-42-9  5.56  ++..................................................
098   81-32-6  5.62  ++..................................................
125   81-48-10 5.64  ++..................................................
108   81-37-1  5.67  +++.................................................
077   82-46-3  5.72  *++.................................................
070   82-45-2  5.81  **++................................................
039   82-43-2  5.82  **++................................................
002   78-92    5.96  ***++...............................................
014   82-1     6.10  ****+++.............................................
```

Fig. 4.12 Tukey test of differences between variety means at the second scoring of the trial at Egham in 1982.

43	81-40-10	1.19	
46	81-41-5	1.29	.
22	82-38-1	1.44	..
04	78-88	1.48	...
12	78-183	1.49	....
11	78-178	1.54	.....
01	78-180	1.56	.....
45	81-41-3	1.56	.....
17	80-38-1	1.61	.....
31	82-39-3	1.63	.....
36	81-43-3	1.64	.....
26	80-39-4	1.65	.....
35	81-43-1	1.67	.....
55		1.69	.....
24	82-38-7	1.70	.....
25	80-39-1	1.71	.....
07	78-246	1.72	.....
44	80-41-2	1.73	.....
49	81-42-5	1.73	.....
61	78-38	1.74	.....
19	81-38-1	1.75	.....
29	81-39-7	1.77	.....
28	81-39-4	1.78	.....
38	82-43-9	1.81	.....
18	80-38-3	1.83	.....
65	82-6	1.85	.....
08	78-240	1.90	.....
20	81-38-3	1.90	.....
37	81-43-9	1.90	.....
52	81-49-6	1.92	.....
64	78-64	1.92	.....
41	80-40-3	1.93	.....
30	82-39-1	1.94	.....
21	81-38-7	1.96	.....
33	80-43-3	1.96	.....
63	78-63	1.97	.....
16	82-2	2.01	.....
40	82-43-10	2.02	.....
53	80-52-2	2.04	.....
54	81-52-1	2.19	++.....
56	80-55-1	2.19	++.....
15	82-3	2.20	++.....
42	81-40-9	2.22	++.....
34	80-43-5	2.24	++.....
39	82-43-2	2.25	++.....
10	78-195	2.30	++.....
47	80-42-1	2.37	*++++.....
09	78-164	2.47	**++++++.....
27	81-39-1	2.52	**++++++++++.....
13	78-187	2.53	**++++++++++.....
50	80-49-3	2.61	***++++++++++++++++.....
06	MALMAISON	2.66	*****++++++++++++++++.....
14	82-1	2.68	*****++++++++++++++++.....
51	81-49-2	2.70	******++++++++++++++++.....
48	81-42-1	2.74	********++++++++++++++++.....
05	78-32-III	2.78	**********++++++++++++++++++.....
62	78-35	2.79	**********++++++++++++++++++.....
57	80-56-1	2.87	**********++++++++++++++++++.....

Fig. 4.13 t test of differences between variety means at the first scoring of the trial at Wisley in 1982.

+ significant at 5% level, * significant at 1% level.

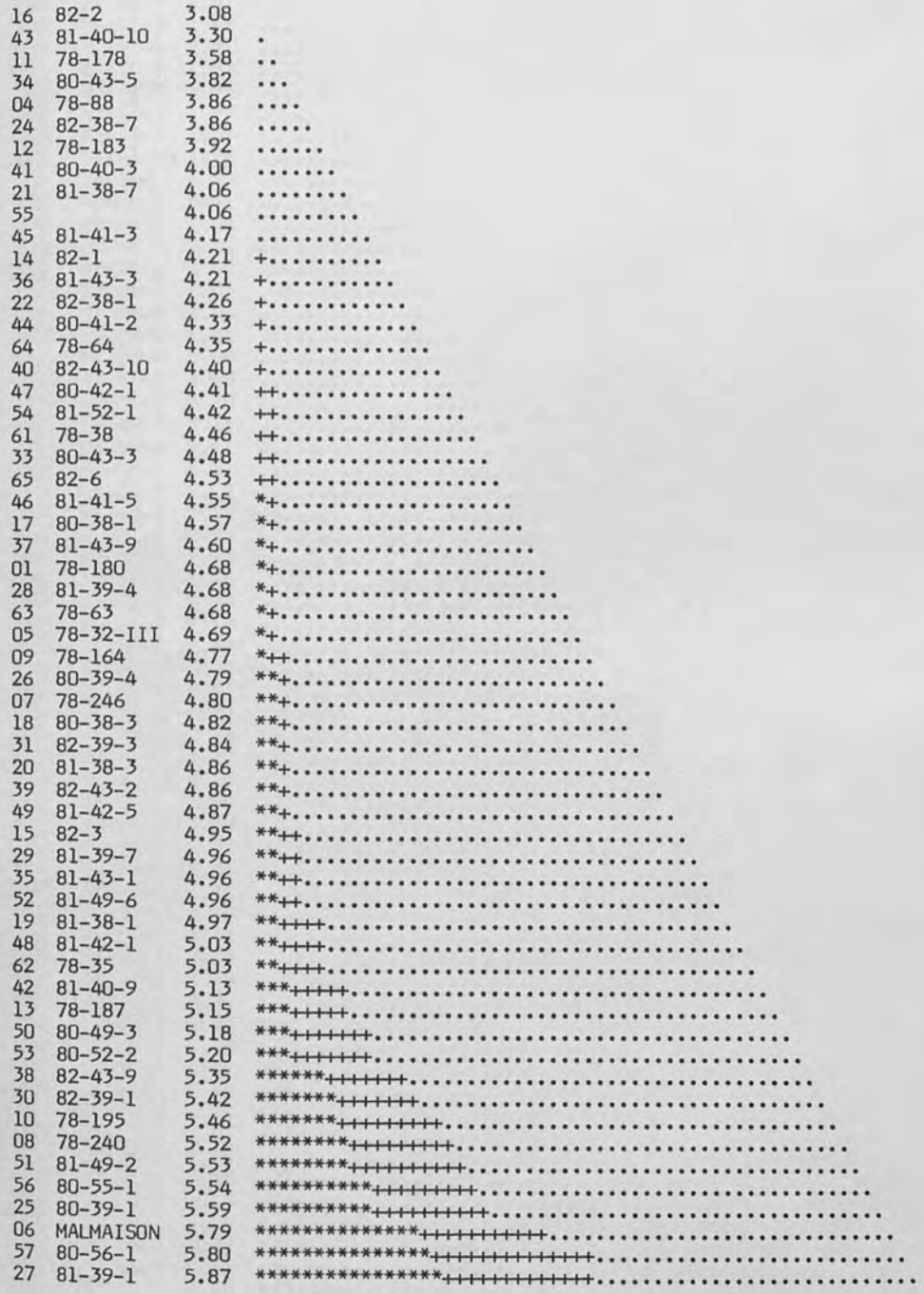


Fig. 4.14 t test of differences between variety means at the second scoring of the trial at Wisley in 1982.

+ significant at 5% level, * significant at 1% level.

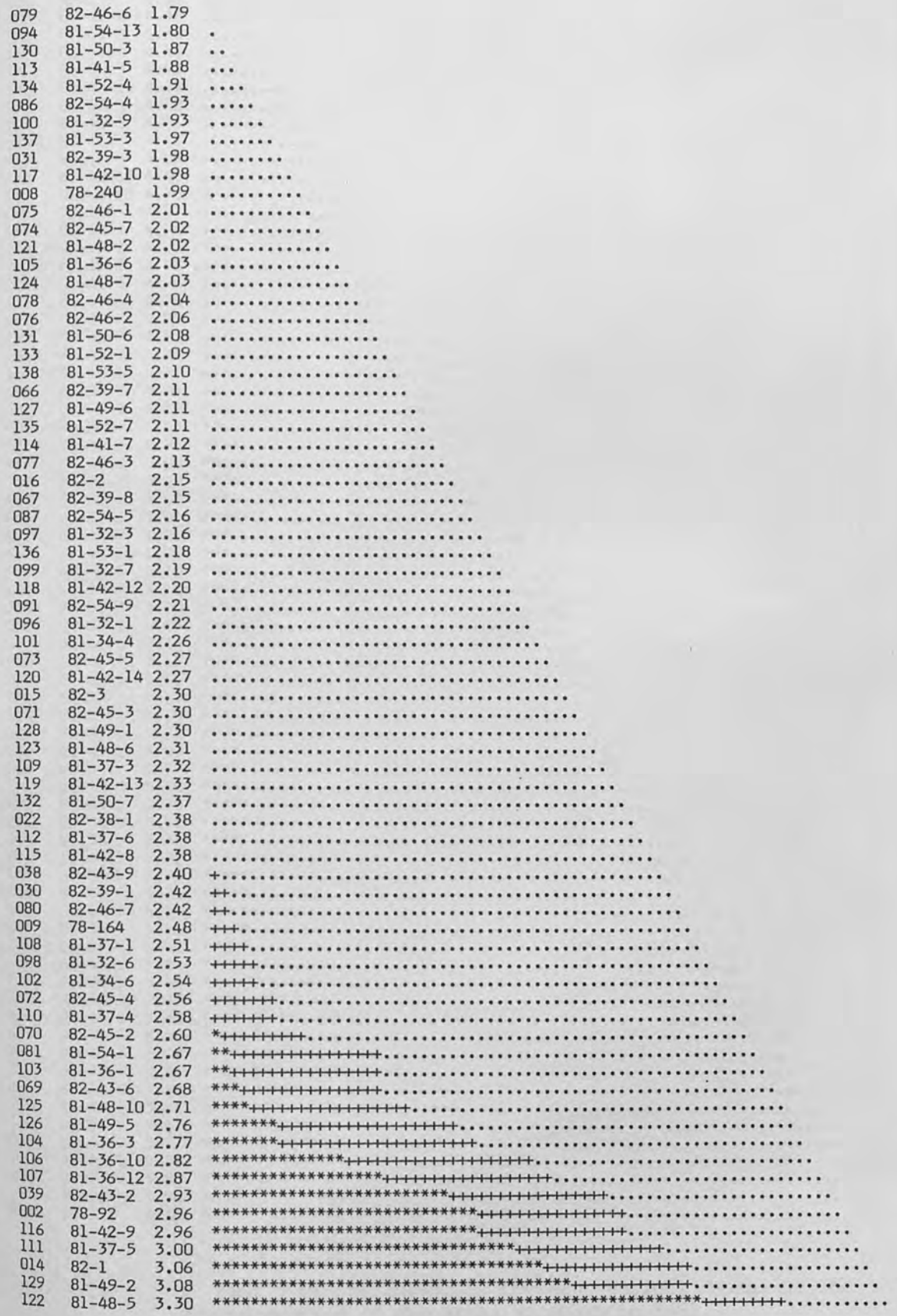


Fig. 4.15 t test of differences between variety means at the first scoring of the trial at Egham in 1982.

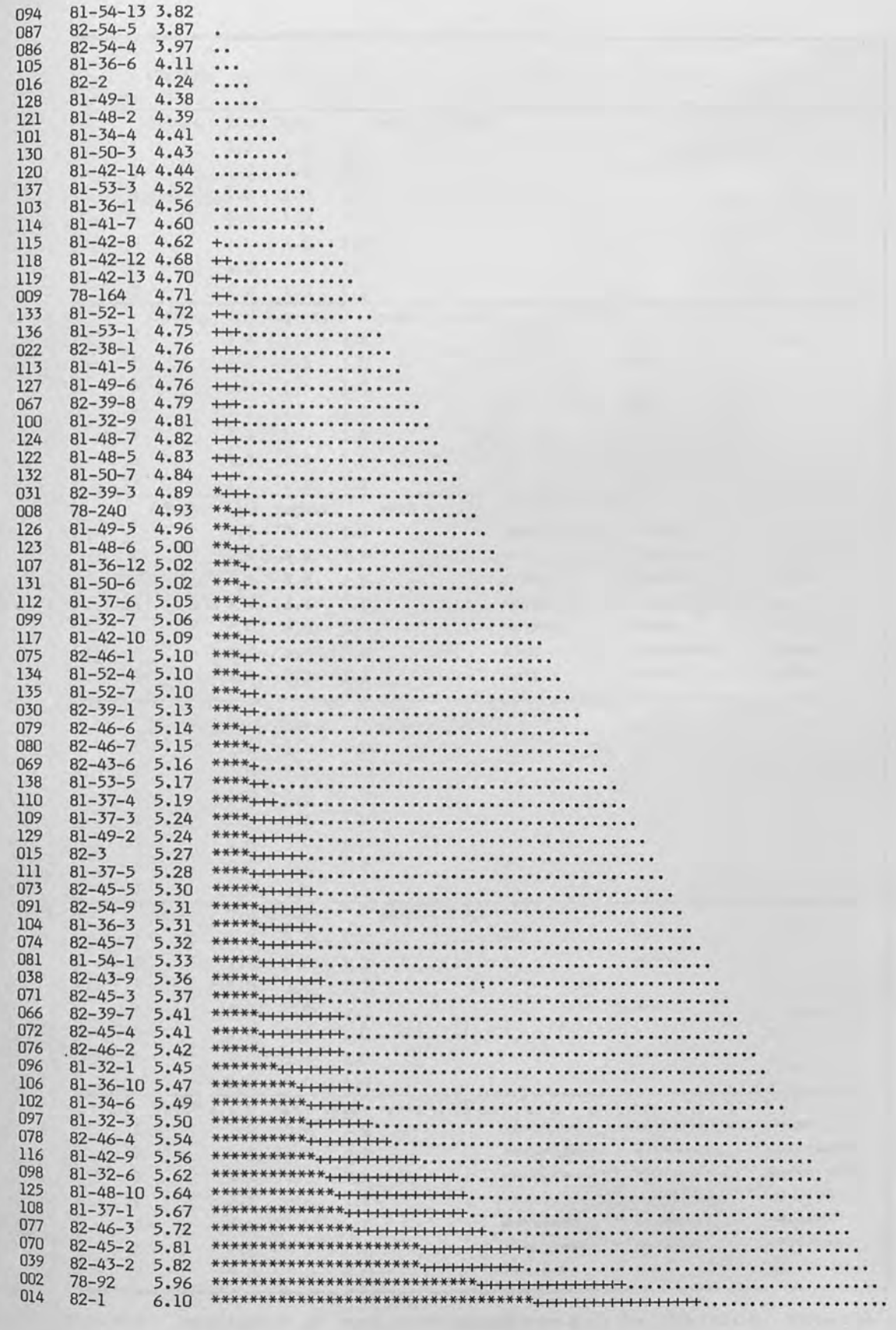


Fig. 4.16 t test of differences between variety means at the second scoring of the trial at Egham in 1982.

ROW:	COLUMN: 1	2	3	1	2	3
Egham, 20th July,			mean = 1.04			
1	1.2	1.0	1.2	*		*
2	1.0	1.2	1.0		*	
3	1.0	1.0	1.0			
4	1.0	1.0	1.0			
5	1.0	1.0	1.0			
6	1.0	1.0	1.0			
7	1.0	1.0	1.2			*
Egham, 3rd August,			mean = 0.56			
1	3.4	1.4	2.6	*****	*	****
2	1.2	2.8	1.0	*	****	
3	1.0	1.4	1.0		*	
4	1.2	1.6	1.0	*	**	
5	1.4	1.0	1.2	*		*
6	1.2	3.8	1.0	*	******	
7	1.2	1.2	1.2	*	*	*
Egham, 11th August,			mean = 3.59			
1	5.8	3.4	4.6	**********	*****	********
2	2.4	4.6	3.8	***	********	******
3	3.2	3.8	3.6	*****	******	******
4	3.4	3.4	2.2	*****	*****	***
5	4.0	3.0	3.0	******	****	****
6	2.6	5.4	3.6	****	*********	******
7	3.0	3.2	3.8	****	*****	******
Wisley, 21st July,			mean = 0.05			
1	1.0	1.0	1.0			
2	1.0	1.0	1.0			
3	1.0	1.0	1.0			
4	1.0	1.0	1.0			
5	1.0	1.0	1.0			
6	2.0	1.0	1.0	**		
7	1.0	1.0	1.0			
Wisley, 3rd August,			mean = 0.29			
1	1.2	1.6	1.0	*	**	
2	1.2	3.0	1.2	*	****	*
3	1.0	1.0	1.2			*
4	1.0	2.2	1.0		***	
5	1.0	1.0	1.0			
6	1.0	2.4	1.0		***	
7	1.0	1.0	1.0			
Wisley, 2nd September,			mean = 4.72			
1	5.0	5.8	3.2	********	**********	*****
2	5.6	4.6	5.2	**********	********	*********
3	4.4	4.2	5.6	*******	*******	**********
4	3.4	5.6	4.8	*****	**********	********
5	4.6	4.4	3.8	********	*******	******
6	5.8	4.2	5.0	**********	*******	********
7	5.0	3.0	6.0	********	****	**********

Table 4.8. Development of rust on the spreader rows in the 1982 trials. Three plants were scored from each of seven of the spreader rows. The scores are means of five leaves on the 1 to 6 scale (1 = no infection). On the right, the same data is presented in histogram form. Each * represents 0.5 units of rust score.

Source of variation:	D.F.	S.S.	M.S.	F
Egham, 11th August				
Columns	2	0.51	0.25	0.27 n.s.
Rows	6	4.73	0.79	0.86 n.s.
Residual	12	11.04	0.92	
Total	20	16.28	0.81	
Wisley, 2nd September				
Columns	2	0.35	0.17	0.15 n.s
Rows	6	1.42	0.24	0.21 n.s
Residual	12	13.63	1.14	
Total	20	15.40	0.77	

Table 4.9 Analysis of variance for the development of rust on the spreader rows in the 1982 trials.
Egham, 11th August and Wisley, 2nd September scoring.
For both trials, differences between the rows and between the columns are no greater than could be expected by chance.

Source of variation:	D.F.	S.S.	M.S.	F.
Variety effect	4	83.66	20.92	4.35 *
Block effect	2	1.44	0.72	0.15 ns
Residual	7(1)	33.63	4.80	
Total	13(1)	118.73		

Table 4.10 Analysis of variance table for the three rows of control varieties at Egham in 1983.
Brackets indicate missing degree of freedom caused by death of all the plants of the variety Victory in the second row. Values of F with 4 treatment d.f. and 7 error d.f. are 4.12 for P = 0.05 and 7.85 for P = 0.01

standard errors of means are given in Table 3.10. Care is needed in the comparison of these means because the experiment was designed for selection from within each progeny. Standard errors in Table 3.10 represent variation within the plots of the breeding lines and do not allow for differences between the different parts of the trial site. The residual mean square in the ANOVA of the control varieties is the mean square for the variety * block interaction. This mean square is therefore a measure of variation within rows, and gives an indication of the differences to be expected between breeding plots in the same row. The block effect represents differences between rows within the site and therefore this mean square gives an indication of differences to be expected between plots in different rows.

4.7 DISCUSSION

While the statistical methods of randomised block designs were originally developed to control environmental effects based on variations in soil, microclimate and similar effects in agricultural trials they have been used in many areas of biological research. They are used to control the variation in disease trials which are superficially very similar to the trials for which they were originally designed. For this their effectiveness can be limited. Latin square, and to a lesser extent randomised complete block, are designs for the control of general trends. The small patches of high and low inoculum potential around exceptional treatment plots and disease foci cannot be resolved by the field-wide error models of randomised complete block or latin square designs.

The significant block effects show that there were differences in rust levels between different parts of the trial sites in 1982. To divide each of the sites into the three blocks of a randomised complete block experiment was not an adequate solution to the problem, and an experimental design with much smaller blocks would have been preferable. This could have been achieved in either of two ways. A lattice design of experiment would have been more effective. Extra control entries in the experiments could have been added to make up the 64 entries needed for a lattice design with eight blocks of eight plots per complete replicate. The use of alpha designs would have allowed a small block

size and a more sensitive experiment without changing the number of entries.

An alternative approach would have been for each of the experiments to have been divided into a number of smaller experiments. The experiments in 1982 could have been adapted to allow only comparisons of progenies and parents within lines. Breeding would have proceeded more efficiently within each independent line (which would be compatible with the aims of the breeding programme) but rigorous comparisons between the lines would not have been possible. Further experiments in later years would have been necessary to compare the best progenies within each line.

Comparison of the results presented above with those of Gawthrop (1980) show that she reported much smaller estimates of standard errors of variety means. These low estimates of standard errors led to a low value for the critical difference in Tukey's test which allowed a very large proportion of all comparisons to be declared significant at the 5% confidence level. The full ANOVA table is only given for the second scoring of the trial at Egham in 1979 (ibid. Table 4.5). This table is presented as a "sample of computer output of a four factor Analysis of Variance". The factors involved are identified by numbers and not by name but can be identified by the number of levels of each. They are:

1. varieties with 74 levels
2. plants with 9 levels
3. leaves with 5 levels
4. blocks with 3 levels

This table does not include columns for the F ratio or its significance. F values for trials in 1978 and 1979 are presented in ibid. Tables 4.12 and 4.13. For the second scoring at Egham in 1979 the variety main effect is tested against the variety * block interaction and declared significant at the 0.1% level. The corresponding F ratio was significant at this level in all the other trials except for the first scoring at Egham in 1979 at which there was no significant variety effect ($F = 1.14$).

The standard error of the variety means should be calculated from the

same mean square (variety * block interaction):

$$S.E.M. = \sqrt{\text{mean square / replication}}$$

The replication for variety means is:

number of blocks * number of plants per plot * number of leaves per plant

Thus for the second scoring at Egham in 1979:

$$S.E.M. = \sqrt{8.88067/(3*9*5)} = 0.256$$

In ibid. Table 4.18 this standard error is given as 0.0081. I am unable to explain this difference. The value of D, the critical value for Tukey's test is calculated from the standard error and is correspondingly too small. Because of this, most comparisons are wrongly declared to be significant, even for the first scoring at Egham in 1979 when the F test could detect no variety effect. The results of Tukey's test for the second scoring at Egham in 1979 are presented in Fig. 4.17 below using variety means from ibid. Table 4.16 and:

$$D = 0.256 * 5.95 = 1.523$$

It will be noted that this figure is similar to those produced for the 1982 trials reported above.

Inspection of ibid. Table 4.18 shows that standard errors used for Tukey's test of the other trials were of a similar magnitude, and that a similar error must occur in each case. It is not possible to recalculate these standard errors from the information given.

In the course of the three years 1981 to 1983 and five experiments, five different methods were used to achieve uniform inoculum across the site. The use of spreader rows and/or artificial inoculum seems necessary to obtain a reliable antirrhinum rust epidemic early in the season. Earliness is more important in breeding work than in trials to compare varieties. In breeding it is a great advantage if selection for rust

```
34          1.43
18          2.14  .
66          2.85  ..
58          3.02  +..
19          3.11  +...
62          3.14  +....
30          3.36  +.....
11          3.41  +......
14          3.51  +.......
57          3.55  +........
38          3.88  ++........
25          3.92  ++........
64          4.04  ++.........
45          4.05  ++.........
39          4.08  ++.........
42          4.14  ++.........
31          4.16  ++.........
61          4.29  ++.........
20          4.41  +++........
26          4.45  +++........
65          4.45  +++........
48          4.51  +++........
27          4.54  +++........
17          4.64  ++++.......
23          4.66  ++++.......
37          4.66  ++++.......
22          4.67  +++++......
40          4.68  +++++......
21          4.70  +++++......
16          4.72  +++++......
28          4.77  +++++......
35          4.78  +++++......
55          4.83  +++++......
63          4.85  +++++......
47          4.86  +++++......
54          4.88  +++++......
29          4.90  +++++++....
3           4.91  +++++++....
32          4.91  +++++++....
51          4.91  +++++++....
15          4.92  +++++++....
5           4.94  +++++++....
13          4.94  +++++++....
59          4.94  +++++++....
60          4.94  +++++++....
2           4.95  +++++++....
44          4.97  +++++++....
1           5.00  +++++++....
4           5.00  +++++++....
6           5.00  +++++++....
7           5.00  +++++++....
8           5.00  +++++++....
9           5.00  +++++++....
10          5.00  +++++++....
12          5.00  +++++++....
24          5.00  +++++++....
33          5.00  +++++++....
36          5.00  +++++++....
41          5.00  +++++++....
43          5.00  +++++++....
46          5.00  +++++++....
49          5.00  +++++++....
50          5.00  +++++++....
52          5.00  +++++++....
53          5.00  +++++++....
67          5.00  +++++++....
```

Fig. 4.7 Tukey test of differences between means at the second scoring of the trial at Egham in 1979. (Means taken from Gawthrop, 1980)

resistance and for horticultural quality can be made at the same time. Also, crosses made earlier in the season when the plants have stronger flowering spikes and the weather is more favourable are more likely to set seed than those made later.

The presence of spreader rows throughout the trial area was considered important in order to ensure the uniform development of the epidemic. Early establishment of very susceptible plants, which can be removed when an epidemic has been started, seems the best approach. The use of moderately resistant spreader rows as in 1983 does not cause a useful infection early in the season. The epidemic that develops is too late for the assessment of breeding material. If very susceptible spreader rows are allowed to remain until they die of rust, the inoculum potential produced will mask any effect of rate reducing resistance. Reinfection from within plots of (moderately) resistant treatments will be negligible when compared with the great influx from the spreader rows. Since the reinfection cycle is the basis of rate reducing resistance, the value of the latter will be underestimated in such circumstances. This can be partly avoided if the spreader rows are only used to build up inoculum early in the season and the epidemic has to continue on the treatment plots.

Deliberately allowing rust to overwinter on infected plants on or near the trial site might be a way of getting inoculum on to the site early in the season. If the rust overwintered on the breeding lines, or if the rust from the breeding trials overwintered on other plants (susceptible to the relevant strains of rust) then the development of rust strains especially virulent to the breeding lines might be encouraged. The selection pressure and population size would be much less than could be expected to follow the release and widespread cultivation of a commercial variety. If the resistance cannot withstand the accumulation of adapted pathotypes on the experimental plot, it is perhaps unlikely to last long in widespread cultivation. However, it seems unprofitable to encourage new strains of pathogen to evolve in parallel with the breeding of new varieties.

It was originally deliberate policy to keep the antirrhinum rust trials on the same land each year. The overwintering of the rust is still

poorly understood. The use of the sick plot technique would have been relevant if spores survived in the soil, or via the unknown stage of the perfect life cycle on nearby trees. The tendency for less and less rust to occur each year would indicate that there was no great carry-over in or near the trial site. The increasing problems with wilt diseases may however indicate a build up of some strains of these under continuous antirrhinums.

4.8 CONCLUSIONS

The presence of spreader rows throughout the trial area is important in order to ensure the build up of inoculum. Early establishment of ample very susceptible material, which is then removed when the epidemic is under way seems most appropriate to these antirrhinum trials. The use of moderately resistant spreader rows as in 1983 does not allow very rapid build up of rust early in the season. The epidemic that develops is then too late for the assessment of breeding material. On the other hand, if very susceptible spreader rows are allowed to survive until they die of rust, then the inoculum potential will be so high that any rate reducing resistance effects are masked. Inoculum transfer between spreader and test rows will provide a very high inoculum potential in the test rows. Any reinfection from (moderately) resistant material back onto itself will be masked by the great influx of spores from the spreader rows. Since the cyclic reinfection from itself is the basis of rate reducing resistance, the true value of such resistance will be badly underestimated. This may be avoided if the spreader rows are used to build up infection early in the season and then removed, so that the epidemic has to continue to develop in the experimental plots.

The experimental methods used allowed selection to be made for the best plants within lines, and showed that differences beween lines and control varieties were statistically significant. Greater precision would have been obtained by experimental designs that allowed smaller blocks and greater statistical control of environmental variation.

CHAPTER 5
FLOWER MORPHOLOGY AND VARIATION

5.1 INTRODUCTION

The flowers of Antirrhinum species are specialized for pollination by large bees. The corolla is cylindrical with a sac-like swelling at the base in which nectar accumulates. There are five corolla lobes, three forming the lower lip and palate. The palate closes the corolla tube against all but the strongest and heaviest insects. The four anthers and the stigma are located just above the palate where they contact the top of any insect able to open the throat of the flower. The shape of the corolla tube provides a spring action to keep the flower closed. Bumble bees are the main pollinators as their strength and weight are needed to work the mechanism.

Flower types within the Scrophulariaceae range from the specialized closed tubes of Antirrhinum and Linaria to simple open and nearly actinomorphic flowers of Verbascum. Some genera closely related to Antirrhinum such as Anarrhinum have closed tubes, but the palate and closing mechanism are not as tightly closing as in the Antirrhinum flower.

Aberrant forms of Antirrhinum and Linaria flowers have been known for a long time. The history of the "Peloria" form of L. vulgaris first described by Linnaeus has been reviewed by Gustafsson (1979). All the flowers of the original "Peloric" form had an actinomorphic corolla with five nectaries corresponding to the five corolla lobes, and five equally long stamens. Peloria was collected in Sweden in 1742 and described by Linnaeus in 1744. It was of special interest to him because the presence of such radically different flowers on a plant that was clearly Linaria was contrary to his ideas of species and his classification system based on flower form. The debate took on a theological aspect when Linnaeus suggested that this was a new species that had arisen since the original creation. His expectations of Peloria were diminished when specimens were found that had both normal and peloric-like flowers on the same plant.

"Peloria" and "Pelorism" became accepted terms for actinomorphic variants of normally zygomorphic flowers. In his book "The variation of animals and plants under domestication" (1868), Charles Darwin discussed peloric forms of a number of genera including Linaria and Antirrhinum. He found that peloric forms could be reproduced by seed. When a peloric snapdragon was crossed with a normal form, all the progeny were normal but after self-pollination he obtained 37 peloric plants out of 137 plants examined. Darwin, being unaware of Gregor Mendel's work was unable to give a satisfactory explanation of this. Clearly pelorism in Darwin's material was determined by a recessive allele at a single locus. Other parts of Darwin's book were studied by Mendel, and Darwin cited pages of a paper where Mendel's name is mentioned. If either of these great men had been aware of the other's work the history of the science of genetics might have been very different. However, peloria in Linaria was associated with the rediscovery of Mendel's work when De Vries studied it and included his results in "Die mutationstheory" (1901-1903). De Vries listed many species with zygomorphic flowers of which peloric forms were sometimes found.

Expression of pelorism is often influenced by environmental conditions. Darwin and modern workers (Stubbe 1966) found a tendency towards a weak manifestation in the hybrid between peloric and normal forms of Antirrhinum. Stubbe described a series of alleles at a locus (cyc) controlling flower shape. The phenotypes of the homozygotes range from normal (wild type) through a series of intermediates in which the palate structure is reduced and the corolla lobes become more similar, to the extreme of a nearly actinomorphic form.

Bumble bees of various species are the main pollen vectors of A. majus. Their behaviour is therefore of great importance to the functioning of the flower, and has been important in the evolution of its structure.

5.2 MATERIALS

Observations described in this chapter were made on the breeding trials described in Chapters 3 and 4 above.

Care was taken during the breeding programme to maintain good horticultural quality. With this aim, many plants of poor general appearance were discarded that might otherwise have been used for crossing. The breeding programme began from plants of commercial varieties, and good horticultural quality, including the absence of aberrant forms, was one of the criteria for their selection. Even so, a wide variety of obvious deformities were observed, and some of these are described below.

5.3 POLLINATING INSECTS

Bumble bees of various species were the commonest pollinating insects on the trial plots. Other insects were only able to enter normal flowers when flowering was nearly over and the flowers were old and relaxed. Most flowers were already pollinated at this stage and probably did not have much nectar in them.

Bees differed in their approach to Antirrhinum flowers: nototribic and sternotribic visits were seen. They visited newly opened flowers at the top of each flowering spike and largely ignored the older flowers lower down. The youngest flowers visited were hardly open and needed a considerable effort from the bee to open them. The F1 generation in the glasshouse were self-pollinated working down the spikes from the top. The youngest flowers used were fully self-fertile at a similar stage of development to the youngest flowers visited by bees on the plots.

Some flowers were observed with holes bitten through the corolla. No insects were seen making these holes, but bees are known to make such holes in red clover and other flowers. In the Antirrhinum flowers holes were in two places:

1. In the lower corolla lobe (Plate 6f)

Some flowers had holes in the lower corolla lobes. To make such a hole

can be of no benefit to any nectar or pollen-gathering insect as it leads directly back to the outside of the flower.

2. In the basal swelling

More commonly, flowers had holes in the swelling at the base of the corolla tube. A bee biting here would gain direct access to the nectar without opening or pollinating the flower. Some bumble bees were observed regularly going to the base of flowers, and moving on to another if there was not a hole already there.

The open tubed flowers of the variety "B" grown as a control were visited by a wider range of insects (Plate 6e).

In 1982 and 1983 some of the flowers in the trials and in the glasshouse were infested with the larvae of a small beetle, about 4mm long (Plates 9e,9f & 9g). This was identified by the British Museum as the Antirrhinum Beetle, Brachypterolus vestitus Kies. The larvae were found in unopened flower buds. They first ate the developing anthers and then the other flower parts. In most cases, damage was confined to the inside of the flowers and was not apparent without opening the throat of the flower. Adult beetles could be found crawling over the plants.

5.4 MORPHOLOGICAL ABERRATIONS OF THE WHOLE PLANT

1. Stunted plants

Some plants made only slow growth, while others had shortened stems. Often the leaves were twisted and uneven. Such plants were considered useless for the breeding programme and often did not flower.

2. Leaf arrangement

A wide range of leaf arrangements was observed, both in the breeding lines and in some established varieties. The leaves are usually opposite below, and become alternate above. In some plants the lower leaves were in whorls of three. The upward extent of the whorled or paired arrangement also varied. In some plants the arrangement of the lower leaves continued up into the inflorescence. This arrangement broke down gradually as one of the two or three leaves at each level

gradually became separate, to give an uneven spacing as intermediate to the alternate arrangement. Variation in the leaf and flower arrangements were not considered in making selections for the breeding programme.

3. Paired leaf midrib

A few leaves were observed with two midribs, and ending in two points. They are not easy to notice among the leaves of a well grown plant, and were mostly found while scoring leaves for rust infection. Four such leaves were found among 3070 leaves examined from the 1983 trial.

4. Cold-induced variegation (Plate 10)

During November 1981, the plot of progeny 80-34-5 in the trial at the Botanic Supply Unit was noticed to contain variegated plants. The leaves at the tips of all the shoots of affected plants were very pale. It was very obvious, and would have been noticed if it had been expressed earlier in the season. The change was apparently induced by the onset of cold weather. When potted up and brought into a warm glasshouse these plants did not produce more than the occasional fleck of green tissue in the new growth. The change was thus irreversible. 10 plants were affected out of 30. Plants given to Dr. Stevens of the Botany Department at Royal Holloway College showed no sign of virus infection when examined using the transmission electron microscope.

5.5 ABERRATIONS OF FLOWER INITIATION

1. No flowering

Some vigorous plants showed no sign of flowering. They remained short and compact. Often they became very bushy with many short branches. Sometimes the branches appeared to be foreshortened, with many leaves and short internodes.

2. Bracts but no flowers

Some plants produced obvious flowering shoots, but had no flowers on them. These shoots had the usual small bracts of A. majus but without flowers. After a period of inactivity a flower spike could also resume growth, producing bracts but not flowers. Usually the bracts

were large and clearly functioning as leaves. They were still sessile (typical of the bracts but not the leaves of Antirrhinum) and not as large as the lower leaves. This was observed in the trials, but was most frequent when plants were lifted, potted and brought into the glasshouse. Sometimes flowering started again higher up.

3. Large bracts

On a few plants the bracts were as large as the leaves. This is of horticultural importance as the bracts hide the flowers and thus ruin the display. Plate 6b shows an intermediate condition. Only plants with bracts of normal size were used for breeding.

4. Shoots in the bract axil

After making the crosses the flowering axis was cut off above the flowers that had been hand pollinated. The aim was to direct the flow of nutrients to the pollinated flower, and to avoid confusion between the hand pollinated capsules and any other capsules setting seed. After this had been done side shoots would sometimes develop from the axils of the bracts, either of the pollinated flowers, or of other flowers that had been cut off. The new side shoot could be either vegetative or a secondary inflorescence.

5. Terminal flowers

Sometimes the inflorescence axis ended in a flower. Usually the end of the inflorescence is hidden amongst some aborted flower buds. Terminal flowers often showed near radial symmetry. (see 5.6, section 8 below)

5.6 ABERRATIONS OF THE COROLLA TUBE

Abnormalities of the corolla are mostly relatively small distortions of normal growth. However, on an ornamental plant, even a slight abnormality of the corolla can be ugly. These are therefore important horticulturally. Variations in the size and shape of the flowers were considered to be a component of "horticultural quality" of the normal plant. Plate 6a shows flowers which were considered too narrow.

1. Double palate

Some plants had flowers with a large palate and lower centre corolla lobe with a prominent centre division. This is rather ugly.

2. Curling lower corolla lobes (Plate 6d)

This may affect the outer two lobes, the centre lobe, or all three. Often a slight curling at the edges was associated with environmental stress. Thus some curling was seen on plants flowering late in the season or that were pot-bound in the glasshouse. Plate 6c shows the range of flower shapes and degrees of curling of the corolla lobes of pot-bound plants of the control variety Malmaison. A protruding centre lobe was commonly seen on healthy, unstressed plants.

3. Browning (Plate 6d)

In 1981, when planting was late in the season, flowering continued well into the autumn. Many of the yellow flowers showed a distinct browning of the edges of the corolla lobes. This may have been an environmental effect. Many yellow or white flowers in the 1983 trial showed local browning of the palate. This was associated with superficial damage probably caused by bees visiting the flowers.

4. Split corolla tube (Plate 11)

This was one of the defects first noticed in the F1 generation, and present in the later generations. The corolla tube was split along the middle of the top. Usually only a few flowers on each plant were affected although flowers of some plants and progenies were more severely and more commonly affected. There was also variation in the extent to which the flower was split. Sometimes the split was only at the distal end of the corolla tube, while more often, it extended to the base. The split corolla is a well known and prominent horticultural fault of antirrhinum.

5. Divided corolla tube (Plate 12)

Some flowers had corollas which bore little resemblence to a tube. Their corolla was divided into narrow strips down to the base. In some cases this was associated with smaller flowers. This flower type was most common in progeny 82-32-2 grown in 1983.

6. Open tube with curly corolla lobes

On many of the plants of line 48 there was no palate, and the associated folding of the corolla was lost. Instead the tube ended in four curly corolla lobes. Some asymmetry remained as the top and bottom lobes tended to curl outwards, while the side lobes tended to curl inwards. One plant of this phenotype from progeny 81-48-7 was self-pollinated, and the resulting progeny (82-48-4) grown in the 1983 trial. All the plants of this progeny showed the abnormal phenotype. These flowers collected water from rain and irrigation. The weight caused the plants to collapse.

7. Staminode-like growths outside the corolla tube

These are not prominent and were not noticed until 1983 although if they had been common they would have been noticed earlier in the breeding programme. Some flowers of progeny 82-53-3 had structures similar to the filamentous staminodes (see 5.7 section 2 below) on the outside of the corolla tube. Other flowers of lines 32 and 53 had strips of petal on the outside of the corolla tube. These were produced from ridges on the outside of the corolla tube, and lay flat along the tube. They did not exceed the tube in length.

8. Radial symmetry (Plate 13)

Some radially symmetrical flowers were produced by 82-53-3, the progeny that produced the extra organs outside the corolla tube (section 7 above). These were similar to the peloric flowers described by Stubbe (1966). Plants would produce one of these peloric flowers among their normal flowers. In one flower the normal upper lip was present above an open peloric type tube.

In occasional progenies, the last flower on a spike was sometimes produced in the terminal position. When this happened, the flower was often nearly radially symmetrical or the asymmetrical structure of the palate was imperfectly formed.

9. Persistent corolla

Some plants were slow to drop their corollas after fertilization. This was a serious horticultural defect as the dead corollas hanging on the plant were unsightly. An additional problem was that in damp

conditions, the dead corollas provided a site of infection for moulds especially Botrytis cinerea. Normally, the corolla drops off soon after fertilization. If it had not dropped when the ovary began to swell the corolla was held by the developing capsule which soon became larger than the inside of the corolla tube. There were two causes of retention of the corolla after seed had been set:

1. Delayed or poor dehiscence of the corolla.
2. Short thick styles (see 5.8 below) which were strong enough to support the corolla on the flower after normal dehiscence.

5.7 ABERRATIONS OF THE ANDROECIUM

1. Protruding stamens (Plate 7f)

The normal Antirrhinum flower has filaments of two lengths. Over elongation of the longer pair carried the anthers out of the corolla tube of some flowers. Sometimes this showed before the flower opened.

2. Staminodes (Plates 7, 8 & 9)

When flowers of the F1 plants were opened for self-pollination, some were found to contain simple spatulate staminodes. In the F1 these were all small. They did not interfere with pollination, and were not visible until the flower was opened. They were additional to the four stamens.

In the trials of later generations staminodes were present in many more bizarre forms. Often they could only be seen when the flower was opened for hand pollination, but the larger forms protruded from the flower. In extreme cases many petal-like staminodes protruded through the throat of the flowers. Expression of these staminodes was very varied. When apparently normal plants were dug up from the plots in 1981, and taken into a warm glasshouse during the autumn, some of them produced many of these staminodes. During the summer of 1982 plants were found producing many staminodes in some flowers and none in others.

Some of the flowers producing staminodes were dissected to examine the insertion of the staminodes, and their relationship to the other flower parts. Staminodes and stamens are both attached near the base of the

corolla tube. In some cases the petaloid staminodes were forked although others were apparently independently inserted into the corolla tube. They may have been in groups corresponding to the individual stamens of the normal flower. In no case were more than four fertile anthers observed.

The staminodes were of three different types:

1. Petaloid Petal-like (Plate 7d)
2. Filamentous Filament-like (Plate 8e)
3. Styloid Style-like (Plate 8h)

The petal-like staminodes were again of three types:

1. Spatulate Simple flat structure (Plate 8e)
2. Complex Complex folded structure (Plates 7d & 9b)
3. Petal - stamen intermediate type (Plates 9c & 9d)

Simple, petal-like staminodes were the most common. They were like a filament but flattened off into a small elongated piece of petal, or like a narrow strip of petal. The complex petal-like staminodes were a development of the simpler forms and some appeared to imitate part of the folding pattern of the Antirrhinum corolla in miniature. While the complex folded shape was never fully formed, the staminode viewed from some angles was like the hinge area of one side of the corolla together with one upper corolla lobe (Plate 9b). The petal-stamen hybrid type had a large petal-like part, but with a solid yellow swelling which was possibly anther tissue (Plate 9d). None of these was able to produce pollen, but the appearance suggested an anther.

Filament-like staminodes resembled the normal Antirrhinum filament, but without an anther (Plate 8e). Sometimes they were hirsute while the normal filaments are glabrous.

Style-like staminodes were almost identical with the stigma and style, even having a differentiated receptive surface, but lacked the green colour of the true style (Plate 8h).

5.8 ABERRATIONS OF THE GYNOECIUM

The only abnormality of the gynoecium noticed was a thickening and shortening of the style of some plants, especially of line 82-32-2 grown in 1983 (Plate 7g). Fertility did not seem to be affected as these plants were producing large seed capsules. However this was a serious horticultural fault as it prevented the corolla from dropping after fertilization. The visual effect was therefore similar to a persistent corolla.

5.9 DISCUSSION

A wide range of abnormalities was observed in the breeding material. The causes are likely to be as diverse as the plants themselves.

The stunted plants may have been infected by virus or have been damaged as seedlings. Plants that as seedlings were attacked by damping off fungi, or that were slow to germinate, would have been smaller than the rest when they were planted out onto the plots. These small plants could very easily be damaged during planting. Other, previously healthy, plants could also have been badly transplanted. Whatever the cause, the stunted plants were considered useless for breeding. Many of these plants had very few flowers from which to breed. Plants lacking vigour clearly have no future as a variety.

When scoring for rust-resistance, the smallest plants often seemed to have the least rust. This could be the result of either a physiological connection between small size and rust-resistance, or genetic linkage between resistance genes and loci with harmful physiological interactions. Rapidly growing leaves may perhaps be more susceptible than those which are growing more slowly. The age of the individual leaves may affect their susceptibility to rust infection. The older leaves may be more rust-resistant than younger leaves (Chapter 6). There are fewer young leaves on a slow growing plant than on a vigorous plant. This may cause the stunted plants to appear resistant because of the absence of young, healthy and rust-susceptible growth.

The primitive flower type within the Scrophulariaceae is an open five-lobed corolla tube with four stamens. Two of the filaments are longer than the other two. The flowers are borne on a panicle. In the Antirrhineae the inflorescence is reduced to a raceme while the corolla tube becomes increasingly specialized to form the familiar "snapdragon" structure. Many of the floral abnormalities that have been observed in this breeding programme and by other workers, can be explained if this background is considered along with a loss of precision of developmental control.

In this breeding programme, all the parental material was from commercial varieties. These were crossed with the aim of producing a reassortment of the genes controlling rust-resistance. Segregation for colour was also expected. The inheritance of colour in Antirrhinum has been well understood for a long time, and it was in A. majus that the relationship between Mendelian factors and biochemical pathways was first demonstrated as the mechanism by which genes control flower colour (Wheldale & Bassett, 1914). There was little apparent variation in flower morphology in the parent varieties which all had the normal flower form. However, antirrhinum varieties seem not to be very uniform. Colour off-types are quite common and observation of control and spreader rows suggests up to 3% of plants may be off-type in some varieties. This may represent residual heterozygosity from the original breeding programmes or could be due to seed contamination or cross pollination accumulating during seed multiplication.

The variation of bract size parallels variation within the wild species of the genus Antirrhinum. Table 5.1 shows the bract sizes and pedicel lengths of the European species based on the descriptions given in Flora Europaea. Most of the species with large bracts have long pedicels which carry the flowers into the open where they are visible to pollinating insects. Many of these species do not have the dense flower spike of A. majus, but have smaller flowers distributed over a more prostrate plant. These forms may be adapted to growing in and through a sward of other plant species, with the flowers within a mixed canopy. The flowers of A. majus and similar species are held above the spike and above any other low growing herbs. The spectacle of the dense flower spike is probably more effective in attracting bees and

Species	Pedicel length (mm)	Bract size
A. valentinum	7-20	
A. sempervirens	5-10	bracts similar
A. pulverulentum	5-10	to foliage-
A. pertegasii	5-10	leaves
A. microphyllum	10-20	
A. molle	3-20	
A. charidemi	3-12	similar to smaller leaves
A. grosii	3- 6	lower bracts similar to
A. braun-blanquetii	3- 6(-12)	foliage-leaves, upper smaller and narrower
A. siculum	c. 5	lower bracts transitional to foliage-leaves, upper about equal to pedicels
A. hispanicum	2-20	bracts similar or much smaller than foliage-leaves
A. meonanthum	1- 4	
A. barrelieri	1- 4	bracts smaller
A. graniticum	3-15	than foliage-
A. australe	1- 5	leaves
A. latifolium	3- 8(-15)	
A. majus	2-10(-15)	

Table 5.1 Pedicel length (mm) and bract size of wild species of Antirrhinum section Antirrhinum, as described in Flora Europaea.

must also have encouraged the early cultivation of this species. Forms of A. majus with large bracts are anomalous. They certainly reduce the impact of the flower spike to the human eye. Large bracts might also reduce its attraction for bees, or hinder their movement between flowers.

The growth of shoots in the axils of bracts was unexpected but could be explained if some meristematic tissue normally remains at the base of the peduncle. After hand pollination, the top of the flower spike was cut off. This would suddenly remove suppression due to apical dominance. At the same time the demand for metabolites was greatly decreased by removing the growing region at the top of the inflorescence and the developing seed capsules (if present) below the hand pollinated flowers. Because of this removal of sinks, the supply of metabolites suddenly far exceeded the demands of one or two developing seed capsules. The purpose of removing unwanted flowers was to direct metabolic resources to the developing hybrid seeds, but a side effect might have been to stimulate repressed meristematic tissue into unexpected growth. Thus a side shoot was produced following the sudden removal of suppression by apical dominance and the simultaneous increase in metabolite availability. It is interesting to note that some of the more primitive members of the Scrophulariaceae (for example some Verbascum species) have paniculate inflorescences.

The occurrence of more or less actinomorphic flowers at the end of the flowering spike is perhaps an indication of the developmental processes. The terminal position is symmetrical, while the normal lateral flowers grow in an asymmetric developmental environment. If, for example, the gradient of auxin flow away from the main stem apex plays a part determining the normal asymmetry of the Antirrhinum flower, then a more symmetrical structure might be expected when the apex itself develops into a flower.

Major genes have been described that affect flower shape in Antirrhinum (Stubbe, 1966). Some of these have variable expression. If the aberrations observed in the breeding programme were caused by major genes, they would be evident in Mendelian segregation ratios. The number of plants affected were not counted for most of the aberrant

types occurring in this material. Most deformities have occurred spasmodically throughout the breeding programme with the exceptions of the curly petal phenotype in line 48 and the cold-induced variegation in line 80-34-5. Expression was usually highly variable. Plants chosen for their conformity on the plot produced abnormal flowers when brought into a warm glasshouse. Plants producing good flowers early in the season produced staminodes in their second flowering period. There were differences between spikes on the same plant at the same time and between flowers on the same spike.

The large environmental variation in expression would make genetic analysis difficult. If any of the abnormalities was due to a single major gene then is should be possible to trace it back to either a mutation event or a parent line. In this breeding programme all parental material came from good horticultural stock; but established varieties are not free from these defects. The majority of these events are probably not oligogenic but may be caused by the interactions of a number of loci with each other and with new genetic backgrounds. Most Antirrhinum varieties are strongly inbred and their genetic structure will reflect this. An outbreeding population is often phenotypically stabilized by partial dominance at many heterozygous loci. Genes are selected that can produce a stable phenotype in a wide range of genetic backgrounds. In an inbred population selection favours gene combinations that are phenotypically stable in one particular genetic background and phenotypic expression of genes or gene combinations is not tested in any other background. When inbred lines are crossed together, as at the beginning of this breeding programme, coadapted gene complexes are broken up. Genes are expressed in new combinations and new pleiotropic interactions can occur. Heterosis is the advantageous expression of these interactions. The opposite effect is hybrid dysgenesis when the new gene interactions are disadvantageous. This may be causing some or all of the abnormalities observed.

The "curly" phenotype appears similar to a variant form of Linaria vulgaris Miller, described by Morander (1980). Since the stand he described had been known since the 1960s, he suggested that the anomaly might be due to genetic aberration.

Many of the other abnormalities produced in the breeding programme were influenced by environmental conditions. Environmental stress or natural changes in season, as when plants were dug up and brought into a glasshouse produced abnormalities on plants that had previously been selected as being of good horticultural quality. These abnormalities may be the result of developmental regulation breaking down under slightly unusual conditions.

In the wild, Antirrhinum species are only minor components of the vegetation (Gawthrop, 1980 for Californian species; Rothmaler, 1956 for Iberian species) and distances between plants are relatively large. An efficient agent of pollen distribution and a self-incompatability system are needed to maintain an outbreeding population in these conditions. An unspecialized pollinating insect visiting flowers unspecifically would be unlikely to move directly from one Antirrhinum plant to the next and its pollen load would be diluted or lost by visiting flowers of other species in between Antirrhinum plants. It is therefore an advantage to Antirrhinum species to have one specific pollinator specialized in the one species of plant. The specialized pollinator would move from one plant to the next without stopping for other species in between. The social bees probably have the most highly developed foraging habits of all flower visiting insects. Individual worker bees have a long adult life and usually restrict and adapt their activities to one nectar or pollen source while it remains available. They are therefore an ideal group to act as specific pollinators of the type envisaged above.

The structure of an Antirrhinum flower reserves the nectar and pollen for large bees because other smaller and lighter insects are unable to gain access. This gives individual bees a reward for specifically seeking out Antirrhinum plants and not visiting other flower species where there is competition with other insects. The exclusive relationship between Antirrhinum and bumble bees is mutually advantageous. The plant gets the efficient transfer of its pollen to other plants of the same species over the range of an individual bee. The bees get a source of nectar and pollen free from competition with other insects. The plant is committed to this relationship by the long term evolutionary adaption of its flower shape. The bee makes a short

term behavioural adaption.

Other insects besides bees are sometimes able to get nectar from Antirrhinum flowers. Small insects are able to creep into old and relaxed flowers. However bumble bees are the main pollinators.

Red clover (Trifolium pratense L.) is also pollinated by bumble bees in nature, but hive bees are used as pollinators in commercial seed production. They have difficulty in tripping the flowers and quickly learn to bite a hole in the base of the flower to obtain the nectar. On subsequent visits the nectar is taken through the same hole. Such "robbing" visits do not pollinate the flowers and are a source of loss for seed producers. They can be reduced by using hives of bees that are new to red clover and have not learned to bite the flowers. However, the bees are quick to learn. Similar behaviour of bumble bees on cultivated antirrhinums is described above. The lower corolla lobes appear to have some effect in preventing such robbing behaviour. Insects biting through the corolla lobes to avoid the palate mechanism do not obtain access to the nectar. To reach the nectar by biting, insects must leave the front of the flower for the bottom which is in shadow in the centre of the flower spike. This requires a greater modification of normal flower visiting behaviour.

Plate 6 (opposite)

a) Flowers which were considered too narrow.

b) Acceptable flowers, but with bracts which were larger than usual. If the bracts were any larger they would be unsightly. Only plants with bracts of normal size were used in the breeding programme.

c) Six flowers of the variety Malmaison. Environmental stress has caused variation in the shape of the flowers and curling of the corolla lobes.

d) Flowers in the autumn showing curling of the corolla lobes and browning of the older flowers.

e) Commercial variety with Penstemon type flowers being visited by a small beetle.

f) A flower with a hole through the lower corolla lobes. Holes like this were probably made by bees. Holes in this position are of no benefit to the bee as they lead back to the outside of the flower. Holes in the base of the corolla allowed bees to take nectar without the effort of opening the corolla tube.

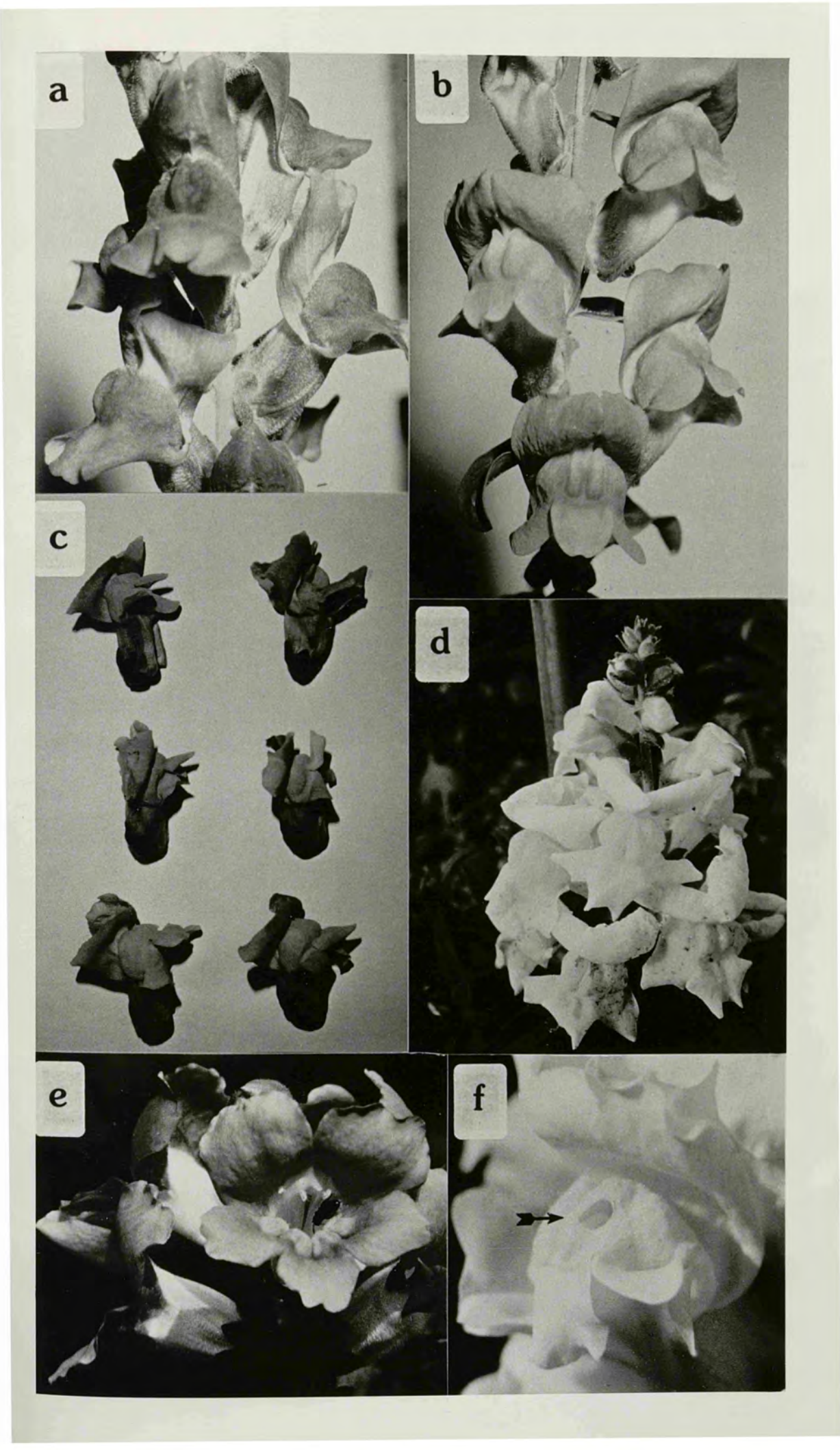

a
b
c
d
e
f

Plate 7 (opposite)

a) A flower with many large petal-like staminodes protruding from the corolla tube.

b) The corolla and androecium of a flower with two petal-like staminodes.

c) Detached flower with many large petal-like staminodes protruding from the corolla tube.

d) The corolla and androecium of the flower in (c). The corolla tube is in two parts at the top left. Seven petal-like and petal-anther hybrid staminodes were inserted into the base of the corolla tube where the anthers would be expected.

e) Three petal-anther hybrid organs, one normal stamen and one forked petal-like staminode forming the androecium of one flower.

f) A bud in which abnormal elongation of the filaments has carried two of the anthers out of the corolla tube.

g) Developing capsules of plant with short and unusually thick styles. The capsules developed normally and fertility was not affected. These styles are strong enough to hold the dead corolla tube after it has separated from the receptacle. The retained dead corollas were removed from these flowers before the photograph was taken.

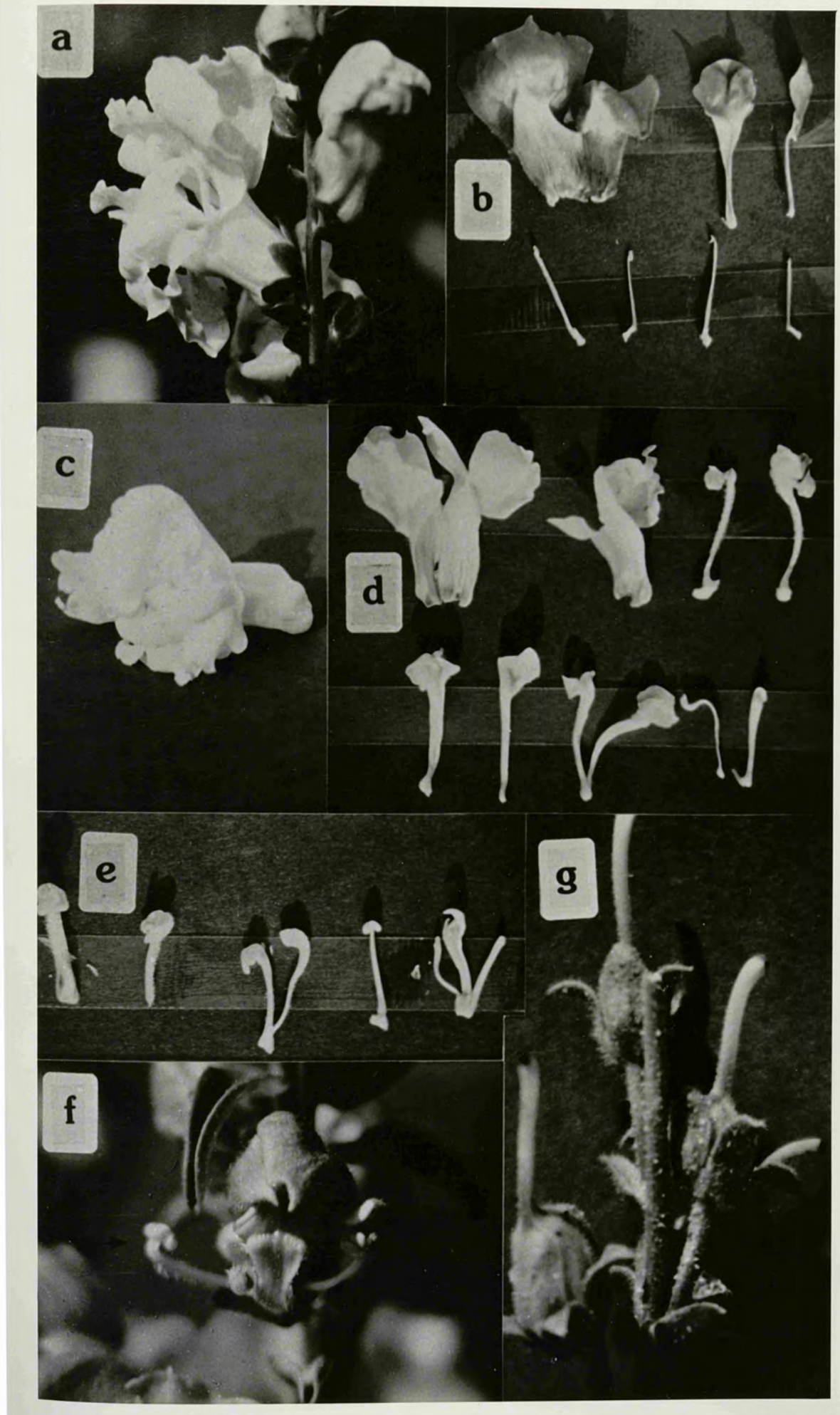

a
b
c
d
e
f
g

Plate 8 (opposite)

a) The four anthers and the stigma of a normal flower showing their relative positions.

b) Insertion of staminodes into the corolla. Notice that several staminodes tend to insert together in groups. Even when there are many staminodes they tend to arise as four groups, which may correspond to the four stamens of the normal flower.

c) A normal flower with the lower half of the corolla tube cut away to show the insertion of the stamens. The hairs at the base of the filaments close off the nectary. There is space for the nectar between the lower filaments and in the basal sac of the corolla tube.

d) A small anther with abnormal filament development.

e) Simple filamentous and petal-like staminodes.

f) Staminode occupying the position of the "fifth stamen" at the top of the flower, as found in some of the more primitive genera of the Scrophulariaceae. Such staminodes lie above the style and are not seen unless the flower is cut open.

g) Stamen with a double filament and very small anther.

h) Style (above) and style-like staminode (below) from the same flower. The style was green but the staminode was white.

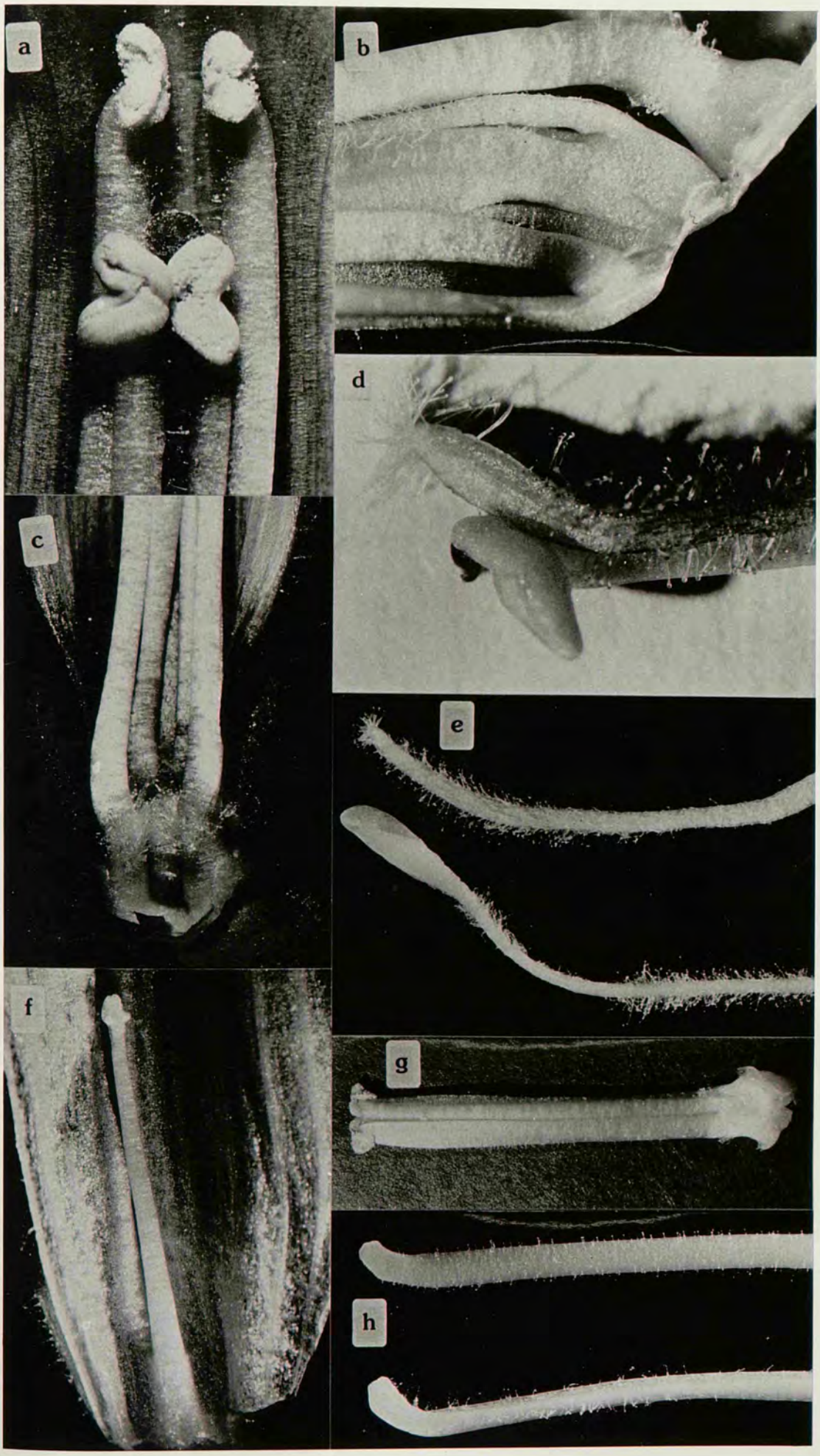

a
b
c
d
e
f
g
h

Plate 9 (opposite)

a) Branched staminode from Plate 7e.

b) Petal-like staminode. Note the resemblance to the upper and lower corolla lobes of a normal flower.

c) Anther with petal-like extensions.

d) Anther-like swelling of an otherwise petal-like staminode.

e) Damage caused to a normal flower by larvae of the antirrhinum beetle Brachypterolus vestitus Kies.

f) Larvae of the antirrhinum beetle Brachypterolus vestitus.

g) Adult antirrhinum beetle Brachypterolus vestitus.

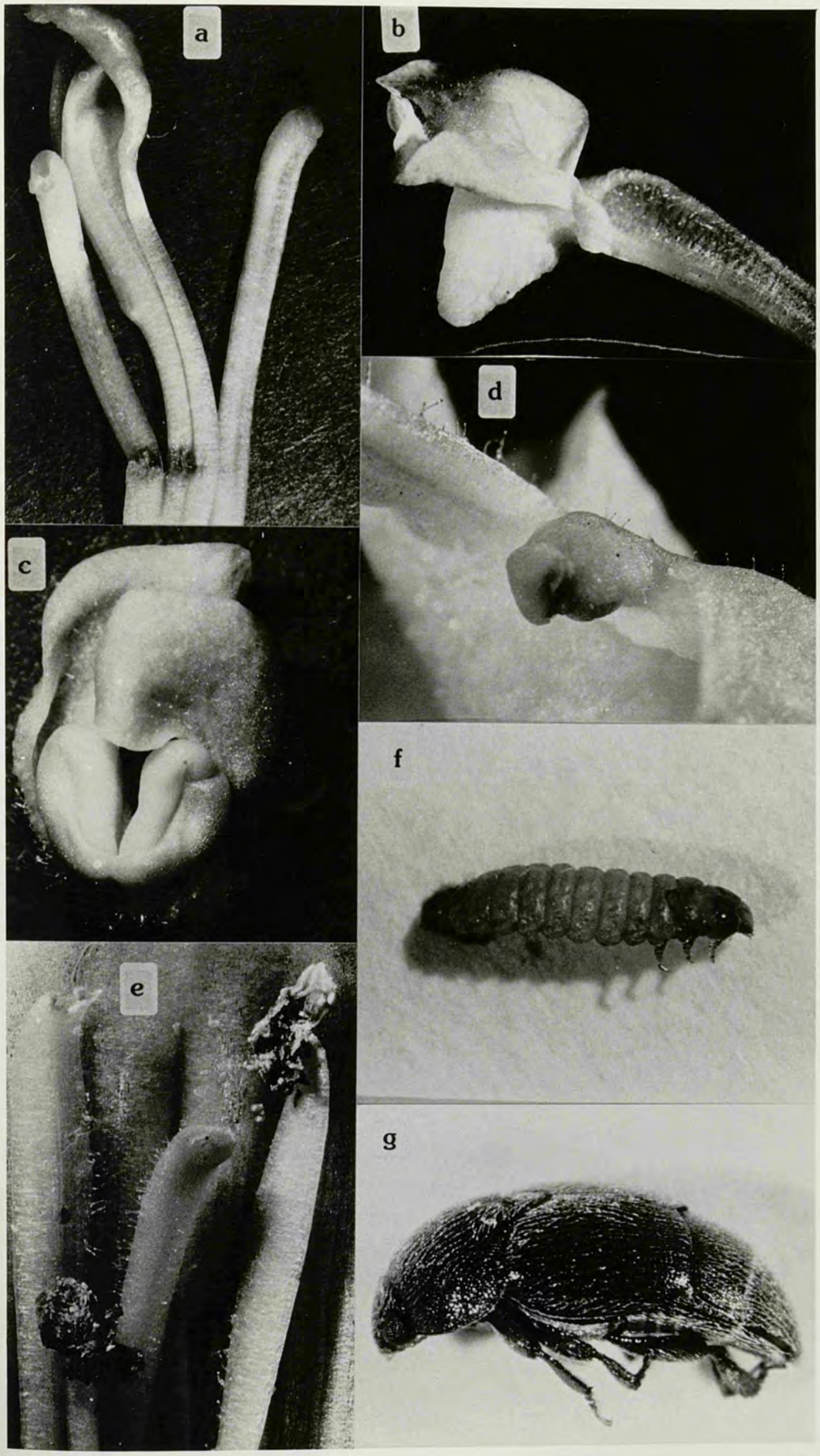

a
b
c
d
e
f
g

Plate 10. Antirrhinum plant showing cold induced variegation.

Plate 11. Single flower with split corolla tube.

Plate 12. Single flower with divided corolla tube.

Plate 13. Flower spike with one actinomorphic flower.

CHAPTER 6
THE RUST

6.1 INTRODUCTION

Little has been published on the overwintering of the antirrhinum rust. When the rust was first reported from Britain, Green (1933 & 1934) pointed out the lack of knowledge about the life cycle of P. antirrhini and recommended that all affected plants should be burnt. Infection of antirrhinum seedlings was found during January 1934 and depressed the early hope that the rust might not overwinter easily in Britain. There is no detailed report of the mode of overwintering of antirrhinum rust in the United Kingdom, although a considerable body of experience has accumulated. The rust is generally considered to overwinter in the uredinial stage on antirrhinum plants. Aronescu-Savulescu (1938) and Kochman (1938) both report the germination of urediniospores overwintered in severe conditions. Aronescu-Savulescu concluded that in Romania the rust infection carried over the winter in the urediospore stage and occasionally as mycelium in host tissue.

Other Puccinia species producing teliospores overwinter at this stage and then infect an alternate host. The teliospores of P. antirrhini have been observed to germinate and produce basidiospores by Hockey (1921), Mains (1924), Kochman (1938), Wahl (1949) and Lehoczky (1954). These basidiospores have not been known to germinate and no infection of any plant has been produced from either teliospores or basidiospores. This negative evidence cannot prove that the sexual life cycle is not involved in overwintering (at least occasionally). However, the rapid and world-wide spread of antirrhinum rust suggests that no other species is necessary for the rust to survive. There is of course the possibility that the urediniospore stage may be able to maintain the rust all the year round in some climates but that an alternate host is necessary elsewhere. Alternatively, the alternate host may be a very widespread weed or common garden plant.

Walker (1954) germinated urediniospores on a dilute extract from antirrhinum leaves. Spores were longest lived at low temperatures and

low humidities. At 5 Celsius and 25% moisture they survived for up to 116 days. At 30 Celsius or at 25 Celsius and 75% relative humidity, spore viability decreased over the first 21 days and no spores germinated after 40 days. Barbe (1967) stored spores in the artificial conditions of glass vials maintained at 4 to 6 Celsius and found a steady loss of viability over 16 months, although at room temperature dry spores lost all viability in four months. Other reports suggest a life of as little as 6 weeks (Pethybridge, 1934; Yap, 1969; Doran, 1921).

If there is an alternate host, this is unlikely to have changed during the switch from wild to cultivated Antirrhinum species. The search for an alternate host should therefore be directed to Californian species and especially to Californian species that are in widespread garden cultivation. These criteria were taken into consideration in selecting species from those growing at the Botanic Supply Unit at Egham. These species were inoculated with teliospores in the spring in order to investigate the possibility of their acting as the alternate host.

If the rust overwinters on antirrhinums then it should be possible to demonstrate infectivity from old plants early in the spring. The most susceptible plants are not necessarily the best for the rust to overwinter on. If the host is killed or severely weakened by the rust, then the chance of the rust surviving the winter is clearly reduced. The amount of rust infection developed in late autumn will be the important factor. For this reason both resistant and susceptible plants were overwintered in order to demonstrate infectivity in the following spring.

The susceptibility of plants to disease can vary with the age of the host plant or of the organs of the host plant. It is often noticed that the start of antirrhinum rust epidemics is at about the same time as the start of flowering. This could be due to some physiological change in the maturing plant that makes it more susceptible to rust, some change in the environment that facilitates epidemic development (for example if bees were to spread the rust) or simply the need for the epidemic to build up from a low overwintering inoculum.

The selection of rust-resistant plants by field trials is time consuming and liable to a large degree of environmental variation. If rust-resistance could be assessed in young seedling plants, selection could be made much more quickly. Even a preliminary screening which enabled the most rust-susceptible plants to be eliminated before the trials were planted would be valuable in its effect of concentrating resources on the better plants. It was envisaged that plants might be grown in the glasshouse in the normal way and inoculated in time for rust development to be assessed before planting out. Final selection would still take place in the field as described in Chapter 3. Such techniques have been developed for breeding programmes in other species. Usually they depend on all or nothing responses to infection, often controlled by single genes. Such tests can be performed with very small samples of host tissue, often a single leaf is sufficient. Young antirrhinum seedlings were inoculated with rust in order to investigate the feasibility of such a procedure. Such an experiment also demonstrated susceptibility of very young plants to rust. Such young plants are not exposed to high levels of inoculum in the normal practices of antirrhinum growing.

The age of the individual leaves might be important in determining the reaction of the plant to rust. This was investigated by comparing the reaction of all the leaves of plants just coming into flower to inoculation with rust spores.

The evidence for genetic change in the history of antirrhinum rust is reviewed in Chapter 2 above. The antirrhinum trials have demonstrated a wide range of host susceptibility. A corresponding range of variation within present rust populations has not been demonstrated although there are indications that it is present.

Two recent authors report direct demonstrations of the variability of P. antirrhini, each inoculating a range of antirrhinum plants with a range of rust isolates.

Barbe (1967) inoculated cultivars of A. majus with two rust isolates collected from wild plants of A. multiflorum and A. virga in

California and six isolates from cultivated A. majus in New Zealand, Australia, Zimbabwe, England and California. The isolates from the wild Californian species would not grow on any of the A. majus cultivars that he tested. By dividing the reactions of the cultivars to the other isolates into three categories, i.e. immune with no rust, resistant with some rust and susceptible with much rust, he was able to differentiate between rust isolates. Six differential varieties were found. All rust isolates were differentiated from all others with the exception that the isolates from New Zealand and South Africa could not be separated from each other.

Gawthrop (1980) inoculated detached leaves placed on plastic mesh floating on water in crystallizing dishes. She used 80 plants but many of them were related to each other, so it is unlikely that the full range of A. majus genotypes was sampled. Plants were inoculated with four rust isolates from England, two from France, one each from South Africa, California and Australia. However, each plant was tested against only two or three rust isolates. Because of this, it is probable that many potential differential plants escaped detection. A minimum of three distinct rust pathotypes are needed to explain her data. At least two of these occurred in England.

6.2 OVERWINTERING WITH AN ALTERNATE HOST

Species tested were chosen from those growing at the Botanic Supply Unit that were perennials native to California or species closely related to Californian species. The rust susceptible A. majus variety Malmaison was also included.

Malmaison plants from the spreader rows of the 1980 trial plot remained in situ until the 16th December when some were put in net bags to be kept as the source of teliospores used to inoculate possible alternate hosts in the spring. Samples of the spores were examined microscopically to check that teliospores were present. One bag was kept until the spring in a glasshouse where the temperature varied from about 5 to 15 Celsius. Another bag was put outside, but under sufficient shelter to prevent rain falling directly onto it.

In the spring (18th March 1981) teliospores and urediniospores were scraped off the antirrhinum stems kept overwinter. The stems which had been in the glasshouse were mostly dry and dusty, but those which had been outside were wet and rotten. Spores were mixed with 0.2% sterile agar solution and applied with a small paintbrush to young growth of the possible host species in the grounds of the Botanic Supply Unit. This concentration of agar was sufficiently dilute to remain liquid indefinitely and when painted onto a microscope slide dried to leave no residue visible to the naked eye. It was hoped that a small amount of agar would help retain the spores on the target host.

Three inoculations were made to each host:

1. control agar solution
2. spores overwintered in the glasshouse
3. spores overwintered outside

Possible host species tested were:

Antirrhinum majus cultivated variety Malmaison
Sequoia sempervirens
Larix occidentalis
Quercus suber
Pinus ponterosa
Pinus contorta
Arctostaphylos nevadensi
Vacinium ovatum
Gautheria procumbens
Mensiesia farogonia
Vaccinium corymbosum
Gawtheria shallon

The Malmaison plants were young glasshouse-grown plants and were inoculated in the glasshouse and kept under plastic sheet isolation tubes. Inoculated branches of the other species were covered with paper bags similar to those used to cover flowers during hand pollination (Chapter 3). It was hoped that these would prevent inoculum from being washed off the inoculation sites. The bags also served to identify the inoculation sites. Bags were removed and the plants inspected at the end of April.

When the plants were examined no lesions were found on any of them that were not also on the untreated leaves of the same plant.

6.3 OVERWINTERING ON ANTIRRHINUM PLANTS

Rust infected Malmaison plants from the trial were moved to a location where they could stand for the winter on 16th December 1980. These plants died during the winter. On 20th March 1981 spores were collected from these plants and applied to two Malmaison plants in the glasshouse. The Malmaison test plants were covered with plastic bags for one week and then with plastic isolation tubes.

No rust developed in the spring of 1981 on any of the Malmaison plants inoculated with spores overwintered in the glasshouse, outside or on the dead transplanted infected plants.

The overwintering of rust on infected antirrhinums was investigated again in the winter of 1981 - 1982. 12 Malmaison plants were dug up from the trial plot at the Botanic Supply Unit on 25th November 1981 and planted in five-inch pots. The three most infected plants were stood outside for the winter. All leaves bearing rust lesions were removed from the other nine plants and the plants randomly assigned to three groups. Plants of accession number 80-51-5 were also potted up and kept outside. There were thus five groups, each of three overwintering plants:

1. Malmaison in a heated glasshouse. All leaves bearing rust lesions were removed.
2. Malmaison in an unheated glasshouse. All leaves bearing rust lesions were removed.
3. Malmaison kept outside. All leaves bearing rust lesions were removed.
4. Malmaison kept outside. All leaves were left on the plants.
5. 80-51-5 kept outside. All leaves were left on the plants.

The plants of groups 1, 2 & 3 were examined at regular intervals in December 1981 and January 1982. All rusted leaves were counted and removed to determine how long latent rust infections would take to appear.

By removing all the lesion bearing leaves from infected plants it was hoped to determine how long the latent period could last. The number of leaves removed at different dates is shown in Table 6.1. On all three groups of plants new lesions continued to appear in midwinter. Unfortunately the plants standing outside were broken off during severe snow but still developed new rust lesions on the few leaves remaining at the base.

Observation of the plants in the heated glasshouse was stopped in January because there were few of the original leaves left, and the stems were badly infected producing large quantities of urediniospores.

The rust lesions that developed on the plants in the heated glasshouse during January 1982 appeared to be of two types. At this time the plants were undergoing active vegetative growth and some lesions appeared on the new leaves as distinct compact lesions similar to those which developed on inoculated plants. These lesions must have resulted from infections within the glasshouse. Spores could have come either from leaf infections before they were removed, or from stem infections that could not be removed without destroying the plants. On the older leaves patches of rust appeared that were up to 6mm diameter, with spores breaking through the epidermis in a number of small sori. These may have been the result of latent infections which had been developing within the leaf tissues for some time.

The plants kept outside were exposed to severe winter conditions. During part of December they were covered by a snowdrift. Snowfall during December snapped the stems of two of the Malmaison plants of group 3. However, the plants survived until the spring when they still showed signs of rust infection.

The viability of the rust on the overwintered plants was demonstrated in two ways in the spring. Young glasshouse grown Malmaison plants were inoculated with urediniospores collected from these plants. The Malmaison plants were first sprayed with distilled water. Spores were transferred by rubbing lesions with cotton sticks and then rubbing the sticks against leaves of the test plants. Test plants were covered with plastic bags to ensure very high humidity and returned to the glasshouse.

		Number of leaves bearing lesions removed				
	Date:	25/11/81	5/12	15/12	6/1/82	14/1
	Plant					
GROUP 1						
HEATED	1	5	17	68	12 <a>	
GLASS-	2	19	70	69	28 <a>	
HOUSE	3	5	30	109	10 <a>	
GROUP 2						
UNHEATED	1	9	18	0	8	17
GLASS-	2	5	12	3	7	13
HOUSE	3	8	3	6	17	38
GROUP 3						
	1	5	3	1		1
OUTSIDE	2	14	11	1 <b>		1
	3	12	16	0 <b>		2
CHI SQUARE <c>		1.63	81.3	-	3.95	-
d.f.		2	2	-	1	-

Table 6.1 Numbers of leaves developing rust lesions during the winter of 1981 - 1982. All rusted leaves were removed at the start of the experiment and at times of inspection.

<a> These plants had rust on the stems but few old leaves left. Observations were discontinued as it appeared that new lesions were arising from spores from the stems.

<b> Plants 2 and 3 were badly broken by snow.

<c> Contingency Chi-squared test.
Null hypothesis: that the totals of number of leaves in each treatment group are equal.
95% level is chi-square = 3.84 with 1 degree of freedom and 5.98 with 2 degrees of freedom.

After 24 hours the plastic bags were changed for isolation tubes. Only one half of each leaf on the test plants was inoculated, so that infection as a result of inoculation could be identified as heavy infection confined to half of each leaf (Plate 14). Young Malmaison plants were also stood in pots amongst the overwintered plants. After two weeks they were brought into the glasshouse and covered with plastic isolation tubes. They were then inspected at intervals to see if rust developed on them. Other plants were stood in other parts of the grounds as a control group.

The plants overwintered outside had active lesions throughout the winter. The results of experiments to demonstrate the infectivity of these plants are given in Table 6.2. The leaves of Malmaison plants artificially inoculated from these lesions in February and March developed heavy rust infection. Some Malmaison plants stood in pots among the infected plants also developed rust. Thus the overwintered plants were still infective in the spring. It should be noted that these plants were exposed to the rigours of the winter weather while standing in pots.

Plate 15 shows a two year old antirrhinum plant growing in a private garden. Plants like this can survive for a considerable time with a low level of rust infection and a few flowers and have the potential to carry-over rust from one season to the next.

6.4 INOCULATION OF YOUNG SEEDLINGS

From the varieties grown in field trials (Gawthrop, 1980), three were chosen to span a wide range of rust-resistance. They were:

1. Malmaison: rust-susceptible
2. Kimosy Orange: moderately rust-resistant
3. Leonard Sutton: very rust-resistant

Seed was sown at two weekly intervals in 5.5 inch diameter pans. Sowing dates were 10th February, 24th February and 10th March 1982. Thus seedlings of three ages were available at the time of inoculation on 6th April and the effect of seedling age could be studied on plants subjected to the same inoculum treatment.

METHOD OF INFECTION	PLANT	DATE <a>	RESULT
ARTIFICIALLY INOCULATED:<b>			
from Malmaison	1	16/2/82	(heavy rust infection
	2	16/2/82	(with more than 10
	3	3/3/82	(lesions per half leaf
	4	3/3/82	(inoculated
from 81-51-5	1	16/2/82	(heavy rust infection
	2	16/2/82	(with more than 10
	3	3/3/82	(lesions per half leaf
	4	3/3/82	(inoculated
PLACED OUTSIDE:			
among infected plants	1	16/2/82	no rust developed
	2	16/2/82	no rust developed
	3	3/3/82	three leaves infected
	4	3/3/82	one leaf infected
	5	17/3/82	one pustule
	6	17/3/82	no rust
away from infected plants (control)	1	3/3/82	no rust
	2	3/3/82	no rust
	3	17/3/82	no rust
	4	17/3/82	no rust

Table 6.2 Infectivity of overwintered rust infected plants. Control plants in the glasshouse did not develop rust.

<a> The date given for the plants set outside is the date that they were put out. They were left out for about 2 weeks before being brought back into the glasshouse.

<b> Spores were transferred from the overwintered plants to the test plants using cotton sticks (see text).

Plate 14. Leaf, half of which was inoculated with rust spores collected from plants overwintered outside.

Plate 15. Two year old antirrhinum plant growing in a private garden.

Inoculum was collected from five plants of the rust collection and four of the test plants from the overwintering experiment using the cyclone spore collector. The initial inoculum collected was applied at full strength and after dilution to one part in five. An inoculation using water with no spores was made as a control.

Care was taken that the application of inoculum was the same for each pot of seedlings. A plastic tube was constructed to fit around the plant pots, and a hole made 150mm up in the side for a quickfit venturi spray. The spray was held in position by a retort stand and driven by the pressure side of the vacuum pump. Each pot was sprayed with 2.5ml of spore suspension which took about 15 seconds. The spray was allowed to settle for 45 seconds from the start of spraying.

After inoculation, the pots were stood in plastic trays and watered well from below. The were covered with plastic trays to ensure high humidity for spore germination. The overnight temperature ranged between 12 and 15 degrees Celsius. The covering trays were removed on the following morning.

Each combination of three sowing dates, three varieties and three inoculum levels was replicated three times and thus there were 81 pots in total. The pots of each sowing date were kept together on the glasshouse benches, but the treatments were randomly arranged within these groups.

Infection produced on individual plants and leaves of all the treatments was uneven. The number of leaves and rust lesions on each of 10 plants of each treatment combination were assessed 23 days after inoculation. No rust developed on plants with the control (no spores) inoculation treatment. The results of all other plants are summarized in Table 6.3. There was an even number of leaves on most plants, reflecting the paired arrangement of leaves on young antirrhinums. There were plants without any apparent rust infection in the most highly infected treatments although these plants were from uniform cultivated varieties. The inoculation method as used was not achieving uniform infection of all susceptible material and therefore was not suitable for use as a preliminary assessment for rust-resistance of the individual plants in a

breeding programme. Reliable estimates of rust-resistance/susceptibility could not be obtained for each plant. Varietal differences were observable at the level of comparison between pots, but these did not correspond with the results of field trials. Among the oldest seedlings Kimosy Orange developed more rust than Malmaison. It is possible that the expression of resistance in young seedlings is different from that in the mature plant. If this is so, then no assessment made at the seedling stage would be able to predict the results of field trials of mature plants.

The disjoint distribution of the results of the seedling inoculation experiment precludes analysis using statistical methods that assume an underlying normal distribution.

Infections were observed on the cotyledons, but the younger plants generally showed less infection than the older plants.

6.5 INFECTION OF MATURE LEAVES

In order to study the effect of the age of the individual leaves on a mature plant, 13 pairs of leaves from each of two Malmaison plants were inoculated with a suspension of rust spores. The two plants were grown in pots in the glasshouse until the first flower buds on the main stems were about 5mm long. Plants were chosen for the experiment that had their leaves arranged in pairs. (Cultivated antirrhinums show a range of leaf and flower arrangement from whorls of three to paired below and alternate above.) On 15th April 1983, 13 pairs of leaves were taken from each plant and numbered from the top (excluding bracts). One or two pairs of the very oldest leaves were left at the bottom of each plant. Each pair of leaves was placed on agar in a separate petri dish. A small paint brush was used to inoculate the leaves with an urediniospore suspension obtained from a single rust isolate. Inoculation was carried out in a randomised order. Dishes were kept in a cold room (at approximately 10 Celsius) overnight and then on a laboratory bench, again in randomised order.

The number of lesions developed on each leaf was counted after 11 days

and again 20 days from inoculation.

The number of lesions developed on each leaf is shown plotted against leaf position (leaf pairs counting down the plant) in Fig. 6.1 (scored 11 days after inoculation) and Fig. 6.2 (scored after 20 days). Correlation and regression coefficients calculated from this data are given in Table 6.4. Fig. 6.1 shows very different results for the two plants at the first scoring. Leaves from the top of one of the plants developed much more rust than those at the bottom, or at any point on the other plant. Results for the two plants have therefore been analysed independently. Fig. 6.2 shows that after 20 days the two plants had developed similar levels of rust. The difference observed after 11 days could have been produced by a small difference in the speed of rust development between the two plants. If the rust developed quicker on the younger leaves, and quicker on the leaves of one plant than on the other, and the lesions were counted while the lesions were developing then results similar to those obtained might be expected. The first pair of leaves from each plant developed only one rust lesion each. These leaves were not fully grown, and there may have been a nutritional effect reducing rust development (and life of the leaf tissues) in isolated leaves. However, in all cases there was significant correlation between leaf position (age) and the amount of rust developed, with the younger leaves developing the most rust. Under the conditions of this experiment younger leaves are more susceptible to rust.

6.6 EVIDENCE OF RUST VARIABILITY IN TRIAL RESULTS

In the antirrhinum trials between 1978 and 1983 some varieties were grown in each trial that had already been grown in earlier trials in order to allow a degree of comparison to be made between the trials. If rust strains differed between sites or between seasons the relative resistance of different varieties might vary between trials. Thus comparison of the results of growing varieties in different environments might demonstrate the presence of differing rust strains.

Finlay and Wilkinson (1963) published an analytical technique for

Age group	Variety	Inoculum level	Plants scored	Leaves scored	Lesions counted	Leaves /plant	Lesions /plant	Lesions /leaf
1	1	1	30	222	154	7.40	5.13	0.69
55 days		2	28	170	22	6.07	0.79	0.13
old	2	1	17	94	208	5.53	12.24	2.21
		2	20	110	74	5.50	3.70	0.67
	3	1	30	198	89	6.60	2.97	0.45
		2	30	200	31	6.67	1.03	0.16
2	1	1	24	122	49	5.08	2.04	0.40
41 days		2	30	165	14	5.50	0.47	0.08
old	2	1	18	82	25	4.56	1.39	0.30
		2	20	94	1	4.70	0.05	0.01
	3	1	2	6	5	3.00	2.50	0.83
		2	3	8	0	2.67	0.00	0.00
3	1	1	30	64	6	2.13	0.20	0.09
27 days		2	18	46	0	2.56	0.00	0.00
old	2	1	2	4	2	2.00	1.00	0.50
		2	6	15	0	2.50	0.00	0.00
	3	1	16	42	0	2.62	0.00	0.00
		2	11	24	0	2.18	0.00	0.00

Table 6.3 Results from spraying young seedlings with urediniospore suspension.

Varieties: 1. Malmaison 2. Kimosy Orange 3. Leonard Sutton

Scoring	Plant	Correlation	Regression	Significance
1	1	-0.551	-0.267	*
1	2	-0.461	-0.096	ns
2	1	-0.544	-0.253	*
2	2	-0.704	-0.319	**

Table 6.4 Correlation and regression coefficients for regression of number of lesions developed against leaf position for detached leaves inoculated with rust.

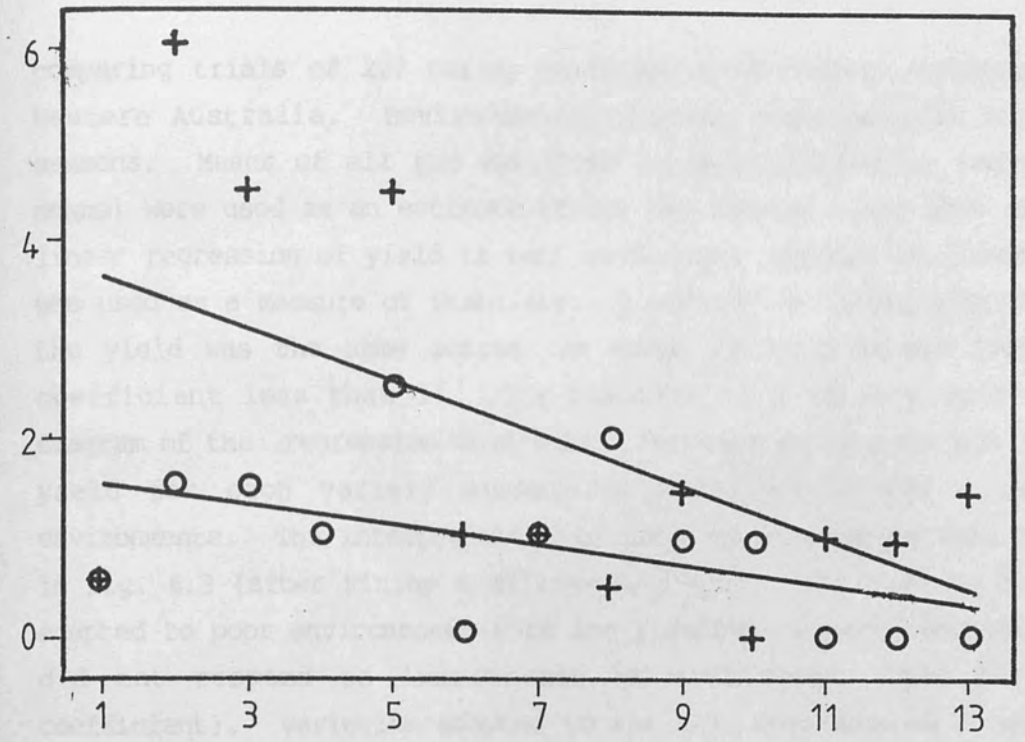


Fig. 6.1 Number of lesions developed after 11 days (mean of two leaves) plotted against leaf position on plant.

\+ Plant 1 o Plant 2

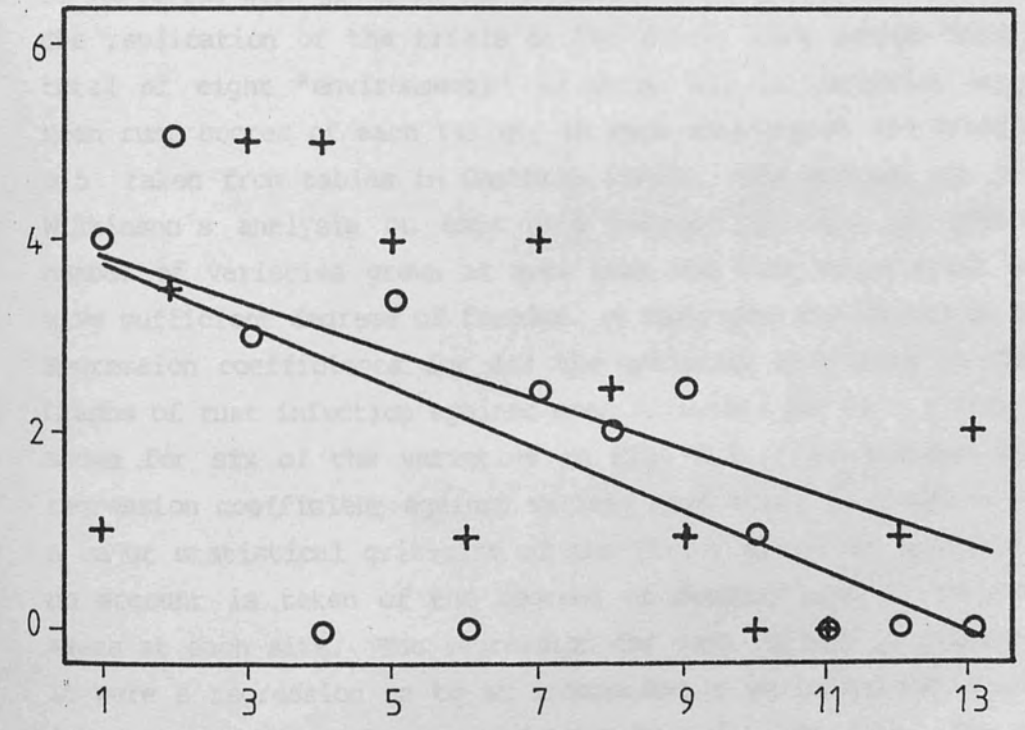


Fig. 6.2 Number of lesions developed after 20 days (mean of two leaves) plotted against leaf position on plant.

\+ Plant 1 o Plant 2

comparing trials of 227 barley varieties in different environments in Western Australia. Environmental changes were between sites and seasons. Means of all the varieties in each environment (environment means) were used as an estimate of the environment. For each variety a linear regression of yield in each environment against environment mean was used as a measure of stability. A variety was considered stable if the yield was the same across the range of environments (regression coefficient less than 1). The position of a variety on a scatter diagram of the regression coefficient for each variety against the mean yield for each variety summarized behaviour across a range of environments. The interpretation of this for the barley data is given in Fig. 6.3 (after Finlay & Wilkinson, 1963). For example, varieties adapted to poor environments were low yielding (variety mean small) and did not respond to improvement in environment (low regression coefficient). Varieties adapted to the best environments often failed altogether in poor environments, and therefore had low mean yields with high regression coefficients.

11 varieties were grown in the antirrhinum trials in both 1978 and 1979. The replication of the trials at two sites, each scored twice gives a total of eight "environments" in which all 11 varieties were grown. Mean rust scores of each variety in each environment are given in Table 6.5 taken from tables in Gawthrop (1980). She did not use Finlay and Wilkinson's analysis on this data because she did not consider the number of varieties grown at more than one site to be great enough to give sufficient degrees of freedom. I have used the method on her data. Regression coefficients for all the varieties are given in Table 6.6. Graphs of rust infection against mean infection for each environment are shown for six of the varieties in Fig. 6.4. The scatter diagram of regression coefficient against variety mean score is given in Fig. 6.5. A major statistical criticism of the Finlay Wilkinson analysis is that no account is taken of the degrees of freedom used in estimating the means at each site. The regression for each variety is presented as if it were a regression on to an independently estimated variable. This is not true: the means are estimated from the same data. The number of values estimated in this way is equal to the number of sites. Thus the residual degrees of freedom from the regression calculations should be reduced by this number. However there is no other constraint making

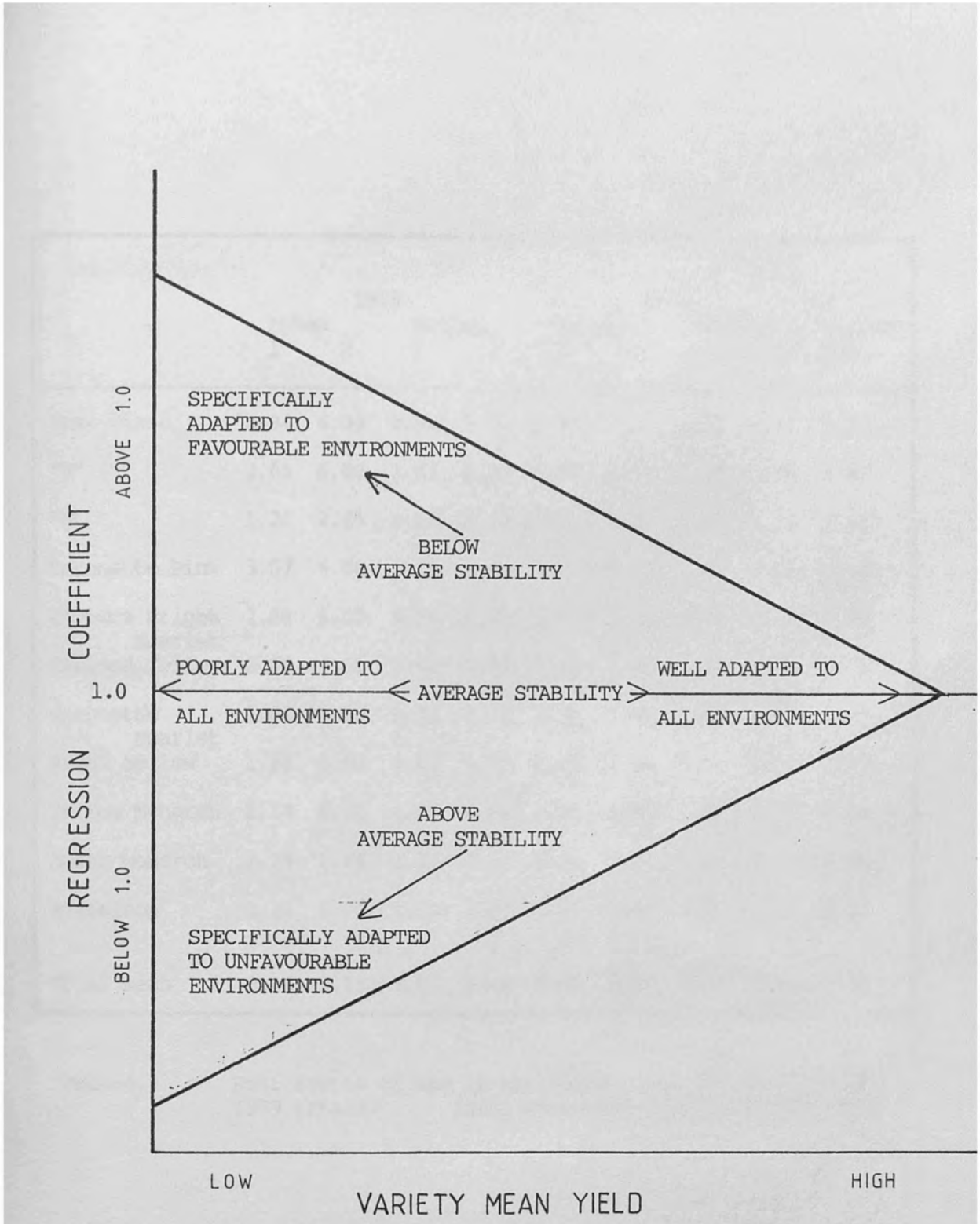


Fig. 6.3 A generalized interpretation of the variety population pattern obtained when variety regression coefficients are plotted against variety mean yields (after Finlay & Wilkinson, 1963).

	1978				1979				
	Egham		Wisley		Egham		Wisley		Variety
	1	2	1	2	1	2	1	2	Mean
Pink Pixie	3.94	6.00	2.90	5.00	1.04	1.37	1.80	2.94	3.12
"B"	2.65	6.00	3.37	4.92	1.36	1.95	1.69	2.56	3.06
"E"	1.36	2.65	1.57	2.14	1.01	1.21	1.24	1.51	1.59
Coronette Pink	3.57	6.00	3.11	4.90	1.38	2.21	2.59	3.61	3.42
Carioca Bright Scarlet	2.88	6.00	3.16	4.66	1.41	2.10	2.96	4.01	3.40
Carioca Yellow	2.60	4.65	2.57	4.08	1.13	1.64	2.21	3.21	2.76
Coronette Scarlet	1.81	6.00	2.31	4.05	1.34	2.12	1.86	2.32	2.73
Regal Yellow	1.68	6.00	3.85	5.00	1.09	2.18	1.74	2.72	3.03
Yellow Monarch	2.14	6.00	4.04	6.00	1.07	1.84	1.70	3.15	3.24
Amber Monarch	1.25	1.64	2.81	3.14	1.04	1.09	1.15	1.39	1.69
Malmaison	3.18	6.00	3.93	5.00	1.36	2.56	2.45	3.71	3.52
Trial mean	2.46	5.18	3.06	4.44	1.20	1.84	1.94	2.83	2.87

Table 6.5 Rust scores of the 11 varieties grown in both 1978 and 1979 trials. (Data extracted from Gawthrop, 1980)

	correlation coefficients										
Pink Pixie											
"B"	0.94										
"E"	0.93	0.99									
Coronette Pink	0.98	0.95	0.96								
Carioca B S	0.91	0.92	0.96	0.97							
Carioca Yellow	0.95	0.93	0.95	0.99	0.99						
Coronette S	0.86	0.95	0.98	0.91	0.92	0.89					
Regal Yellow	0.85	0.97	0.96	0.89	0.90	0.89	0.93				
Yellow Monarch	0.88	0.97	0.95	0.91	0.90	0.92	0.89	0.98			
Amber Monarch	0.51	0.61	0.52	0.48	0.45	0.53	0.40	0.67	0.75		
Malmaison	0.94	0.97	0.97	0.97	0.96	0.97	0.91	0.96	0.96	0.60	
Trial mean	0.95	0.99	0.99	0.97	0.96	0.87	0.94	0.97	0.98	0.62	0.99
	1	2	3	4	5	6	7	8	9	10	11

	VARIETY NAME	regression coefficients ESTIMATE	S.E.	r
1	Pink Pixie	1.24	0.17	0.95 **
2	"B"	1.20	0.07	0.99 **
3	"E"	0.40	0.03	0.99 **
4	Coronette Pink	1.07	0.10	0.97 **
5	Carioca Scarlet	1.04	0.13	0.96 **
6	Carioca Yellow	0.85	0.09	0.97 **
7	Coronette Scarlet	1.08	0.16	0.94 **
8	Regal Yellow	1.26	0.13	0.97 **
9	Yellow Monarch	1.40	0.12	0.98 **
10	Amber Monarch	0.38	0.20	0.62 ns
11	Malmaison	1.09	0.06	0.99 **

Table 6.6 Values of correlation and regression coefficients of variety score against environment mean for the 11 control varieties grown in both 1978 and 1979.

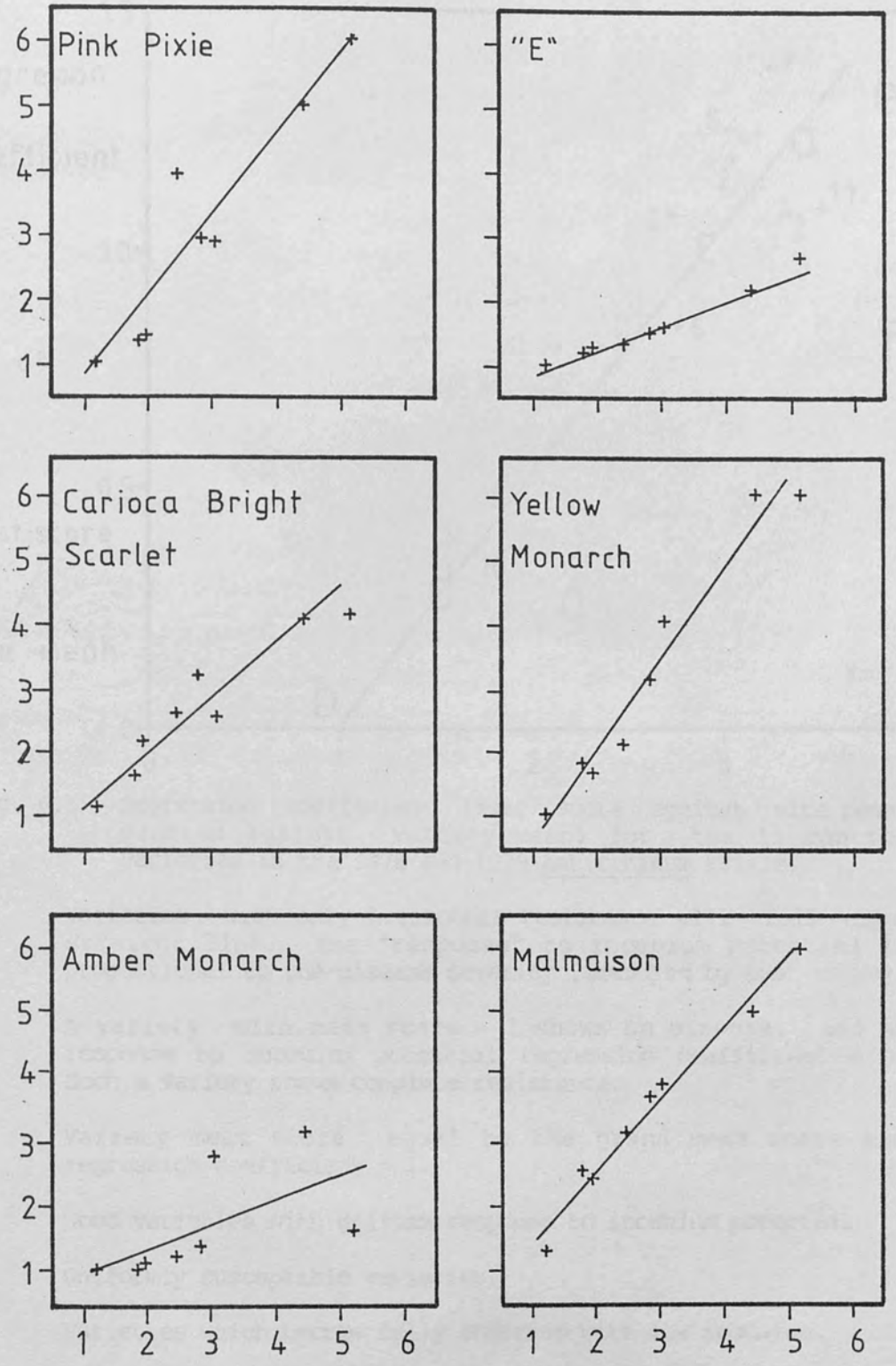


Fig. 6.4 Graphs of mean rust infection for each of six of the control varieties against trial (environment) mean score.

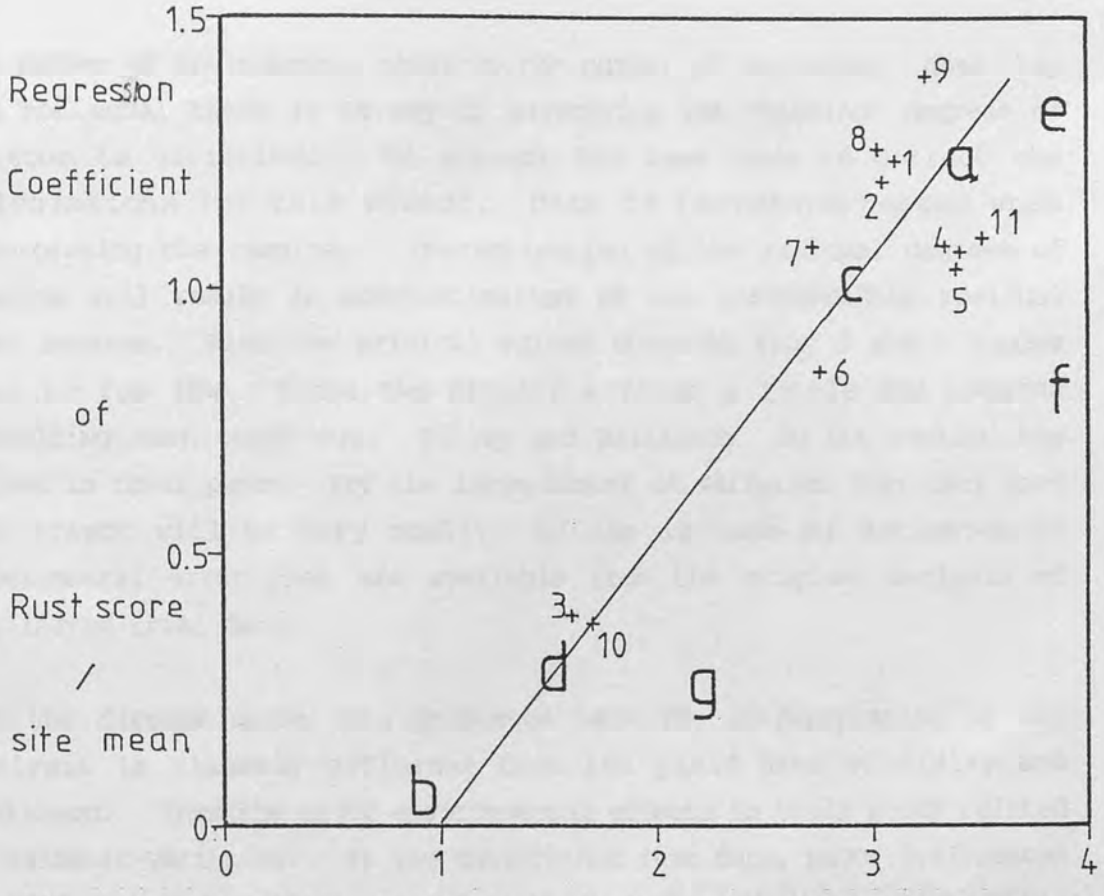


Fig. 6.5 Regression coefficient (rust score against site mean) plotted against variety mean, for the 11 control varieties in the 1978 and 1979 Antirrhinum trials.

a) Varieties with only horizontal resistance will fall in a straight line. The "response" to inoculum potential is proportional to the disease severity (measured by rust score).

b) A variety with mean score = 1 shows no disease, and no response to inoculum potential regression coefficient = 0. Such a variety shows complete resistance.

c) Variety mean score equal to the grand mean score and regression coefficient = 1.

d) Good varieties with uniform response to inoculum potential.

e) Uniformly susceptible varieties.

f) Varieties which become fully infected with low inoculum.

g) Varieties with both high and low scores unrelated to general means. Varieties with vertical resistance may fall in this category.

Varieties:

1)	Pink Pixie	5)	Carioca Scarlet	9)	Yellow Monarch
2)	"B"	6)	Carioca Yellow	10)	Amber Monarch
3)	"E"	7)	Corinette Scarlet	11)	Malmaison
4)	Coronette Pink	8)	Regal Yellow		

the number of environments equal to the number of varieties. When they are not equal there is no way of allocating the "missing" degrees of freedom to varieties. No attempt has been made to correct the calculations for this effect. Care is therefore needed when interpreting the results. Overestimation of the residual degrees of freedom will result in underestimation of the corresponding residual mean squares. Also the critical values obtained from f and t tables will be too low. These two effects will go a little way towards cancelling each other out. Finlay and Wilkinson do not mention the effect in their paper. For the large number of varieties that they used the effect will be very small. No use is made of estimates of experimental error that are available from the original analysis of replicated trial data.

For the disease score data presented here the interpretation of the analysis is slightly different from the yield data of Finlay and Wilkinson. Thus the major environmental effects in their study related to climatic variation. In the antirrhinum rust data, major differences in environment are likely to represent inoculum potential and possible differences between rust strains. The theoretical interpretation of the regression coefficient against varietal mean graph is also slightly different. This is emphasized by the form of the actual results (Fig. 6.5). There is a strong trend towards a linear relationship between regression coefficients and variety means. This is because both are related measures of resistance. The variety mean score is the measure that has usually been used to present the results of the antirrhinum trials. The regression coefficient is in effect a measure of response to inoculum potential, and thus a measure of resistance. A strong relationship between regression coefficients and variety means in Fig. 6.5 is therefore to be expected from this data. It is also a feature of the analysis (related to the missing degrees of freedom discussed above) that random data would show some tendency towards such a relationship.

Nine of the 11 varieties were very similar in this analysis. Two varieties, "E" and Amber Monarch stand out as different from the others. (Figs. 6.4 & 6.5)

The nine similar varieties had similar amounts of rust infection in each trial, and because they constitute most of the data, correlation between rust development in each environment and average rust development in that environment is high and the regression coefficients are close to one.

Variety 3 ("E") is strongly correlated with the means like the other varieties. The difference is that the regression coefficient and mean score are much lower. This is the effect that would be expected of horizontal resistance. Increased inoculum potential (as measured by rust infection of the other varieties) has given only a slight increase in rust infection.

For variety 10 (Amber Monarch) the correlation with the site mean score is less than for any of the other varieties. In six of the eight environments, Amber Monarch shows resistance similar to that of "E", but in two environments (both the scorings of the 1978 trial at Wisley) Amber Monarch has a higher level of infection. This can be explained as the effect of vertical resistance, and is what would be expected if a strain of rust able to attack this variety was present in that one trial only. The environment mean scores do not measure the effective inoculum potential for this variety. This conclusion is not dependent upon exact estimates of confidence limits, and so is not heavily influenced by the problems in the allocation of degrees of freedom outlined above. Thus comparison of these control varieties indicates that rust strains were not uniform throughout the 1978 and 1979 trials.

6.7 DIFFERENCES BETWEEN RUST ISOLATES

Rust isolates were cultured from infected antirrhinum leaves from different parts of England and from the experimental plots. The maintenance of rust isolates followed the procedures of Gawthrop (1980). Plants of the rust susceptible variety Malmaison were grown in pots in a separate section of the glasshouse to act as host plants for the rust isolates. Individual plants were sprayed with distilled water and the infected leaves from the rust source rubbed against the underside of the leaves. The plants were covered with plastic bags to maintain a high

humidity for the optimum germination of rust spores. The next day the plastic bags were removed and the plants covered with "incubation tubes" to contain the rust spores and to prevent contamination from other isolates. (Plate 16) Rust lesions developed after approximately two weeks in the summer or four weeks in the winter. Rust isolates were maintained by subculture onto new plants.

The tubes were made from thin sheet plastic joined with "UHU" glue to form cylinders 600mm high fitting tightly around the pots. The top of the tube was covered with muslin. Two holes covered with fine mesh (pore size 0.25mm) provided ventilation.

For the inoculation of test leaves, spores were collected from the plants using a Quickfit cyclone driven by an electric vacuum pump. The cyclone was fitted onto 100ml flasks containing a small amount of water. The spores collected in the water and were kept in suspension by mounting the flasks on a mechanical shaker until the suspension was needed.

Inoculations of leaves in petri dishes were made using a Quickfit glass venturi sprayer driven by a hand squeezed rubber bulb. Care was taken to give each test dish similar levels of inoculum. Inoculum density was checked by spraying sections of oxoid micropore filter paper and counting spores in the marked squares.

Detached leaves were placed with their upper surface on sterile 0.4% agar in petri dishes. The lower surfaces of the leaves were inoculated with rust, and then the dishes turned over. The leaves were held up by surface tension and the adhesive properties of the agar. The leaves were therefore the normal way up during incubation, and the lower surface exposed to air kept humid by evaporation from the agar surface. Detached leaves survived for some weeks in these conditions. Some leaves developed callous tissue at the detached end of the petiole. In a few cases roots grew from the callous tissue.

An alternative method was tried which more closely followed the methods of Gawthrop (1980). This was to place the leaves on plastic mesh floating on distilled water in a petri dish. This method was found to

give less satisfactory results and was discontinued.

Inoculated leaves were placed in a cold room maintained at about 10 Celsius for the first 24 hours. They were then kept on a laboratory bench until rust developed or the leaves died.

Plants used in these experiments were chosen to cover a wide range of rust-resistance from diverse sources.

The result of the first isolate tests are shown in Table 6.7. Two of the antirrhinum accessions were tested on both agar and water. The leaves suspended over water did not live as long as those on agar. Dishes filled with agar were therefore used in all the later experiments.

The three antirrhinum accessions at the top of Table 6.7 did not differentiate between the rust isolates. As expected Malmaison leaves were susceptible to all three rust isolates. The breeder's line 79-103 is probably to be considered moderately susceptible to all three isolates, although the infection level was low and uneven. This may have been caused by the physiological condition of the leaves not being right for rust development. 78-117 developed no rust.

The three accessions at the bottom of Table 6.7 differentiated between all three rust isolates. Two of the accessions developed rust when inoculated with the two isolates from Norfolk but not when inoculated with the isolate from Surrey. Accession 78-111 developed rust from only one of the Norfolk isolates, and so provided a differential between them.

The results of the second rust isolate experiment are given in Table 6.8. The four rust isolates used were produced by inoculation with spores from single lesions collected from the trial ground at Egham in December 1981. There are some missing combinations in this data caused by leaves dying or succumbing to moulds before the rust developed. The information is summarized in Table 6.9, which treats each rust isolate/plant combination as rust developed, rust not developed or uncertain. Combinations which developed only a few rust lesions were

Plate 16. Plastic bag and inoculation tube used to maintain rust isolates.

Test variety	<a>	Source of rust isolate: Egham Surrey				Mileham Norfolk				Litcham Norfolk				Summary
78-32	A	0	7	1	3	10	3	4	2	6	8	5	1	+ + +
Malmaison	A	4	0	6	3	4	9	3	7	9	4	5	5	
	W	1	0	0	0	2	2	3	0	4	0	0	0	
	W	0	0	0	1	1	4	*	*	0	0	0	0	
79-103	A	0	0	1	1	0	6	0	0	0	0	0	0	+ + ?
breeder's line	A	0	0	3	*	2	3	1	0	2	0	0	0	
	W	*	1	0	1	0	*	*	0	*	0	0	0	
	W	0	0	0	0	*	0	0	*	*	3	0	0	
78-117	A	0	0	0	0	0	0	0	0	0	0	0	0	- - -
A.asarina L.	A	0	0	0	0	0	0	0	0	0	0	0	0	
78-198	A	0	0	0	0	5	0	0	3	1	0	0	5	- + +
Flame Frontier	A	0	0	0	0	5	2	2	1	3	4	0	0	
78-111	A	0	0	0	0	2	2	0	1	0	0	0	0	- + -
garden plants	A	0	0	0	0	1	0	0	0	0	0	0	0	
origin	A	0	0	0	0	0	1	0	3	1	3	1	2	- + +
unknown	A	0	0	0	0	0	3	5	0	0	0	0	2	

Table 6.7 Number of rust lesions developed on detached leaves of plants of six antirrhinum varieties inoculated with three isolates of antirrhinum rust. Four leaves of each variety were placed in a petri dish. Each variety/isolate combination was replicated in two dishes with four leaves in each dish.

<a> Leaves of all varieties were placed in dishes containing 0.4% agar. Additional replicates of two of the varieties were also supported over water. This is indicated by "A" or "W" in this column.

Test Plant	Source of rust isolate							
	81-48-9		81-50-6		81-36-10		81-36-5	
Malmaison	+	2	+	3(6)	+	(4)	0	3(5)
81-44-5	0	0	0	0	2	1	0(4)	3
81-49-2	+	+	+	+	+	+	M	+
81-42-9	2	2	0	0	2	2	7(+)	2(5)
Malmaison	+	+	+	+	2(4)	6(8)	0	1
81-33-2	0	+	0	0	0	0	+	+
81-41-6	0	2	2	0	0(1)	0	3(+)	+
81-35-3	0	0	1	0	1	1	4(+)	+
Malmaison	+	7	+	2	0	3(7)	0(2)	1
81-48-9	+	+	10	M	0	M	0	1(2)
81-50-6	2(+)	0(+)	+	M	+	+	+	+
81-48-9	M	M	+	+	M	M	+	+

Table 6.8 Number of rust lesions developed on detached leaves of plants of ten antirrhinum varieties inoculated with four rust isolates collected from the trial at Egham in 1981. The information is summarized and interpreted in Table 6.9.

+ indicates 10 or more lesions.
Brackets indicate number of yellow spots where this is greater than the number of developed lesions.
M indicates missing values caused by leaves which died or succumbed to moulds before rust developed.

Test Plant	Source of rust isolate				Groups of rust isolates distinguished	
	81-48-9	81-50-6	81-36-10	81-36-5		
Malmaison	+	+	+	+		
81-44-5	-	-	+	+	(1 & 2)	(3 & 4)
81-49-2	+	+	+	+		
81-42-9	+	-	+	+	(1, 3 & 4)	(2)
81-33-2	+	-	-	+	(1 & 4)	(2 & 3)
81-41-6	+?	?	?	+		
81-35-3	-	+?	+?	+		
81-48-9	+	+	-?	+?		
81-50-6	+	+	+	+		
81-48-9	?	+	?	+		

Table 6.9 Summary of information from Table 6.8

\+ rust developed - no rust developed
? uncertain result

The plants of 81-44-5 and 81-33-2 together allow all four rust isolates to be distinguished.
81-42-9 distinguished 81-50-6 from the others.

considered uncertain. Three of the test plants allow all four rust isolates to be distinguished. One test plant (81-35-3) confirms the differences between two of the isolates but is uncertain for the other two. The rust isolates distinguished three (or possibly four) different combinations of resistance in addition to the universal susceptible among the test plants. Four different rust isolates could theoretically distinguish 16 test plant types including universal resistant and susceptible types.

It was not possible to repeat the individual tests sufficiently to demonstrate that these results are repeatable. If the results are not repeatable using the same isolates and plants, then the value of these results is small. However there does appear to be a wide range of variation in both host and parasite.

Problems arose mainly in the maintaining of rust isolates which did not seem to retain their vigour in the glasshouse. It is noteworthy that although the rust isolates in their tubes were kept in a section of the glasshouse adjoining the section in which the Malmaison rust-susceptible plants were grown, only one rust lesion was found on the glasshouse plants during the entire project. Rust development may be helped by fluctuating environmental conditions, especially temperature. The glasshouse environment may have been more stable than the ideal environment. Humidity was probably higher than optimum during the daytime in the glasshouse. Humidity in the inoculation tubes was even higher, and yet the thermal insulation of tubes and glasshouse prevented heavy dew forming on the plants. Free moisture is necessary for spore germination and infection.

The results of testing the leaves of different ages show that rust does not always develop when a healthy leaf of a susceptible plant is inoculated.

6.8 DISCUSSION

The negative results of the test for an alternate host are not surprising but need to be interpreted with great care. The plants were

growing out of their native environment and the method of overwintering and inoculating the spores artificial. If the normal life cycle was that teliospores germinate and produce basidiospores in the spring and the basidiospores are dispersed to an alternate host then infections of the alternate host would be widely scattered and difficult to find. The inoculation technique was used in order to produce a higher level of infection in a known place where it could be observed. The fact that no infection was observed could be due to the conditions and timing of the experiment being unsuitable for the development or observation of the hypothetical stages of the P. antirrhini life cycle.

Between 1978 and 1983, six antirrhinum rust trials were conducted on the same land at the Botanic Supply Unit. Rust epidemics became less severe and occurred later in the season in the later trials, probably because the level of rust-resistance was higher. This suggests that there was no overwintering of inoculum on or near the site. It is unlikely that spores survived the winters on the soil or on trees and shrubs nearby.

The infectivity of the plants overwintered in pots demonstrates that Puccinia antirrhini can survive the winter in the urediniospore cycle on its normal host, Antirrhinum majus, in Southern England. The continued appearance of rust lesions on plants from which infected leaves were removed suggests that the latent period of development between infection and the rupture of the epidermis can be greatly extended in the winter months. Some of this effect must be due to the lower temperatures slowing metabolism and growth. Inoculated plants in the glasshouse normally took two weeks to develop rust lesions in the summer and four weeks or more in the winter. However, the sudden appearance of some larger infected areas on the plants brought into the heated glasshouse suggests that the normal development of lesions may be delayed by a regulated extension of the latent period. This would allow the rust to overwinter as mycelium in leaves which show no visible signs of rust infection. Winter conditions are not suitable for the normal life cycle. Temperatures are well below optimum for infection. The broken epidermis of sporulating lesions leads to the death of surrounding tissue and therefore of the lesion itself. Sporulating lesions are thus not very viable in winter conditions but there would be selective advantage in extending the latent period of development.

Antirrhinum plants frequently overwinter in private gardens and such plants often have a small amount of rust infection. The low level of infection can be explained in terms of the age of the leaves of such plants. Also as isolated plants only a small proportion of the spores produced will germinate on suitable host tissue (see Chapter 7 below). Thus these plants often persist for a long time with a low level of rust, and a few flowers. These plants can provide ideal conditions for antirrhinum rust to overwinter.

The seedling inoculation experiment was undertaken to test the possibility of testing plants for rust-resistance at an early seedling stage. The differences of infection of seedlings of the same variety in the same pot shows that the method used was not suitable for this purpose. This may be due to uneven distribution of the inoculum. In the experiments inoculum was applied as a fine spray and allowed to settle on the plants. Further development of the technique would require the development of a more uniform application technique.

It is uncertain to what extent the experiment shows a higher level of resistance in the younger plants. For all the plants there was a period of growth between inoculation and the appearance of the disease. No measure of the size of the plants (number of leaves) was made at inoculation, so there is no way of correcting the data to give "number of lesions per leaf inoculated". For detailed comparison of the age groups an expression of rust per unit area would in any case be more satisfactory.

The experiment comparing leaves of different ages from mature plant provides clearer evidence of a change in rust-resistance with age. It is possible that the observed effect was caused by physiological differences affecting the survival of leaf tissues of isolated leaves in petri dishes. The rust fungi are obligate parasites living in very close association with the host at the cellular level. It is to be expected that the rust development will be affected by the host physiology, including both natural changes in the ageing leaf, and artifacts of the in-vitro experiment. The experimental results are in broad agreement with experience in the field. Rust epidemics are

commonly observed to start at about the time of flowering. At this time, the plants have the maximum number of young and photosynthetically active leaves. When the plants are examined rust lesions in the top half of the plant are often sporulating more freely than those on old leaves at the bottom of the plant.

The works cited above and in Chapter 2 give a very strong indication that P. antirrhini on the cultivated antirrhinum is far more variable than the designation of physiologic races 1 and 2 might suggest. The behaviour of control varieties (Section 6.6) and rust isolates (Section 6.7) suggests that there were a number of different rust strains present in the antirrhinum trials. The exact nature of this variation has not been adequately demonstrated, but is probably similar to other rust species where the interaction of host and parasite genomes are better understood and the experimental systems of isolate testing more developed. The range of variation within P. antirrhini populations cannot be reliably assessed until a number of experimental procedures have been perfected. Until that is done all results are no more than an indication of what is waiting to be uncovered. Problems in the following three areas have contributed to the unreliability of the results on rust isolates reported above:

1. A method is needed of maintaining healthy rust cultures.
2. The test leaves should all be young, quick-grown, and of uniform size.
3. A better inoculation and incubation method is needed.

The problems of maintenance of rust cultures are unexpected in such a rapidly multiplying disease. It may be that temperature and/or humidity in the glasshouse were too uniform for the rust to complete all stages of the urediniospore life cycle. The humidity in the isolation tubes was higher than in the glasshouse, and the glasshouse tended to be too humid for the optimum growth of antirrhinums. High humidities favoured moulds and mildews. Infection with these probably reduces the success of the rust, while their spores would be carried from culture to culture with the rust inoculum.

Although test plants were multiplied by cuttings, this did not produce the rapid growth of new leaves that would be needed for a comprehensive

testing programme. New growth following removal of leaves or stems did not have the vigour of young seedling plants, while there is no certainty that seedling plants of commercial varieties would be uniform in their response to differential rust isolates. In the field trials some commercial varieties produced colour off-types as frequently as one plant in thirty five. This could be the result of contamination in the seed production stage, or residual variation from the original breeding. In either case, if the varieties are non-uniform in the most important horticultural quality they are likely to be non-uniform in physiological qualities affecting rust development.

The incubation of rust on detached leaves can work only if the leaves survive longer than the time taken for the rust to develop visible symptoms. This condition is just met by healthy leaves. The high humidity needed to keep the leaf alive had the disadvantage of encouraging the growth of saprophytic organisms. A technique using inoculations onto individual leaves of whole plants might be better if sufficient containment of rust spores could be achieved. Inoculation of whole plants might be possible, but a large number of plants would need to be grown from cuttings to provide the necessary test material. More inoculum might also be needed.

6.9 CONCLUSIONS

There is still a lot that is not understood about the physiology of Puccinia antirrhini. The teliospores produced in the autumn may or may not be functional, but in any case are not necessary for survival in southern Britain where the rust is able to overwinter by a lengthening of the latent period of urediniosorus development. In the winter the development of the rust is very much slower than in the summer. This could be simply the direct effect of low temperatures and the reduced physiological activity of the host. However, a mechanism in the rust that slowed development at this time would probably be advantageous as a means of overwintering. Sporulating lesions are rendered liable to desication and insect damage through the ruptured leaf epidermis.

There appear to be changes in susceptibility to rust associated with the

age of plants and individual leaves. These changes could reflect changes in the defence mechanisms of the host, or may simply be the effect of changing nutritional status.

The full range of variability in the rust is not known. Field trials have all shown a range of responses of antirrhinum varieties to rust epidemics. Cultivated A. majus has a considerable genetic variation in its response to rust. There are many indications that P. antirrhini is equally variable. This variation may exist in the form of a number of rust and host genotypes with all susceptible and resistant interactions between them. However, it seems likely that most of the variation is in degrees of resistance and virulence. Thus even in the original major gene conferring resistance to rust "Race 1" the resistance was only complete when a suitable genetic background had been selected. Experiments to explore the range of host-pathogen interactions may need to compare different degrees or probabilities of disease symptoms developing. To obtain useful results in such experiments will require great care in maintaining uniform conditions. In particular, the interaction with physiological condition of the host tissue is difficult to control experimentally. Until the system is better known it would seem unwise to assume that seedling plants of a cultivated variety are of uniform rust-resistance genotype.

CHAPTER 7
EPIDEMIC DEVELOPMENT

7.1 INTRODUCTION

Computer simulations of plant pathosystems have been made for two distinct purposes:

1. To provide accurate forecasts of epidemic development for the management of particular plant diseases.

2. To improve our understanding of epidemic development.

Accurate forecasting has been attempted for a number of pathosystems. (Potato blight Blightcast; maize) Such forecasting involves the accurate determination of the response of the disease to many environmental factors. Waggoner and Horsfall (1969) undertook a long series of experiments on Alternaria solani spore germination in the development of EPIDEM, their computer program to forecast early blight epidemics of tomatoes and potatoes. Using EPIDEM they were able to model the effect that chemicals affecting sporulation might have on a real epidemic. They were also able to model the effects of different weather patterns. An accurate prediction program of this type needs to be based on a detailed knowledge of the effects of environmental conditions at each stage in epidemic development. Measurements of the environment (weather, initial inoculum, microclimate and host development stages) for particular crops or regions can be used to predict epidemic development and to help growers with management decisions. The main factors affecting multiplication of an aerially dispersed plant pathogen are summarized in Table 7.1.

Other simulation programs have been written to help understand the process of epidemic development and the effects that might occur. An intuitive approach may indicate some of the effects that can occur when parameters are changed. (For example, plant breeders and growers alike are aware of the dangers of monoculture and growers may seek to avoid them by rotation.) It is often difficult to anticipate the effects of

Stage in parasite life cycle	Location	Factors affecting further development
spore germination	leaf surface	humidity and temperature in the canopy microclimate
penetration of host	leaf surface and stomata	humidity, temperature, stomatal opening
establishment of infection	leaf tissue	host-pathogen interaction
development during the latent period	leaf tissue	host vigour, host-pathogen interaction
spore release	leaf surface	canopy microclimate - windspeed, humidity, temperature, light
length of infectious period		host vigour, host-pathogen interaction
short range dispersal	air, water film	weather, microclimate within the leaf canopy, host structure
long range dispersal	air above canopy	weather, especially wind and rain

Table 7.1 Factors affecting epidemic development during the life cycle of an airborne pathogen. (original)

any particular management practice and for accurate prediction experience from field conditions is needed. However it is not necessary to know all about every possible effect in order to consider the general effect of varying a few of the conditions. In this area the use of computer simulation can help to sort out what factors might effect epidemic development in real situations. This can help in the development of the theory of epidemiology. An example of the use of computer simulation for the study of epidemiology is the program EPIMUL 76 used by Zadoks and Kampmeijer (1977).

7.2 FIELD OBSERVATIONS

Observations made on the field trials (see Chapter 3 above) will be used in this chapter.

The importance of size and location of plots of host is indicated by the following observations:

1. In 1982 a small plot of the variety Yellow Monarch was grown in an arable field in Norfolk. The plot was about half a mile from the nearest village. Plants were raised from seed at the Botanic Supply Unit with the plants for the main trial and were planted in June 1982. Yellow Monarch is a rust susceptible variety and was used in the spreader rows of the 1982 trial (Chapter 3 above). The plants on this small isolated plot did not develop any rust.

2. Where a few plants are grown in gardens they commonly survive for a long time, often showing low levels of rust infection, and yet continuing to flower.

3. Large displays (and trial plots) are subject to severe epidemics.

7.3 COMPUTATION METHODS AND MODELS

In order to simulate the progress of disease epidemics, a program "EPIGAR" was written in FORTRAN 77 to run on the VAX computer at the

Royal Holloway College Computer Centre. Selected data produced by EPIGAR is saved on to disc, and plotted by the program "EPIPLOT". Epiplot uses the CALCOMP graphics subroutine library to drive a plotter. Full listings of EPIGAR and EPIPLOT are given in Appendices 8 and 9. Some of the variables used, the CALCOMP subroutines called, and an indication of their uses are listed in Appendix 10.

The model used in EPIGAR is based on a square array, with each element in the array representing one patch of host. In its inception, each element of the array is considered to represent a garden. If the model is considered at a different scale, then each patch might be considered as an individual plant or as one leaf. Zadoks & Kampmeijer (1977) developed their model to represent infection on individual plants, but also considered its application to other levels of the pathosystem.) In EPIGAR the size of the array is fixed as 11 X 11 elements. Initial inoculum is represented by one unit of inoculum in the centre element. Time is considered in discrete units. Simulation proceeded for 10 time units.

The hypothetical life cycle is represented in diagrammatic form in Table 7.2. Inoculum arriving at time T causes infection. The pathogen is considered to have a latent period of development of one time unit. The new infection is therefore ready to produce further inoculum in the next time unit (time = T + 1). The pathogen produces new inoculum for one time unit and then dies. Therefore in the third and subsequent time units, it is no longer active. It remains as part of the total disease within the element but does not contribute to the further development of the epidemic. The amount of active disease present is used as the measure of inoculum production.

Dispersal of inoculum between patches is modelled as a function of distance. The distance between elements in the same row or column is calculated from the number of elements. Distance between elements in different rows and columns is calculated using Pythagoras's theorem. The amount of inoculum moving from one element to another is taken to be inversely proportional to the cube of the distance:

$$\text{inoculum transferred} = \text{inoculum produced} / (\text{distance}^{3} * S)$$

The constant of proportionality or scaling factor (S) is one of the variables to be considered in the model. It is supplied as data to EPIGAR.

The dispersal function assumes that inoculum potential decreases with the cube of the distance from the source of inoculum. A distribution of this nature would be produced by the combination of spore divergence (inverse square) and spores settling out of the air mass (proportional to time and hence distance). The spreading out of spores as they disperse from the source of inoculum could be expected to follow the inverse square of distance. An additional effect with spore dispersal is that spores will be removed from the spore cloud. This removal can be the result of simple settlement out of suspension, of washing out by rain, or by impact on solid objects from a moving air mass. The proportion of spores removed in this way might be modelled as proportional to time. Time (in the air) will be proportional to distance travelled. Multiplying the inverse square (divergence) and linear (spore removal) effects would suggest the inverse cube relationship that is used in the model.

The scaling factor represents all factors determining spore transfer between elements. When S is large, the plots are effectively isolated. This factor was intended to represent the physical distances between host units. Simulation results using large and small values of S should be similar to the results of growing host plants at wide and close spacings. The scaling factor used in EPIGAR should be considered as proportional to the cube of the distance between host units.

Inoculum transfer is not calculated for the special case of transfer from an element to itself: the distance would be zero, and would result in the computational nonsense of division by zero. The sum of all possible inward transfers of inoculum is calculated for each element in turn using this formula, and added to the spore production within the element. This gives the inoculum potential within the element. Thus each element that contains active disease, contributes to the inoculum potential in every element of the array.

The new infection produced as a result of this inoculum potential is

calculated by one of two different growth models. The model to use is supplied to EPIGAR as data. The models are an exponential growth model and a logistic growth model. In each case the type curve is followed only approximately due to the discrete time units used in calculation.

For the exponential growth model:

$$\text{new infection} = \text{inoculum potential} * R$$

For the logistic growth model:

$$\text{new infection} = \text{inoculum potential} * R * \frac{(\text{maximum} - \text{present disease})}{\text{maximum}}$$

In both models R is the "multiplication factor", the rate of production of inoculum per unit of active infection and is supplied as data to EPIGAR.

The multiplication factor is easy to consider in terms of biological meaning. It is a measure of the average number of spores produced by each spore landing on the host. It will be affected by all climatic and biological factors affecting pathogen development from spore germination to spore release.

In the logistic model, the maximum disease level is another variable supplied as data. Negative values of this have no biological meaning and are used to signal that the exponential model is to be used. It is possible for the function to overshoot the maximum level set. This can happen when R is large or when there is a great influx of inoculum from other elements (ie when S is small). In order to prevent overshoot, the program compares the new infection level with the maximum. If the new level of total disease is greater than the maximum allowed, then the total disease is set to be equal to the maximum.

7.4 SIMULATION RESULTS

The graphical results of the simulation process, produced by the program EPIPLOT are shown in Figs. 7.1 to 7.12. The form of all these figures is the same and is explained in a caption common to them all.

The values of the parameters of the model for all the simulations reported are shown in Table 7.3.

1. Exponential model

In the absence of inoculum transfer between elements, setting R = 1 maintains a constant level of active inoculum. Thus with infinite S, the active pathogen would remain at the starting condition (1 unit in the central element, 0 everywhere else). The total pathogen in the central element would increase by one unit for each unit of time; and remain at 0 everywhere else. This condition is not shown in the results but Fig. 7.1 (with R = 1, S = 100) shows the results of a low level of inoculum movement between the elements. Results are similar to those described above. However, a low level of disease develops in the elements nearest to the initial inoculum. Return of inoculum from these elements to the centre results in a very slight increase of the active disease in the central element. From a starting value of 1, this has increased to 1.005 at time unit 10.

Lower values of the scaling factor S (more transfer of inoculum between elements) result in the development of epidemics. Notice from the highest line in Fig. 7.2c that after 10 time units with R = 1 and S = 10, the distribution of the pathogen is beginning to flatten out in the centre of the array. Fig. 7.2d shows that the elements near the centre are increasing at a similar rate to the central element after about 7 time units. This is because the increase in active disease is predominantely due to inoculum dispersal from the neighbouring elements, and therefore the inoculum potential is similar for all elements near the centre of the array. Under these conditions, inoculum production within an element has little effect on the rate of infection of that element.

With S further reduced to five (Fig. 7.3), the epidemic develops much faster. The final level of total pathogen in the central element is increased from 32.0 to 395, while the level of active pathogen is increased from 6.2 to 95.0. The pattern of distribution is much broader (compare Figs. 7.2c and 7.3c).

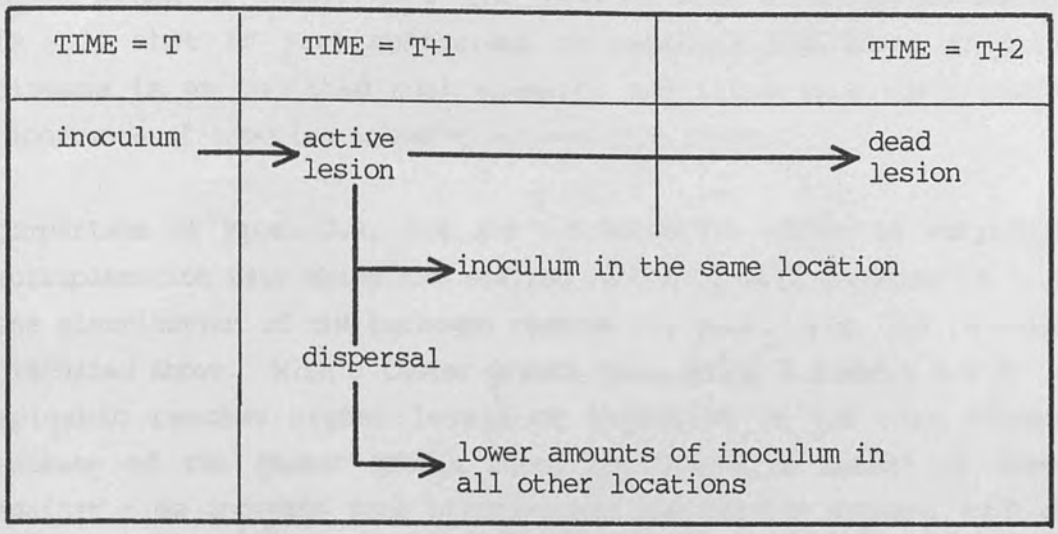


Table 7.2 Disease life cycle model used in the simulation program EPIGAR. Time is modelled in discrete units. The complete life cycle spans three of these time units, for infection, multiplication and death. Active lesions cause new infection within the same location, and a lesser amount of infection in all other locations.

	S	R	Maximum disease	Results in figure no.
Exponential model:				
	100	1.0	-	7.1
	10	1.0	-	7.2
	5	1.0	-	7.3
	10	2.0	-	7.4
	10	0.5	-	7.5
Logistic model:				
	10000	2.0	100	7.6
	10000	3.0	100	7.7
	10	2.0	100	7.8
	10	3.0	100	7.9
	5	1.0	100	7.10
	2	1.0	100	7.11
	1	1.0	100	7.12

Table 7.3 Values of parameters used with the program EPIGAR. The results are presented in Figs. 7.1 to 7.12. The meaning of the various parameters is explained in the text, section 7.3.

These different results are all obtained with a multiplication rate (R = 1) that is just sufficient to maintain the level of active disease in an isolated host element, and illustrate the potential importance of inoculum transfer between host units.

Comparison of Figs. 7.2, 7.4 and 7.5 shows the effect of varying the multiplication rate while the scaling factor is held constant (S = 10). The distribution of the pathogen remains the same. Fig. 7.2 (R = 1) is discussed above. With a faster growth rate, (Fig. 7.4 where R = 2) the epidemic reaches higher levels of infection in the time allowed. Because of the faster growth rate, the graphs of amount of disease against time increase more steeply, and the disease appears much more quickly over the entire area of the array.

Fig. 7.5 shows the decline of the pathogen under conditions unsuitable for disease multiplication. The pathogen has a period of increase in the cells of the array nearest to the initial inoculum, even though in the cell with the initial inoculum, the amount of active pathogen is halved after each time unit.

The only way in which the level of active inoculum can rise and then fall again with the exponential model, is the low level of disease activity surrounding the central element when the epidemic is not viable. This is shown in Fig. 7.5d. Under settings of the parameters more favourable to disease increase, but still not leading to long term viability, this transitory increase would become greater. The amounts of disease involved in this phenomenon must remain smaller than the initial inoculum. Therefore this cannot be considered as a prediction of the appearance and subsequent disappearance of plant disease in any real situation. If the inoculum was allowed greater mobility between elements (small S), then epidemics could be produced even with values of R less than one.

2. Logistic model

In all simulations on the logistic growth model, the maximum has been set to 100. Units of disease can therefore be considered as percentages

of the maximum possible.

Fig. 7.6 (R = 2, scaling factor large) illustrates simple logistic growth as simulated by this model. Comparison with Fig. 7.1 shows the difference between the logistic and exponential models. In both cases the scaling factor is large so that the epidemic is effectively isolated in the centre element. Notice (Fig. 7.6b) that the epidemic development slows down at about 80% of the maximum allowed by the model. At this point the epidemic is limited by lack of fresh host to infect. The associated reduction in the amount of active infection is shown in Fig. 7.6d.

Fig. 7.7 differs from Fig. 7.6 in that R is increased from 2 to 3. The result is that in T = 0 to 7 the active pathogen peaks more quickly in the centre element. From T = 7 onwards there is no active disease in the centre element. In T = 8 to 10 the pathogen is multiplying in the neighbouring elements. The first peak shown in Fig. 7.7d is the pathogen in the centre element. The second rise is the pathogen in the neighbouring elements. This second rise takes place in all the elements around the centre and is the beginning of a general epidemic.

The effect of allowing spread between elements is shown by Fig. 7.8 (S = 10, R = 2). Epidemic development in the centre element is faster although the multiplication rate is unchanged. In this case 80% of the maximum is passed by T = 6 while in Fig. 7.6, 80% is passed by T = 7. Neighbouring elements are accelerating the epidemic development in the centre element. The epidemic starts as an infected area with a peak at the centre element, but by time T = 8 there is little (6.3 units) active pathogen in the centre element. The highest activity is 3 or 4 units from the centre. At this stage the distribution of the disease is in the form of a ring around the original point of infection. By time T = 10, the epidemic has almost passed out of the area of the simulation array. Increasing the value of R causes all this to happen more quickly. This is illustrated by the comparison of Fig. 7.8 where R = 2; and Fig. 7.9 where R = 3. With R = 2, the centre element peaks in time unit 7, while with R = 3, the centre element peaks in time unit 5.

Figs. 7.10, 7.11 and 7.12 illustrate the effect of changing S at low values of R. For these three figures R = 1. Epidemic development is supported by the transfer of inoculum between elements (as for Figs. 7.2 and 7.3 under the exponential model). In these examples, the epidemic develops in a wider and less distinct focus than in Fig. 7.8, because of the less isolated nature of the elements. In Fig. 7.10 (S = 5) the epidemic is reaching its peak in the centre after 10 time units. In Figs. 7.11 and 7.12, the maximum is reached more quickly (7 and 5 time units respectively) and the distribution of the pathogen becomes more uniform across the array. The difference between the inner and outer elements is exaggerated in the model because of the effect of the edge of the array. If the size of the array was increased, then disease development outside the area of the present array would produce inoculum to be dispersed back into the area of the original area. This would increase the development at the edges of the original array, and further flatten the distribution curves.

7.5 DISCUSSION

For these models to be of any use in understanding the development of epidemics, they must be interpreted in terms of biological conditions. In all cases care must be taken that the model represents something like the real situation.

There is a choice of the biological unit of host that is considered as the basic unit of the model corresponding to one element in each array. This may be as small as the area of leaf surface occupied by a single disease lesion. It may be as large as a whole country. In developing the model the unit of most interest was the group of Antirrhinum plants in a garden. The changes in S would model the distance between gardens growing antirrhinums. When plants are self-sown the group may be the same epidemiologically as an individual plant. When they are planted as bedding out the group may represent a large border in a park or other public garden. Different groups may be of different sizes, and of different varieties. If the model is applied with elements representing single leaves, then differences in leaf age and physiology will become important (see Chapter 6). A limitation of the model is that all elements have the same properties. Differences between groups

of plants and of the spacing or age of plants within groups are not represented. This is one area in which the model could be improved to provide a more realistic simulation.

Other physical and biological factors may also be important in determining epidemic development. The detailed factors to be considered will depend on the level of host structure that is represented by each element of the arrays (see above). They will be mainly physical factors of climate and microclimate. Within crop canopies microclimate may be affected by the size and shape of the plants and by the planting density of the crop. Microclimate then becomes a biological factor, and one that is amenable to control by both the plant breeder and the grower. For example the drier microclimate within the canopy of leafless pea varieties reduces disease levels. Such alteration of the plant is unlikely to be acceptable in an ornamental, but the grower may have greater freedom in designing the arrangement of the plants. Thus if plants can be arranged in a larger number of smaller groups or if the individual plants could be spaced out then disease susceptibility should be reduced. The shape of a group of plants will also have an effect. If a given number of plants are grown in a singe row, spore transfer between them will be less than if they were grown in a more compact group.

1. Exponential model

The exponential model used here can only be considered as a very great simplification of any real situation. In particular this model contains no limits to the growth of the disease. Thus with combinations of the parameters that lead to disease development, the disease continues to increase indefinitely. It does provide a very simple theoretical environment in which the effects of inoculum dispersal and multiplication rate on the ability of the pathogen to flourish, can be considered.

With this exponential model, the epidemic can either develop indefinitely or else die out from the beginning if the parameters do not allow maintenance of active pathogen. A multiplication rate of one, and infinite scaling factor (ie no spread to other elements) would be the

only combination of parameters leading to no change in active disease.

The comparison of different values of the scaling factor while the multiplication rate is set at 0.5 (ie for an isolated element, halving of active disease after each time unit) illustrates the way in which the ease of spread can make the difference between an epidemic developing or fading out.

2. Logistic model

The difference between the two models is the incorporation of an upper limit to disease increase in the logistic model. In some of the conditions, disease increases rapidly to reach this limit. After this happens there is no further host tissue available to the pathogen. The active life of the pathogen is one time unit. If the total disease in a host element reaches the maximum level at TIME = T; then from TIME = T + 2 onwards there can be no active disease in that element. Thus the host can be considered as dead. This corresponds to the complete destruction of the Antirrhinum trials of 1978 and 1979 (Gawthrop, 1980). These trials contained many susceptible plants in close proximity. When the disease development is less rapid, it slows down before reaching the maximum. When this happens, there is some disease activity continued in the pathosystem. This is an indication of how a low level of disease can continue to exist in equilibrium with the host. The model does not allow for any replacement or growth of host tissue. There is therefore a gradually decreasing amount of host available to continuing infection. This corresponds to the partial destruction of the trials in 1982, when many plants were killed. If such growth was included in the model, it would add to the host available to this continuing disease. The level of disease could then be maintained at a higher level. It is interesting that under some conditions, a continued disease presence is predicted without such host growth.

The effect of mixing genotypes with different (vertical) disease resistance has not been included in these simulations. Other workers considering different pathosystems have concluded that this will also may a significant contribution to controlling plant disease.

(Borlaug, 1959; Wolfe, 1977)

Epidemiological considerations suggest that disease would be lessened if plants could be grown in small and widely spaced groups (or individual plants) and if genotypes are as diverse as possible for rust resistance. The natural populations of Antirrhinum fulfil these conditions.

Although the program EPIGAR was developed to model antirrhinum rust, both simulation results and the above discussion could apply equally well to other aerially dispersed diseases of plants grown in small groups.

7.6 CONCLUSIONS

Theoretical and highly simplified modelling can reproduce many of the features of the epidemiology of Puccinia antirrhini. It explains why the disease can be expected to be most severe when many plants are grown together. A few isolated plants are more likely to reach a stable situation, with a balance between host and disease.

The distance between gardens growing Antirrhinum can be important in controlling the level of disease in each of the gardens. In particular, isolated gardens will receive a very low level of inoculum early in the season. The probability of escaping the disease is therefore greater for these gardens and often the disease may not develop into a devastating epidemic until after the plants have flowered.

If a given number of plants are to grown in one garden, they will develop less disease if they are widely spread.

These conclusions suggest that the plants will do best if they are grown with a population structure that is closer to the situation of Antirrhinum in its natural habitat.

Figs 7.1 - 7.12

Graphs showing hypothetical disease development. Four graphs are produced for each set of parameters. Axes labelled "position" refer to a cross section through the centre of the simulation array. The 11 cells of the array are labelled from 0 to 10. Time is plotted in discrete time units from time = 1 to time = 10.
The "active pathogen" represents the amount of actively reproducing disease in a cell of the array. The "total pathogen" represents the total of active and dead pathogen in a cell of the array.

Scales for the X axes are the same for all figures.

Scales for the Y axes vary between figures.

Parameters of the model are:

disease multiplication factor	R
scaling factor	S
growth model type	exponential or logistic
maximum disease level	DISMAX (logistic model only)

The four graphs for each set of parameters are:

a) Total pathogen (Y axis) against position (X axis) plotted at TIME: 2 4 6 8 10	b) Total pathogen (Y axis) against time (X axis) plotted for elements: 1, 6 3, 6 4, 6 5, 6 6, 6
c) Active pathogen (Y axis) against position (X axis) plotted at TIME: 2 4 6 8 10	d) Active pathogen (Y axis) against time (X axis) plotted for elements: 1, 6 3, 6 4, 6 5, 6 6, 6

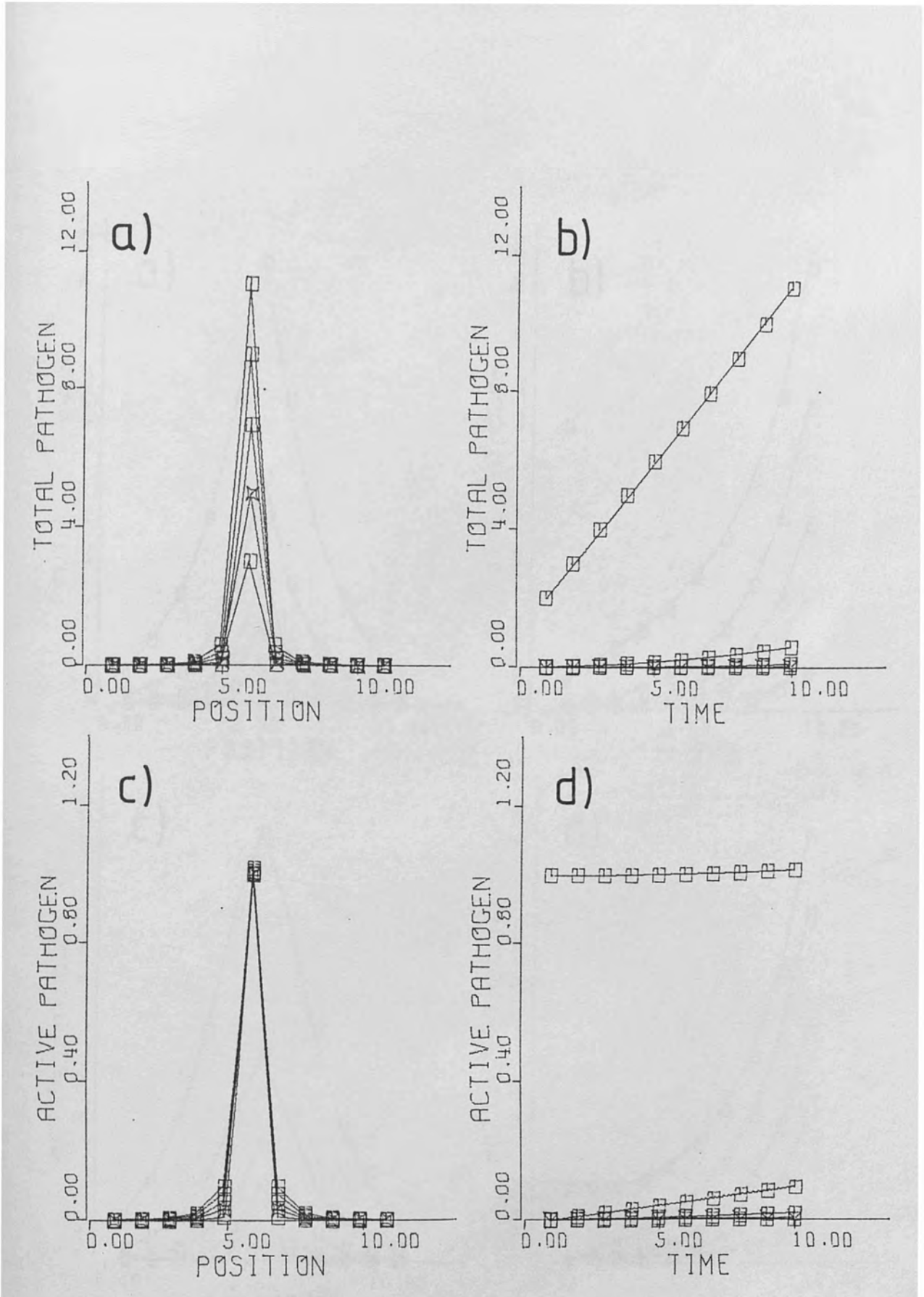


Fig. 7.1 Epidemic simulation using the exponential model.
S = 100 R = 1.0

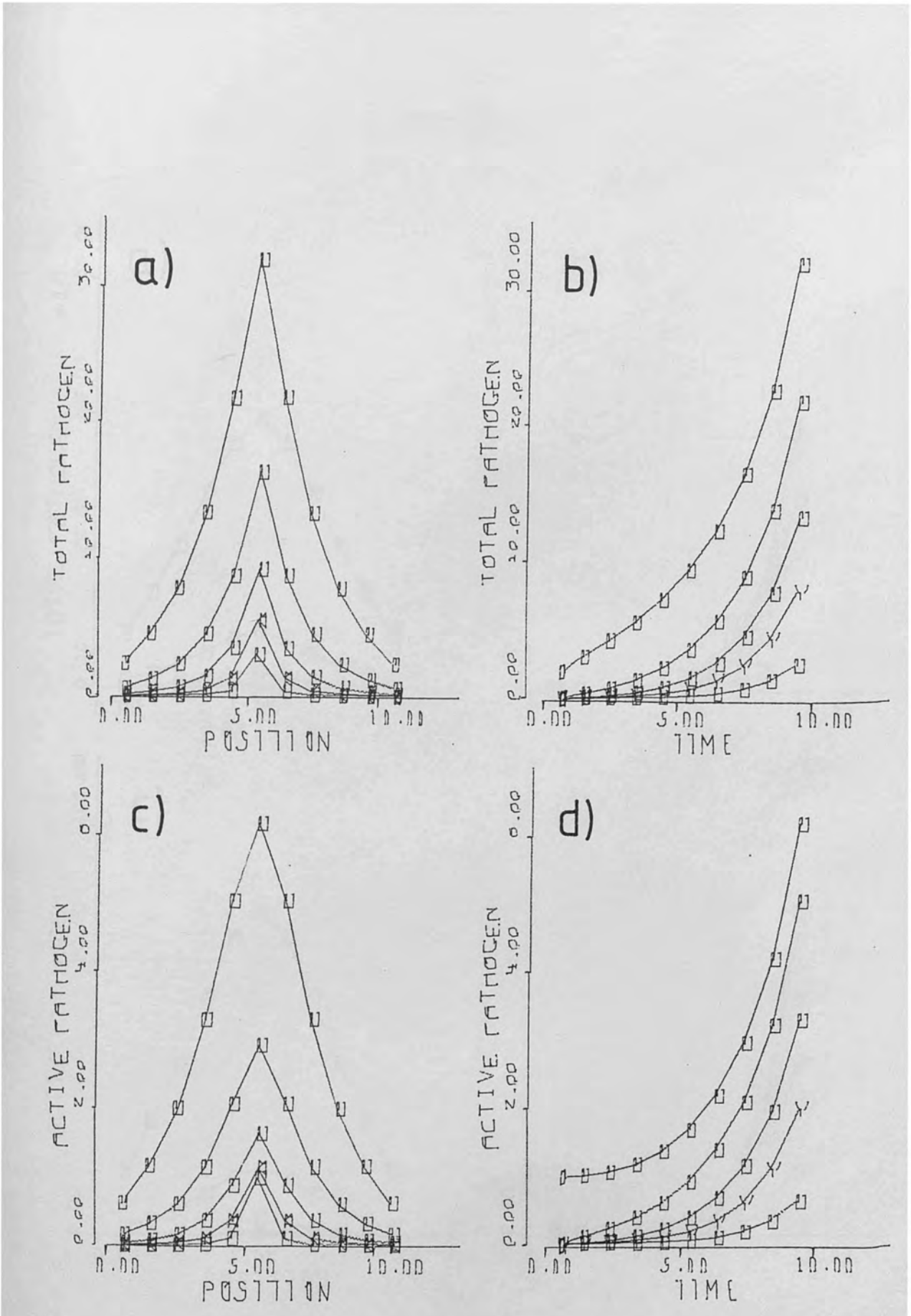


Fig. 7.2 Epidemic simulation using the exponential model.
S = 10 R = 1.0

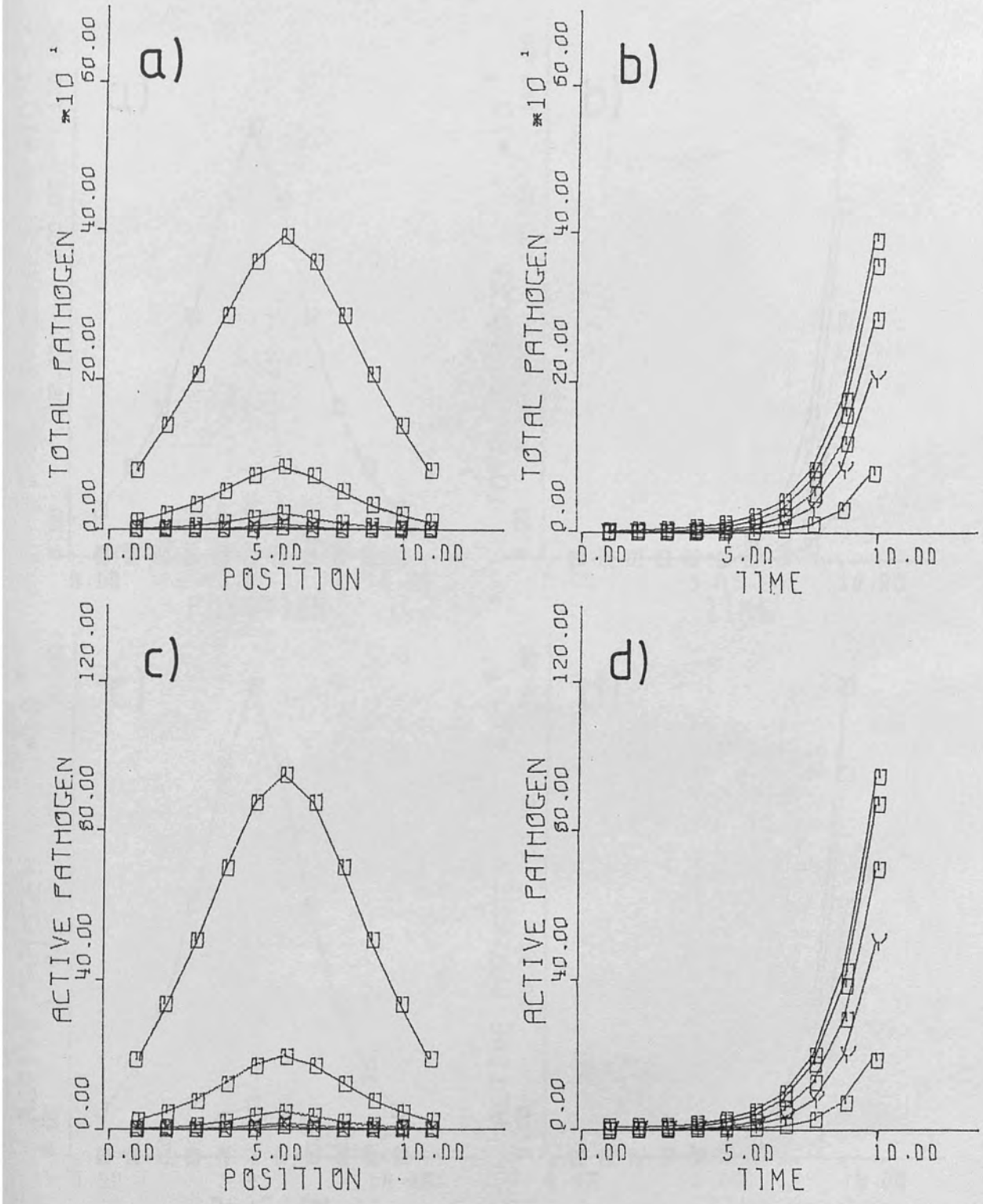


Fig. 7.3 Epidemic simulation using the exponential model.
S = 5 R = 1.0

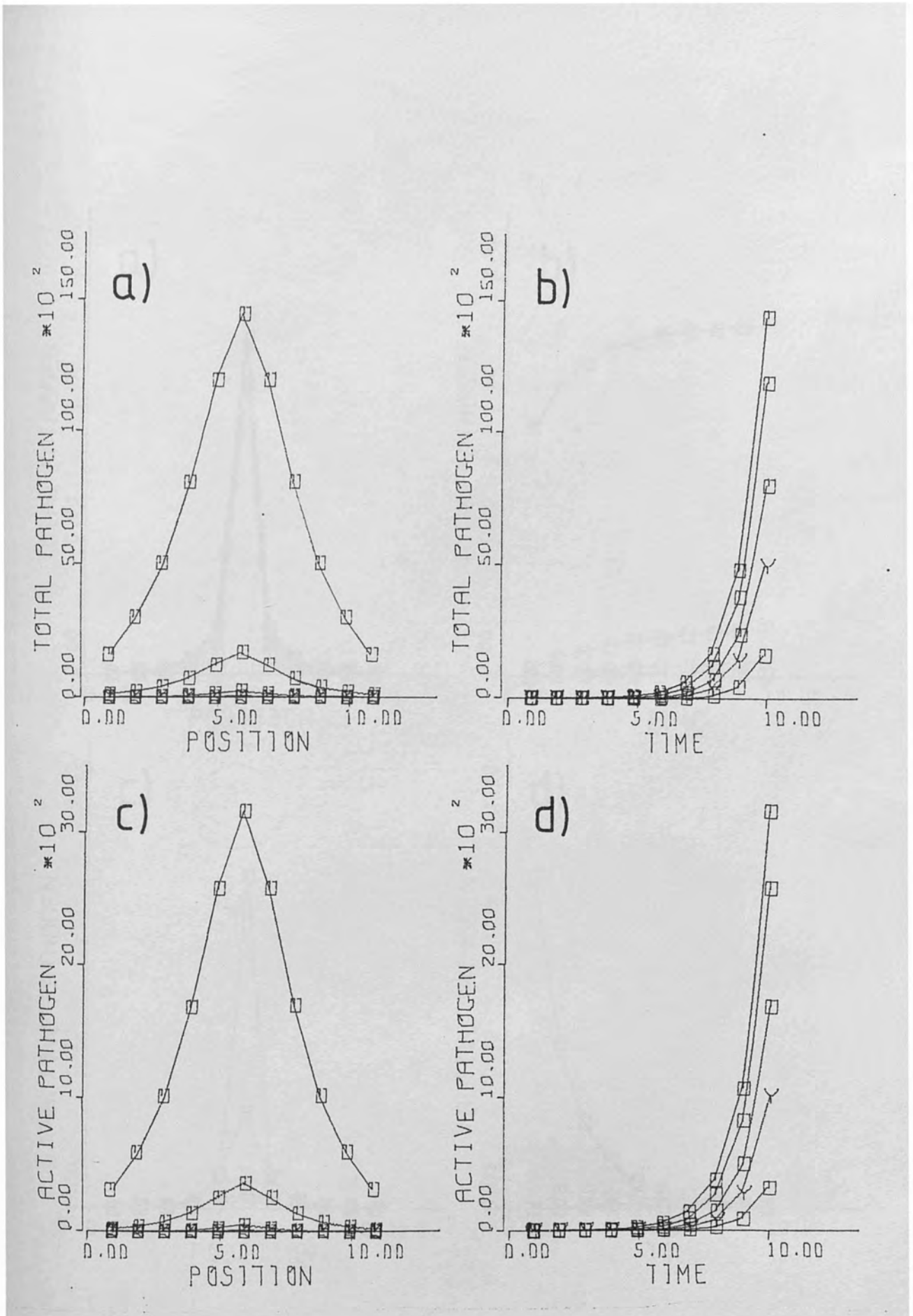


Fig. 7.4 Epidemic simulation using the exponential model.
S = 10 R = 2.0

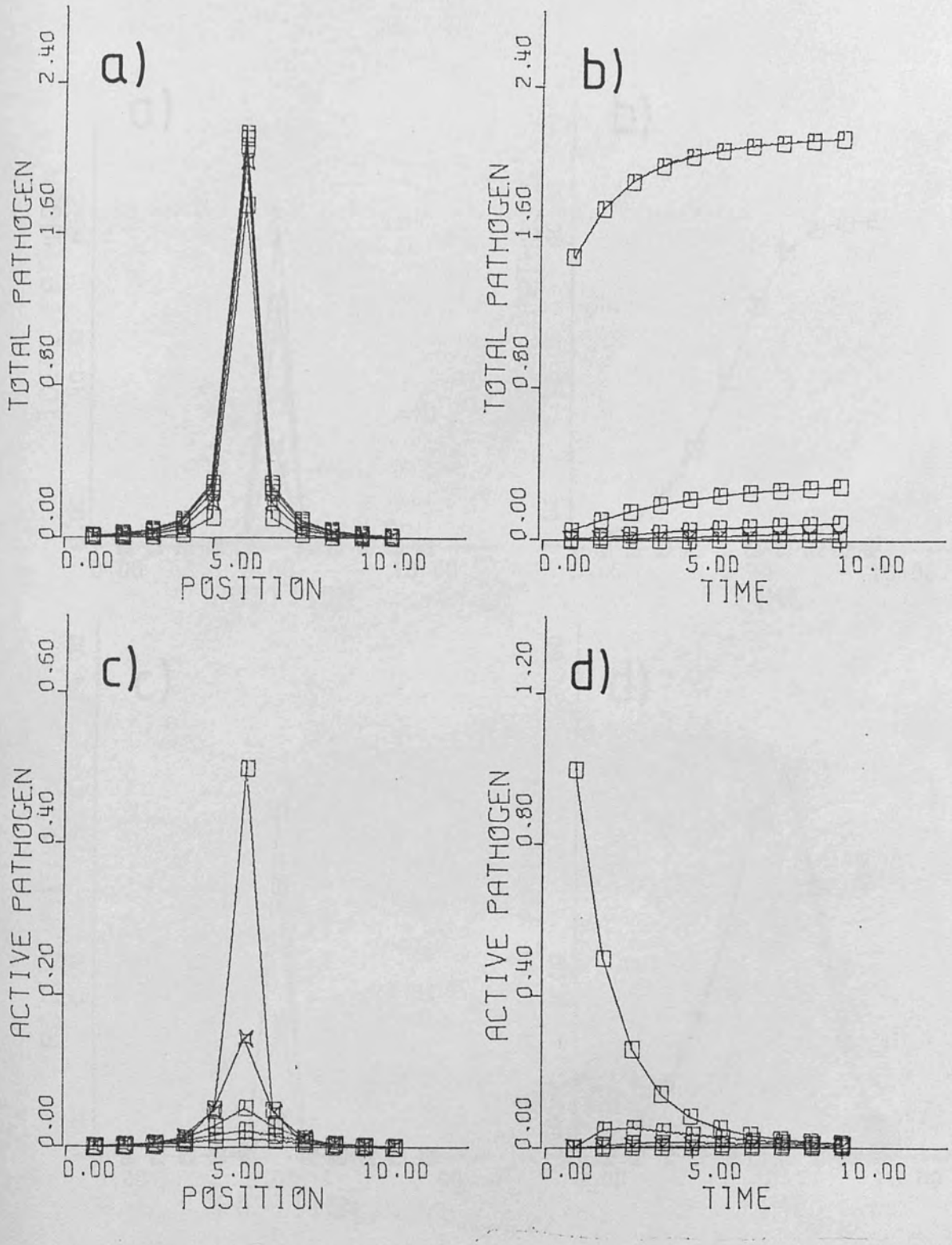


Fig. 7.5 Epidemic simulation using the exponential model.
S = 10 R = 0.5

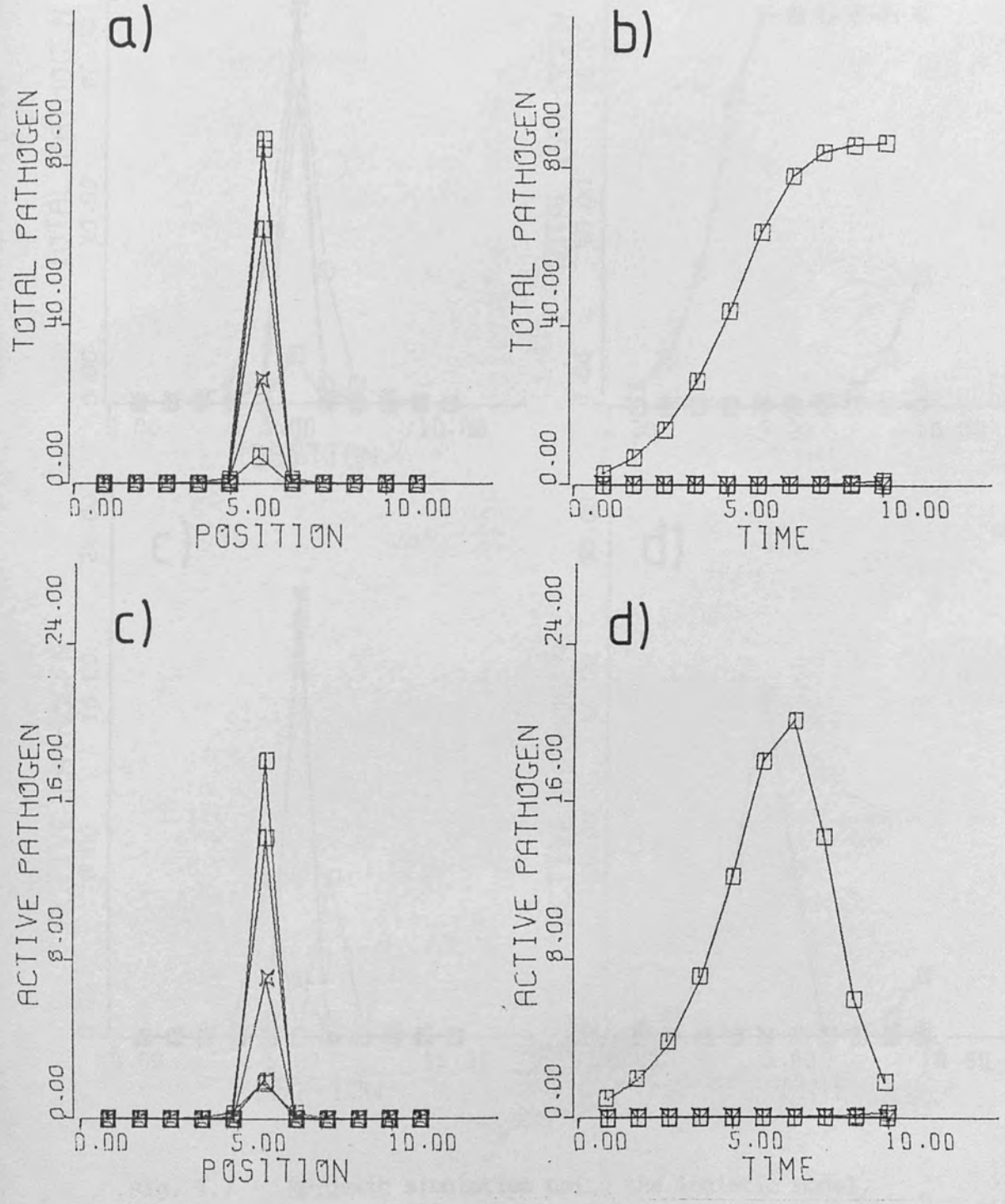


Fig. 7.6 Epidemic simulation using the logistic model.
S = 10000 R = 2.0 DISMAX = 100

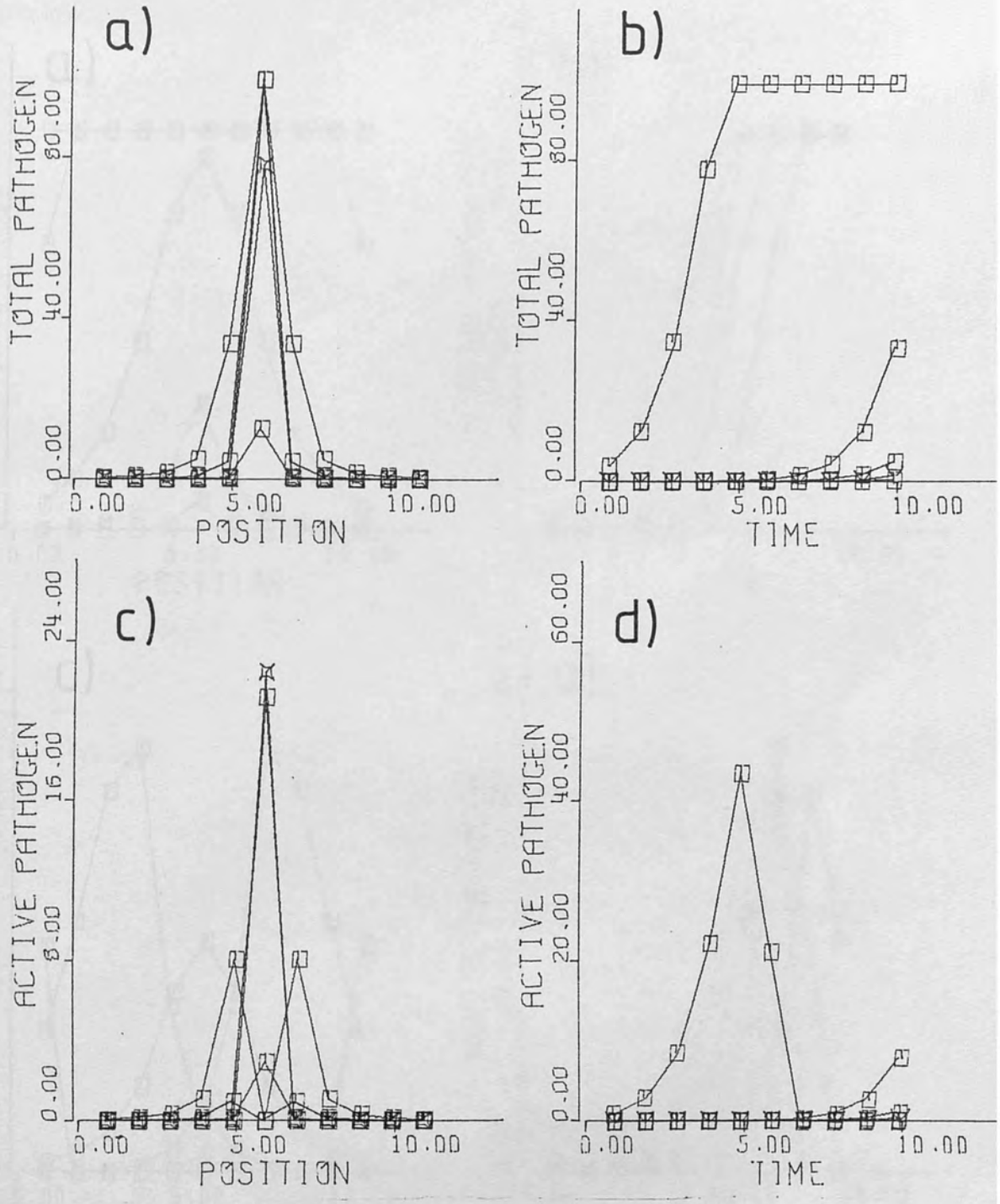


Fig. 7.7 Epidemic simulation using the logistic model.
S = 10000 R = 3.0 DISMAX = 100

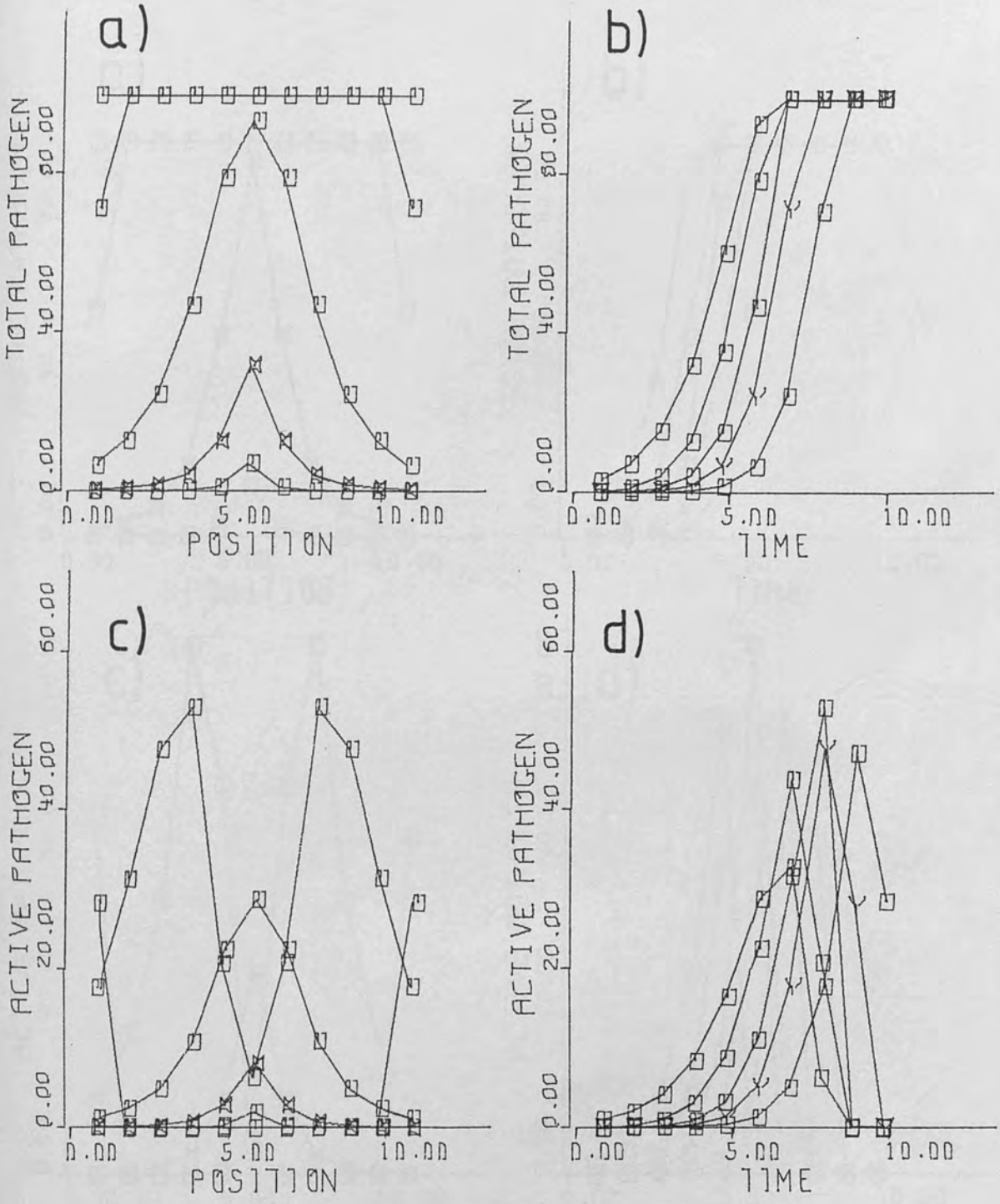


Fig. 7.8 Epidemic simulation using the logistic model.
S = 10 R = 2.0 DISMAX = 100

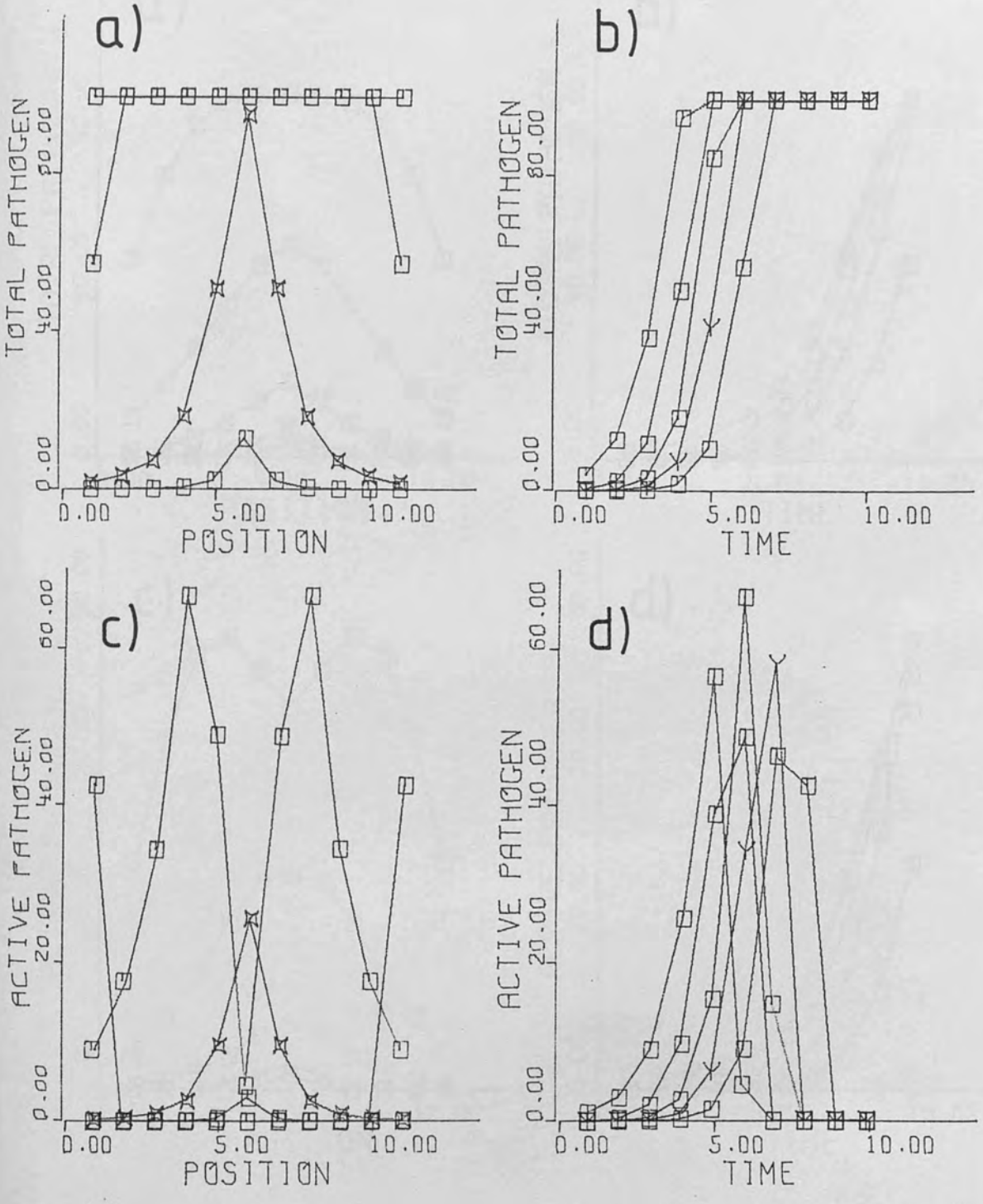


Fig. 7.9 Epidemic simulation using the logistic model.
S = 10 R = 3.0 DISMAX = 100

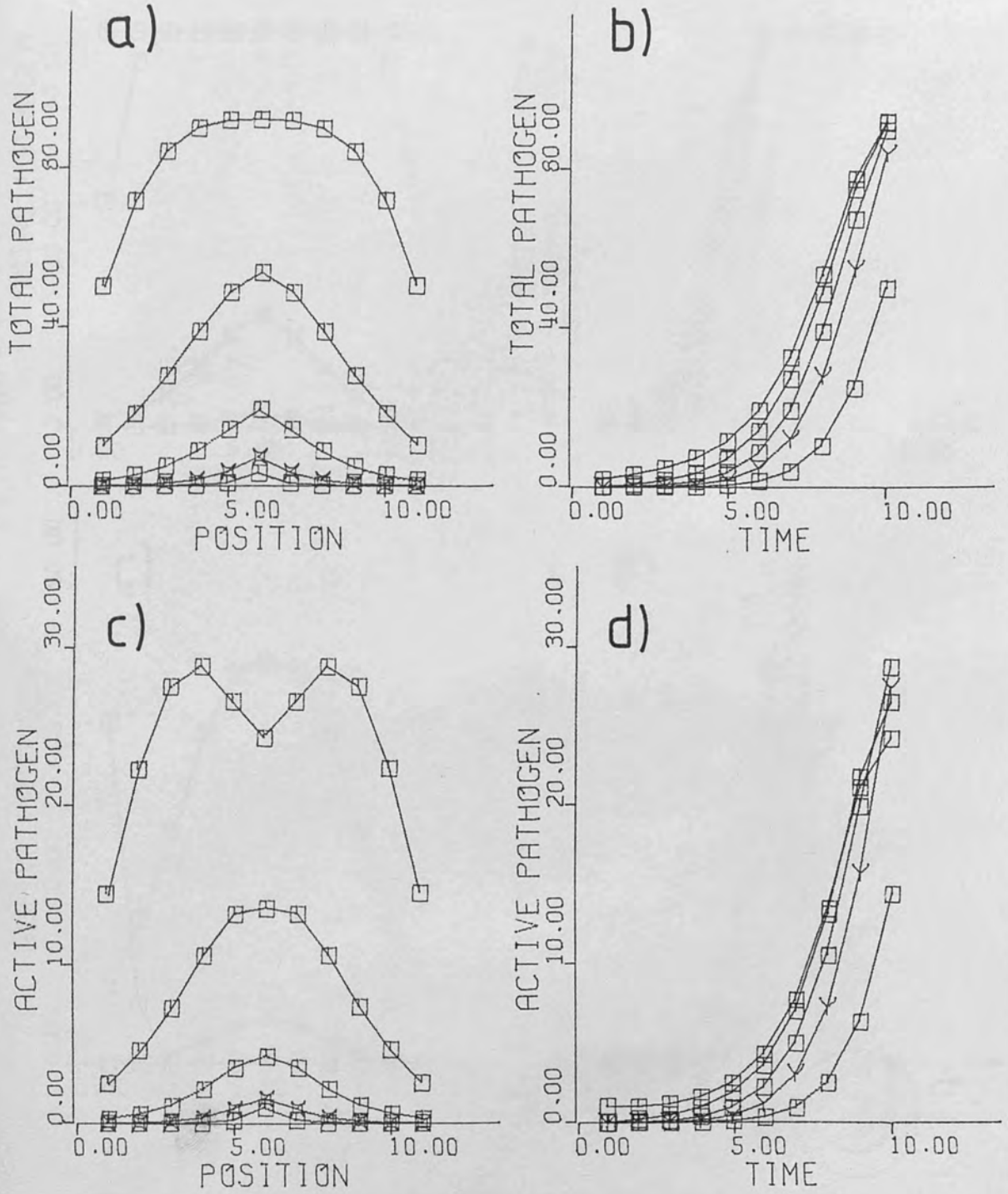


Fig. 7.10 Epidemic simulation using the logistic model.
S = 5 R = 1.0 DISMAX = 100

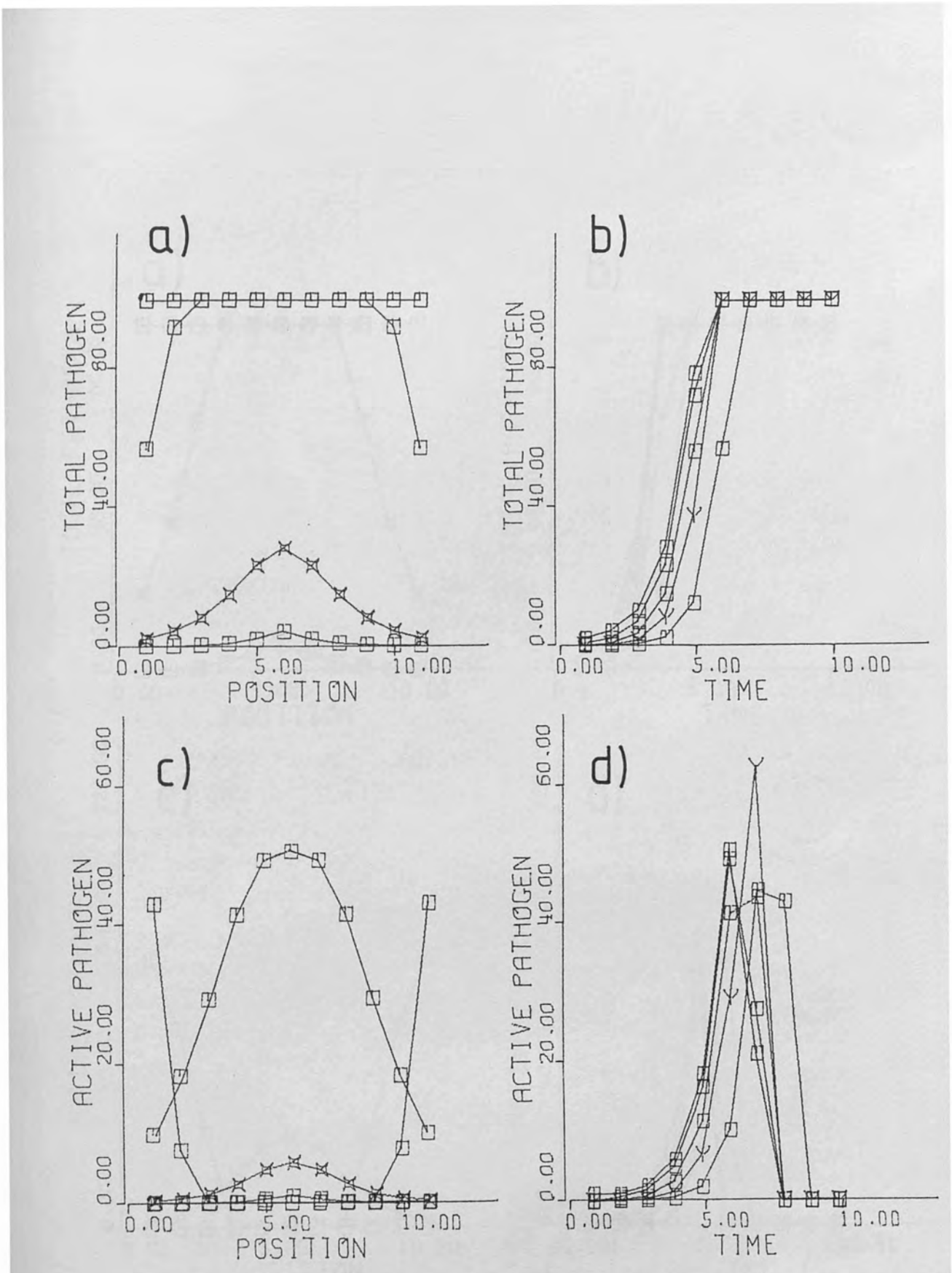


Fig. 7.11 Epidemic simulation using the logistic model.
S = 2 R = 1.0 DISMAX = 100

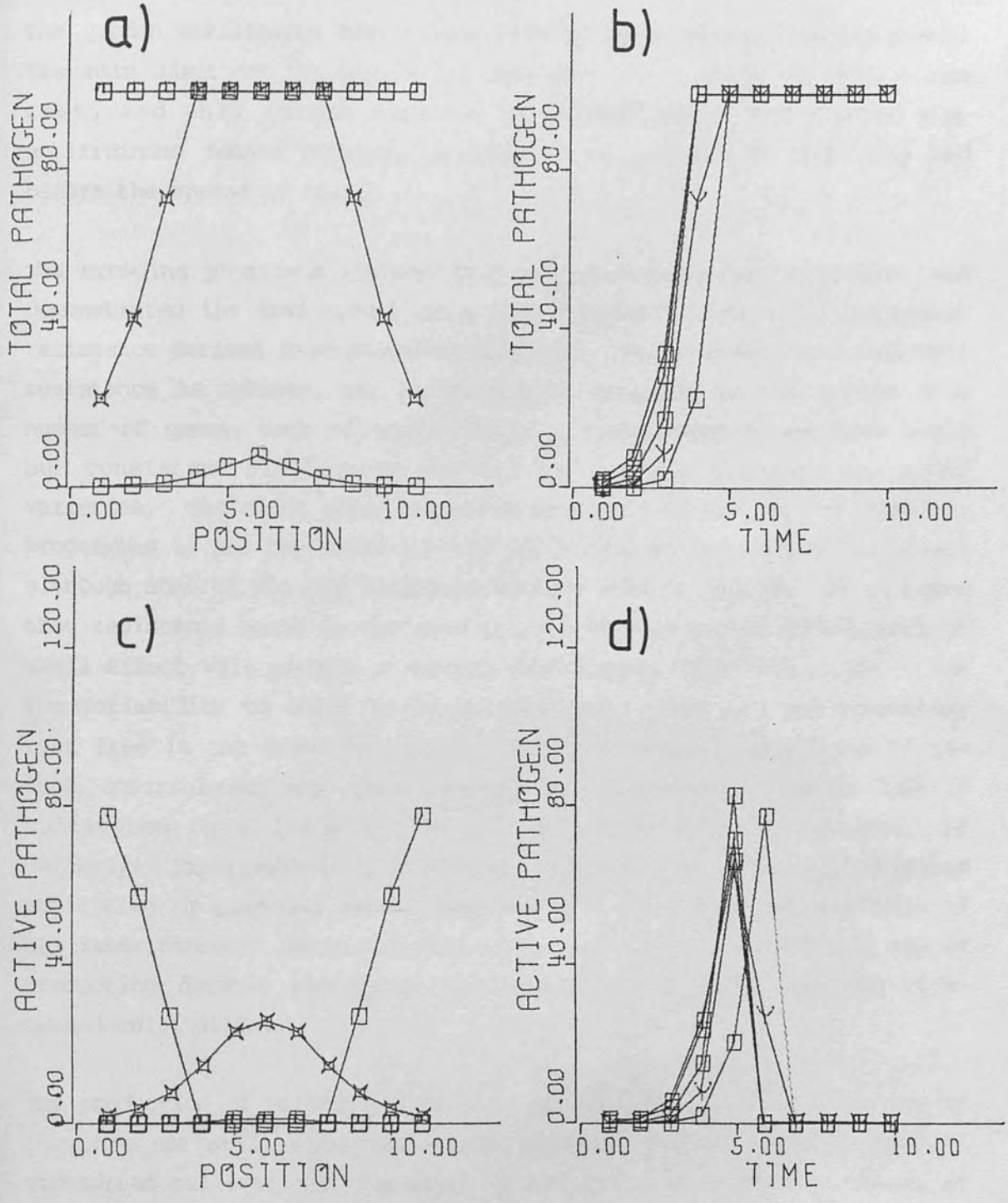


Fig. 7.12 Epidemic simulation using the logistic model.
S = 1 R = 1.0 DISMAX = 100

CHAPTER 8
CONCLUSIONS AND PROSPECTS

The garden antirrhinum has a long history as a popular bedding plant. The main limit to its popularity has been the problem of antirrhinum rust, and only if the disease is sufficiently controlled can antirrhinums remain popular, or recover the importance that they had before the spread of rust.

The breeding programme started from good rust-resistant genotypes, and demonstrated the development of a wide range of lines with comparable resistance derived from different sources. The ultimate source of this resistance is unknown, but is thought to be based on the action of a number of genes, each of small effect. Field observations show small but consistent differences between the various breeding lines and varieties. The major gene for resistance used in the earlier breeding programmes is not the basis for the resistance of the recent varieties, although some of the old varieties perform well in trials. It is hoped that resistance based on the accumulation of a number of genes, each of small effect will produce a durable resistance. The rust probably has the variability to adapt to these varieties. They are not completely rust free in the breeding trials. With the gradual adaptation of the rust antirrhinums may gradually appear increasingly susceptible if cultivated on a large scale. A sudden devastating epidemic is unlikely. Experience in a wide range of crops, some with sophisticated monitoring of pathogen races, coupled to a more detailed knowledge of the inheritance of resistance has shown that there is no certain way of predicting durable resistance or identifying it in advance of widespread cultivation.

The production of perfectly rust-free antirrhinums will be of no use if they are not still effectively rust-resistant after several years of widespread cultivation. However, a few pustules of rust on plants at the end of the season do no harm to a display. Bedding plants are usually grown in mixed colours. This provides an ideal opportunity for disease control using variety mixtures. Variety mixtures have been

recommended for disease control in cereals but farmers are afraid of the consequences of non-uniform ripening and quality. In contrast with this, growers of bedding plants want mixtures of colours. If they can have mixtures of disease resistant genotypes at the same time, this might be an important control of the disease. This is rather a different approach from the introduction of different colours into the same genetic background, which is common in horticultural breeding schemes.

Disease resistance, however good, is of no use unless the plant is acceptable in all other ways too. The first rust-resistant (immune) breeding lines produced in the 1930s were of very poor horticultural quality as a result of inbreeding and selection only for rust-resistance. Further breeding programmes were required in order to produce acceptable commercial varieties. If rust-resistance can be improved without this loss of quality, then useful varieties can be obtained much quicker.

Increased rust resistance should bring two practical benefits to the antirrhinum grower:

1. Increased reliability.
2. A longer flowering season.

The improved reliability will overcome the main problem that has brought about a decline in the popularity of antirrhinum as a bedding plant. The absence of an annual rust epidemic at or soon after the peak flowering time would greatly increase confidence in the plant.

A longer flowering season as plants survive to flower on more side shoots would also be of benefit.

Teliospore production by Puccinia antirrhini on cultivated Antirrhinum majus is probably a vestigial feature remaining from the life cycle on the wild species in California or from earlier in the evolution of this rust. The urediniospore cycle is sufficient for the propagation and overwintering of the rust in the conditions of the United Kingdom. The latent period of development is much longer in winter conditions than in the summer. This may be a simple effect of

temperature and host physiology, or may be an adaptation of the pathogen to allow successful overwintering as mycelium in living host tissue. In either case the effect is the same. Antirrhinum rust can and does survive the winter in apparently healthy or mildly infected antirrhinum plants. Such plants are common in many gardens in the United Kingdom, and carry infection from one season to the next. Removing overwintering plants from individual gardens will still only provide protection against rust in the most isolated places because of the widespread distribution of the rust and occasional long distance dispersal of inoculum. However, if the onset of rust infection can be delayed by any means, and the development of an epidemic slowed by the use of resistant varieties, then an effective level of control can be achieved.

ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. B.M.G. Jones for his help and encouragement and the time that he has so kindly given to this project.

I am also most grateful to other staff at Royal Holloway and Bedford New College, Mr C.D. Brickell the director of the Royal Horticultural Society Gardens at Wisley and his staff, and the staff of the London University Botanic Supply Unit. Special thanks are due to Miss J. Jenkins, Mrs P. Sullivan, Mr P Clawson and Miss R. Mummary for help with planting the trials and collecting the data. The staff at Wisley Gardens and the Botanic Supply Unit raised the plants, helped at planting out and maintained the trials. The staff at the Botanic Supply Unit raised and cared for many plants for the crossing programme in their glasshouses. Mr D. Ward and Miss L. Etherington developed and printed the black and white photographs.

I am grateful to the Computer Services group of the Welsh Plant Breeding Station for the use of their facilities, and their interest in the interactions between neighbouring plots.

I am grateful for the support provided by a S.E.R.C. CASE studentship and the Lensbury Fellowship fund of the Royal Horticultural Society from 1980 to 1983.

I would also like to thank my family and friends for their encouragement and support. I am especially grateful to Miss M.E. Davis for her help in the final production of this thesis and proof reading and checking the many tables.

REFERENCES

Aronescu-Savulescu, A. (1938) Contributions á l´étude de la Rouille du Muflier (Puccinia antirrhini Diet et Holway). Analele Institutuluide Cercetari Agronomice al Românei **10** IX 473-517.

Barbe, C.D. (1964) Snapdragon rust. Bulletin of the Californian Department of Agriculture **53** 181-184.

Barbe, C.D. (1967) Studies on the biology of the snapdragon rust fungus. Ph.D. Thesis. University of California, Davis, California, U.S.A.

Blasdale, W.C. (1903) On a rust of the cultivated snapdragon. Journal of Mycology **9** 81-82.

Blodgett, C.O. & Mehlquist, G.A.L. (1941) Snapdragon rust-resistance trials 1937-1938. Hilgardia **13** 569-581.

Borlaug, N.E. (1959) The use of multibreed or composite varieties to control airborne epidemic diseases of self-pollinated crop plants. Proceedings of the 1st International Wheat Genetics Symposium 12-26.

Bos, I. (1981) The relative efficiency of honeycomb selection and other procedures for mass selection in winter rye (Secale cereal L.) Doctoral thesis, Agricultural University, Wageningen.

Clothier, R.B. (1987) Welsh Plant Breeding Station annual report 1986. 97-98.

Cochran, W.G. & Cox, G.M. (1957) Experimental designs. Wiley, New York. 611pp.

Darwin, C. (1868) The variation of animals and plants under domestication I-II. J. Murray, London. (Cited by Gustafsson 1979).

De Vries, H. (1901-1903) Die mutationstheorie I-II. Veit & Co., Leipzig. (Cited by Gustafsson 1979).

Dietel, P. (1897) Einige neue Uredineen. Hedwigia **36** 297-299.

Doran, W.L. (1921) Rust of Antirrhinum. Bulletin of the Massachusetts Agricultural Experiment Station No.**202** 39-66.

Emsweller, S.L. & Jones, H.A. (1934) The inheritance of resistance to rust in the snapdragon. Hilgardia **8** 197-211.

Fasoulas, A. (1981) Principles and methods of plant breeding. Department of Genetics and Plant Breeding, Aristotelian University of Thessaloniki, Greece, Publication No. **11**

Finlay, K.W. & Wilkinson, G.N. (1963) The analysis of adaptation in a plant breeding programme. Australian Journal of Agricultural Research **14** 742-754.

Fisher, R.A. (1935) The design of experiments Oliver and Boyd, Edinburgh. 252pp.

Flor, H.H. (1955) Host-parasite interaction in flax rust - its genetics and other implications. Phytopathology **45** 680-685.

Galpine, J.K. (1782) A catalogue of the most useful & ornamental hardy trees, shrubs, plants &c. reprinted in 1983 as The Georgian Garden Dovecote Press, Wimborne, U.K.

Gawthrop, F.M. (1980) Resistance to rust (Puccinia antirrhini) in Antirrhinum majus. Ph.D. Thesis. University of London, Royal Holloway College, U.K.

Gawthrop, F.M. & Jones, B.M.G. (1980) Evidence for genetic change in the antirrhinum rust (Puccinia antirrhini). Annals of Botany **47** 197-202.

Goodman, F.M. (1976) Maize. In: N.W. Simmonds (Ed.) Evolution of crop plants pp 128-136. Longmans, London, U.K.

Green, D.E. (1933) A disease of antirrhinums new to Great Britain. Gardeners Chronicle **94** 131.

Green, D.E. (1934) Antirrhinum rust I. A disease new to Great Britain caused by the fungus Puccinia antirrhini (Diet. et Holw.) Journal of the Royal Horticultural Society **59** 119-126.

Green, D.E. (1937a) Contributions from the Wisley Laboratory LXXXI - Antirrhinum rust III. Rust-resistant strains of antirrhhinums. The Journal of the Royal Horticultural Society **62** 214-219.

Green, D.E. (1937b) Antirrhinum rust. The Journal of the Royal Horticultural Society **62** 530-537.

Green, D.E. (1941) Contributions from the Wisley Laboratory LXXXIX - Antirrhinum rust IV. Improvement of rust-resistant varieties. Journal of the Royal Horticultural Society **66** 83-86.

Günther, E. & Rothmaler, W. (1963) Chromosomenzahlen amerikanischer Antirrhinum - Arten. Biologisches Zentrablatt **82** 95-99.

Gustafsson, A. (1979) Linnaeus' peloria: the history of a monster. Theoretical and applied genetics **54** 241-248.

Harshbarger, B. (1949). Triple rectangular lattices. Biometrics **5**, 1-13.

Hockey, J.F. (1921) Germination of teliospores of Puccinia antirrhini. 13th Annual Report of the Quebec Society for the Protection of Plants 1920-21 54-57.

James, W.C., Shih, C.S., Hodgson, W.A. & Callbeck, L.C. (1976) Representational errors due to interplot interference in field experiments with late blight of potato. Phytopathology **66** 695-700.

Jenkyn, J.F., Bainbridge, A., Dyke, G.V. & Todd, A.D. (1979) An investigation into inter-plot interactions, in experiments with mildew on barley, using balanced designs. Annals of Applied Biology **92** 11-28.

Kochman, J. (1938) Choroby lwiej paszczy (Antirrhinum majus): Rdza, Puccinia antirrhini Diet et Holw i plamistość liści Phyllosticta antirrhini Syd. Compte rendu de la Societie des Sciences, Varsovie **31** 136-157.

Lehoczky J. (1954) Az oroszlánszáj rozsdabetegségéröl. A Kertészeti és szölészeti Föiskola Évkönyve **18** 3-39.

Mains E.B. (1924) Notes on the life history of the snapdragon rust, Puccinia antirrhini Diet. & Holw. Phytopathology **14** 281-287.

Mains, E.B. (1935) Rust resistance in Antirrhinum. Phytopathology **25** 977-991.

Mehlquist, G.A.L. & Rahmani, R. (1948) Inheritance of resistance to rust in the snapdragon, Antirrhinum majus. Proceedings of the American Society of Horticultural Science **52** 481-486.

Morander, R. (1980) Linaria vulgaris, gulsporre, med öppna blommor. [An open-flowered form of Linaria vulgaris.] Svensk Botanisk Tidskrift. **74** 9-10.

Munz, P.A. (1968) Supplement to a California flora. University of California Press, Berkeley.

Norwood, J.M., Freeman, G.H. & Crute, I.R. (1982) The evaluation of field resistance to downy mildew (Bremia lactucae) in lettuce. Journal of the National Institute of Agricultural Botany **16** 104-113.

Patterson, H.D., Williams, E.R. & Hunter, E.A. (1978) Block designs for variety trials. Journal of Agricultural Science, Cambridge **90**, 395-400.

Payne, R.W. (Chairman Genstat 5 committee) 1987 Genstat 5 reference manual. Clarendon Press, Oxford. 749pp.

Peltier, G.L. (1919) Snapdragon rust. Bulletin of Illinois Agricultural Experiment Station No. 221 535-548.

Pethybridge, G.H. (1934) Snapdragon (Antirrhinum) rust. Journal of the Ministry of Agriculture **41** 336-340.

Rothmaler W. (1956) Taxonomische monographie der gattung Antirrhinum. Feddes Repertorium specierum novarum regni vegetabilis. Beiheft 136.

Sampson, D.R. (1960) Linkage of a rust-resistance gene with two flower-color genes in Antirrhinum majus. Canadian Journal of Genetics and Cytology **2** 216-219.

Stubbe, H. (1966) Genetic und zytologie von Antirrhinum L. sect antirrhinum. Gustav Fischer Verlag, Jena, G.D.R. 42lpp.

Truter, S.J. & Martin, M.M. (1971) Occurrence of spermogonia and aecia on Antirrhinum majus. Phytophylactica **3** 149.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.K. & Webb, D.A. (1972) Flora Europea vol. 3. Cambridge University Press.

Van der Plank, J.E. (1963) Plant diseases; epidemics and control. Academic Press, New York 349pp.

Waggoner, P.E. & Horsfall, J.G. (1969) EPIDEM, a simulator of plant disease written for a computer. Connecticut Experiment Station, New Haven Bulletin 698 80pp.

Wahl I. (1949) Snapdragon rust (Puccinia antirrhinum D. and H.) in Palestine. Palestine Journal of Botany Jerusalem Series 4 **4** 225-229.

Walker, J. (1954) Antirrhinum rust, Puccinia antirrhini D. and H., in Australia. Proceedings of the Linnaean Society of New South Wales **79** 145-155.

Webb, D.A. (1971) Taxonomic notes on Antirrhinum L. In: Heywood, V.H. Flora Europaea. Notulae Systematicae ad Floram Europaeam spectantes No. 10. Botanical Journal of the Linnaean Society **64** 271-275.

Wheldale, M. & Bassett, H.L. (1914) The chemical interpretation of some Mendelian factors for flower-colour. Proceedings of the Royal Society of London **87** B 300-311.

White, H.E. (1933) Preliminary report on breeding rust resistant snapdragons. Proceedings of the American Society of Horticultural Science **30** 589-590.

Wolfe, M.S. (1977) Yield stability in barley using varietal mixtures and disease control. Cereals Research Communication **5** 119-124.

Yap, A.S.J. (1969) Studies on the epidemiology of Puccinia antirrhini Diet et Howl and Marssonina panattoniana (Berl.) Magn. Dissertation submitted for Master of Agricultural Science, University of Adelaide.

Yarwood, C.E. (1937) Physiologic races of snapdragon rust. Phytopathology **27** 113-115.

Zadoks, J.C. & Kampmeijer, P. (1977) The role of crop populations and their deployment illustrated by the new simulator, EPIMUL 76. In P.E. Day (Ed.) Plant disease and epidemiology.

Zar, J.H. (1984) Biostatistical analysis. Prentice Hall, New Jersey, U.S.A.

APPENDIX 1 List of all accessions grown in the trials.

Accession number	Generation	Entry number in each trial					
		Wisley 1981	Egham 1981-1	Egham 1981-2	Wisley 1982	Egham 1982	Egham 1983
MALMAISON	CONTROL				6		
78-32-1	CONTROL				5		
78-35	CONTROL				62		
78-38	CONTROL				61		
78-63	CONTROL				63		
78-64	CONTROL				64		
78-88	CONTROL				4		
78-92	CONTROL					2	
78-95	CONTROL					3	
78-164	CONTROL				9	9	9
78-178	CONTROL				11		
78-180	CONTROL				1		
78-183	CONTROL				12		
78-187	CONTROL				13		
78-195	CONTROL				10		
78-240	CONTROL				8	8	8
78-246	CONTROL				7	7	
82-1	CONTROL				14	14	
82-2	CONTROL				16	16	16
82-3	CONTROL				15	15	15
82-6	CONTROL				65		
83-1	CONTROL						166
80-38-1	F1				17		
80-38-3	F1				18		
80-39-1	F1				25		
80-39-4	F1				26		
80-40-3	F1				41		
80-41-2	F1				44		
80-42-1	F1				47		
80-43-3	F1				33		
80-43-5	F1				34		
80-49-3	F1				50		
80-52-2	F1				53		
80-55-1	F1				56		
80-56-1	F1				57		
81-32-1	F2		X			96	
81-32-2	F2		X				
81-32-3	F2		X			97	
81-32-5	F2			X			
81-32-6	F2			X		98	
81-32-7	F2			X		99	
81-32-8	F2			X			
81-32-9	F2			X		100	100
81-32-10	F2			X			
81-33-1	F2			X			
81-33-2	F2			X			
81-33-3	F2			X			
81-33-4	F2			X			
81-33-6	F2			X			
81-33-7	F2			X			
81-34-1	F2			X			
81-34-2	F2			X			
81-34-3	F2			X			
81-34-4	F2			X		101	
81-34-5	F2			X			
81-34-6	F2			X		102	
81-34-7	F2			X			
81-35-1	F2		X				
81-35-2	F2		X				
81-35-3	F2		X				
81-36-1	F2			X		103	
81-36-2	F2			X			
81-36-3	F2			X		104	
81-36-5	F2			X			
81-36-6	F2			X		105	105
81-36-7	F2			X			
81-36-9	F2			X			
81-36-10	F2			X		106	

APPENDIX 1 List of all accessions grown in the trials

81-36-12	F2						107		
81-37-1	F2				X		108		
81-37-2	F2				X				
81-37-3	F2				X		109		
81-37-4	F2				X		110		
81-37-5	F2						111		
81-37-6	F2						112		
81-38-1	F2	X				19			
81-38-2	F2	X							
81-38-3	F2	X				20			
81-38-4	F2	X							
81-38-5	F2	X							
81-38-6	F2	X							
81-38-7	F2	X				21			
81-38-8	F2	X							
81-38-9	F2	X							
81-39-1	F2	X				27			
81-39-2	F2	X							
81-39-3	F2	X							
81-39-4	F2	X				28			
81-39-5	F2	X							
81-39-6	F2	X							
81-39-7	F2	X				29			
81-39-8	F2	X							
81-39-9	F2	X							
81-39-10	F2	X							
81-40-1	F2				X				
81-40-2	F2				X				
81-40-3	F2				X				
81-40-4	F2				X				
81-40-5	F2				X				
81-40-6	F2				X				
81-40-7	F2				X				
81-40-8	F2				X				
81-40-9	F2				X	42			
81-40-10	F2				X	43			
81-41-1	F2				X				
81-41-2	F2				X				
81-41-3	F2				X	45			
81-41-4	F2				X				
81-41-5	F2				X	46	113		
81-41-6	F2				X				
81-41-7	F2				X		114		
81-42-1	F2		X			48			
81-42-2	F2		X						
81-42-3	F2		X						
81-42-4	F2		X						
81-42-5	F2		X			49			
81-42-6	F2		X						
81-42-7	F2		X						
81-42-8	F2		X				115		
81-42-9	F2		X				116		
81-42-10	F2		X				117		
81-42-11	F2		X						
81-42-12	F2						118		
81-42-13	F2						119		
81-42-14	F2						120	120	
81-43-1	F2	X				35			
81-43-2	F2	X							
81-43-3	F2	X				36			
81-43-4	F2	X							
81-43-5	F2	X							
81-43-6	F2	X							
81-43-7	F2	X							
81-43-8	F2	X							
81-43-9	F2	X				37			
81-44-1	F2		X						
81-44-2	F2		X						
81-44-3	F2		X						
81-44-4	F2		X						
81-44-5	F2		X						
81-44-6	F2		X						
81-44-7	F2		X						
81-44-8	F2		X						

APPENDIX 1 List of all accessions grown in the trials

81-44-9	F2			X				
81-45-1	F2	X						
81-45-2	F2	X						
81-45-3	F2	X						
81-45-4	F2	X						
81-45-5	F2	X						
81-45-6	F2	X						
81-46-1	F2	X						
81-46-2	F2	X						
81-46-3	F2	X						
81-46-4	F2	X						
81-46-5	F2	X						
81-46-6	F2	X						
81-46-7	F2	X						
81-46-8	F2	X						
81-46-9	F2	X						
81-47-1	F2			X				
81-47-2	F2			X				
81-47-3	F2			X				
81-47-4	F2			X				
81-47-5	F2			X				
81-47-6	F2			X				
81-48-1	F2			X				
81-48-2	F2			X			121	121
81-48-3	F2			X				
81-48-4	F2			X				
81-48-5	F2			X			122	
81-48-6	F2			X			123	
81-48-7	F2				X		124	
81-48-8	F2				X			
81-48-9	F2				X			
81-48-10	F2				X		125	
81-49-1	F2				X		128	128
81-49-2	F2				X	51	129	
81-49-3	F2				X			
81-49-4	F2				X			
81-49-5	F2				X		126	
81-49-6	F2				X	52	127	
81-49-7	F2				X			
81-50-1	F2				X			
81-50-2	F2				X			
81-50-3	F2				X		130	
81-50-4	F2				X			
81-50-5	F2				X			
81-50-6	F2				X		131	
81-50-7	F2				X		132	
81-50-8	F2				X			
81-51-1	F2				X			
81-51-2	F2				X			
81-51-3	F2				X			
81-51-4	F2				X			
81-51-5	F2				X			
81-51-6	F2				X			
81-52-1	F2				X	54	133	
81-52-2	F2				X			
81-52-3	F2				X			
81-52-4	F2				X		134	
81-52-5	F2				X			
81-52-6	F2				X			
81-52-7	F2				X		135	
81-53-1	F2						136	
81-53-3	F2						137	137
81-53-5	F2						138	
81-54-1	F2	X						
81-54-2	F2	X						
81-54-3	F2	X						
81-54-4	F2	X						
81-54-5	F2	X						
81-54-6	F2	X						
81-54-7	F2	X						
81-54-8	F2	X						
81-54-9	F2	X						
81-54-10	F2	X						
81-54-11	F2	X						

APPENDIX 1 List of all accessions grown in the trials

81-54-12	F2	X			
82-32-2	F3				141
82-32-3	F3				142
82-36-1	F3				144
82-38-1	F3		22	22	
82-38-6	F3			23	
82-38-7	F3		24		
82-39-1	F3		30	30	
82-39-3	F3		31	31	
82-39-4	F3			32	
82-39-7	F3			66	
82-39-8	F3			67	
82-39-11	F3			68	
82-42-1	F3				147
82-42-3	F3				149
82-43-2	F3		39	39	
82-43-6	F3			69	
82-43-9	F3		38	38	
82-43-10	F3		40	40	
82-45-2	F3			70	
82-45-3	F3			71	
82-45-4	F3			72	
82-45-5	F3			73	
82-45-7	F3			74	
82-46-1	F3			75	
82-46-2	F3			76	
82-46-3	F3			77	
82-46-4	F3			78	
82-46-6	F3			79	
82-46-7	F3			80	
82-48-1	F3				150
82-48-3	F3				151
82-48-4	F3				152
82-48-5	F3				153
82-48-6	F3				154
82-49-1	F3				155
82-49-4	F3				157
82-53-3	F3				159
82-53-5	F3				160
81-54-1	F3			81	
82-54-1	F3			82	
82-54-2	F3			83	
81-54-3	F3			84	
82-54-3	F3			85	
82-54-4	F3			86	86
82-54-5	F3			87	
82-54-6	F3			88	
82-54-8	F3			89	
81-54-9	F3			90	
82-54-9	F3			91	
82-54-10	F3			92	
82-54-11	F3			93	
81-54-13	F3			94	
82-54-13	F3			95	
82-54-5	F4				163

Appendix 2

Variety mean rust scores for the trial at Wisley in 1982, as produced by analysis of variance with and without neighbours as covariates.

Trial entry number	Accession number	Randomised blocks 12th Aug.	Randomised blocks 30th Sept.	Covariates 12th Aug.	Covariates 30th Sept.
1	78-180	1.56	4.68	1.83	4.90
2	78-92	dead			
3	78-95	dead			
4	78-88	1.48	3.86	1.70	4.06
5	78-32-III	2.78	4.69	2.73	4.69
6	MALMAISON	2.66	5.79	2.72	5.71
7	78-246	1.72	4.80	1.98	5.04
8	78-240	1.90	5.52	1.84	4.13
9	78-164	2.47	4.77	2.37	5.89
10	78-195	2.30	5.46	2.11	5.01
11	78-178	1.54	3.58	1.62	3.54
12	78-183	1.49	3.92	1.69	4.54
13	78-187	2.53	5.15	2.73	5.10
14	82-1	2.68	4.21	2.89	4.03
15	82-3	2.20	4.95	2.16	4.78
16	82-2	2.01	3.08	2.16	2.88
17	80-38-1	1.61	4.57	1.84	4.62
18	80-38-3	1.83	4.82	1.89	4.70
19	81-38-1	1.75	4.97	1.86	5.12
20	81-38-3	1.90	4.86	1.79	4.89
21	81-38-7	1.96	4.06	1.54	3.65
22	82-38-1	1.44	4.26	1.54	4.39
23	82-38-6	dead			
24	82-38-7	1.70	3.86	1.91	4.47
25	80-39-1	1.71	5.59	1.56	5.18
26	80-39-4	1.65	4.79	1.63	4.99
27	81-39-1	2.52	5.87	2.28	5.67
28	81-39-4	1.78	4.68	1.74	4.56
29	81-39-7	1.77	4.96	1.93	4.98
30	82-39-1	1.94	5.42	1.62	5.55
31	82-39-3	1.63	4.84	1.51	4.70
32	82-39-4	dead			

(continued on next page)

Trial entry number	Accession number	Randomised blocks 12th Aug.	Randomised blocks 30th Sept.	Covariates 12th Aug.	Covariates 30th Sept.
33	80-43-3	1.96	4.48	2.04	4.53
34	80-43-5	2.24	3.82	2.05	4.16
35	81-43-1	1.67	4.96	1.63	4.80
36	81-43-3	1.64	4.21	1.58	4.21
37	81-43-9	1.90	4.60	2.15	4.95
38	82-43-9	1.81	5.35	1.50	4.95
39	82-43-2	2.25	4.86	2.31	4.69
40	82-43-10	2.02	4.40	1.90	4.51
41	80-40-3	1.93	4.00	1.80	4.24
42	81-40-9	2.22	5.13	2.12	4.95
43	81-40-10	1.19	3.30	1.49	3.38
44	80-41-2	1.73	4.33	1.65	4.24
45	81-41-3	1.56	4.17	1.79	4.18
46	81-41-5	1.29	4.55	1.68	4.46
47	80-42-1	2.37	4.41	2.21	4.81
48	81-42-1	2.74	5.03	2.84	5.06
49	81-42-5	1.73	4.87	2.08	5.01
50	80-49-3	2.61	5.18	2.26	4.86
51	81-49-2	2.70	5.53	2.27	5.03
52	81-49-6	1.92	4.96	1.78	4.77
53	80-52-2	2.04	5.20	1.98	5.99
54	81-52-1	2.19	4.42	2.33	4.26
55		1.69	4.06	1.78	4.39
56	80-55-1	2.19	5.54	2.19	5.40
57	80-56-1	2.87	5.80	2.73	6.12
58	80-58-1	dead			
59	80-78-1	dead			
60	80-80-1	dead			
61	78-38	1.74	4.46	1.73	4.68
62	78-35	2.79	5.03	2.53	5.97
63	78-63	1.97	4.68	2.17	4.69
64	78-64	1.92	4.35	1.82	4.60
65	82-6	1.85	4.53	1.70	4.54

Appendix 3

Variety mean rust scores for the trial at Egham in 1982, as produced by analysis of variance with and without neighbours as covariates.

Trial entry number	Accession number	Randomised blocks 10th Aug.	Randomised blocks 28th Sept.	Covariates 10th Aug.	Covariates 28th Sept.
2	78-92	2.96	5.96	3.00	5.95
8	78-240	1.99	4.93	2.14	5.02
9	78-164	2.48	4.71	2.42	4.90
14	82-1	3.06	6.10	2.87	5.86
15	82-3	2.30	5.27	2.55	5.14
16	82-2	2.15	4.24	2.00	5.16
22	82-38-1	2.38	4.76	2.52	4.84
30	82-39-1	2.42	5.13	2.60	5.33
31	82-39-3	1.98	4.89	2.16	4.90
66	82-39-7	2.11	5.41	2.13	5.38
67	82-39-8	2.15	4.79	2.24	4.90
38	82-43-9	2.40	5.36	2.59	5.28
39	82-43-2	2.93	5.82	2.69	5.79
69	82-43-6	2.68	5.16	2.41	4.95
70	82-45-2	2.60	5.81	2.69	5.97
71	82-45-3	2.30	5.37	2.38	5.49
72	82-45-4	2.56	5.41	2.59	5.52
73	82-45-5	2.27	5.30	2.33	5.45
74	82-45-7	2.02	5.32	1.96	5.16
75	82-46-1	2.01	5.10	2.12	5.06
76	82-46-2	2.06	5.42	1.75	5.25
77	82-46-3	2.13	5.72	2.15	5.50
78	82-46-4	2.04	5.54	2.06	5.33
79	82-46-6	1.79	5.14	2.08	5.24
80	82-46-7	2.42	5.15	2.39	5.17
81	81-54-1	2.67	5.33	2.54	5.14
86	82-54-4	1.93	3.97	1.82	4.04
87	82-54-5	2.16	3.87	2.01	3.69
91	82-54-9	2.21	5.31	2.34	5.28
94	81-54-13	1.80	3.82	1.74	3.68
96	81-32-1	2.22	5.45	2.35	5.80
97	81-32-3	2.16	5.50	2.38	5.42
98	81-32-6	2.53	5.62	2.41	5.43
99	81-32-7	2.19	5.06	2.17	5.07
100	81-32-9	1.93	4.81	1.97	4.82
101	81-34-4	2.26	4.41	2.24	4.42
102	81-34-6	2.54	5.49	2.53	5.65

(continued on next page)

Trial entry number	Accession number	Randomised blocks 10th Aug.	Randomised blocks 28th Sept.	Covariates 10th Aug.	Covariates 28th Sept.
103	81-36-1	2.67	4.56	2.70	4.80
104	81-36-3	2.77	5.31	2.74	5.41
105	81-36-6	2.03	4.11	2.12	4.13
106	81-36-10	2.82	5.47	2.78	5.28
107	81-36-12	2.87	5.02	2.58	5.14
108	81-37-1	2.51	5.67	2.60	5.93
109	81-37-3	2.32	5.24	2.23	5.12
110	81-37-4	2.58	5.19	2.73	5.47
111	81-37-5	3.00	5.28	2.70	5.31
112	81-37-6	2.38	5.05	2.35	4.98
113	81-41-5	1.88	4.76	1.98	4.64
114	81-41-7	2.12	4.60	2.13	4.61
115	81-42-8	2.38	4.62	2.13	4.58
116	81-42-9	2.96	5.56	2.92	5.53
117	81-42-10	1.98	5.09	2.08	4.89
118	81-42-12	2.20	4.68	2.29	4.83
119	81-42-13	2.33	4.70	2.36	4.72
120	81-42-14	2.27	4.44	2.35	4.56
121	81-48-2	2.02	4.39	2.15	4.53
122	81-48-5	3.30	4.83	3.02	4.82
123	81-48-6	2.31	5.00	2.40	4.96
124	81-48-7	2.03	4.82	2.11	4.73
125	81-48-10	2.71	5.64	2.46	5.55
126	81-49-5	2.76	4.96	2.70	5.05
127	81-49-6	2.11	4.76	2.02	4.63
128	81-49-1	2.30	4.38	2.31	4.64
129	81-49-2	3.08	5.24	2.91	5.21
130	81-50-3	1.87	4.43	1.97	4.46
131	81-50-6	2.08	5.02	2.18	4.88
132	81-50-7	2.37	4.84	2.38	4.81
133	81-52-1	2.09	4.72	2.18	4.72
134	81-52-4	1.91	5.10	1.94	5.17
135	81-52-7	2.11	5.10	2.19	5.07
136	81-53-1	2.18	4.75	2.17	4.70
137	81-53-3	1.97	4.52	1.97	4.40
138	81-53-5	2.10	5.17	1.97	5.22

Appendix 4 Critical values for Tukey's test and least significant difference test for the trials of 1982 using neighbours as covariates.

Error Mean Square	Degrees of Freedom Treatment	Error	Standard Error of Mean	Test	Sig. Level	Tabulated Value	Significant Difference
WISLEY 1st scoring							
0.132	57	108	0.21	Tukey	5%	5.93	1.24
				Tukey	1%	6.59	1.38
				t	5%	1.98	0.58
				t	1%	2.62	0.77
WISLEY 2nd scoring							
0.306	56	93	0.32	Tukey	5%	5.93	1.89
				Tukey	1%	6.59	2.10
				t	5%	1.98	0.89
				t	1%	2.62	1.18
EGHAM 1st scoring							
0.090	72	140	0.17	Tukey	5%	6.04	1.05
				Tukey	1%	6.69	1.16
				t	5%	1.98	0.48
				t	1%	2.62	0.64
EGHAM 2nd scoring							
0.210	72	140	0.26	Tukey	5%	6.04	1.50
				Tukey	1%	6.69	1.77
				t	5%	1.98	0.74
				t	1%	2.62	0.98

APPENDIX 5. Tukey test and least significant difference tests between accession means for the trials in 1982, using neighbours as covariates.

43	81-40-10	1.49	
38	82-43-9	1.50	.
31	82-39-3	1.51	..
21	81-38-7	1.54	...
22	82-38-1	1.54	....
25	80-39-1	1.56	.....
36	81-43-3	1.58	.....
11	78-178	1.62	.....
30	82-39-1	1.62	.....
26	80-39-4	1.63	.....
35	81-43-1	1.63	.....
44	80-41-2	1.65	.....
46	81-41-5	1.68	.....
12	78-183	1.69	.....
04	78-88	1.70	.....
65	82-6	1.70	.....
61	78-38	1.73	.....
28	81-39-4	1.74	.....
52	81-49-6	1.78	.....
55		1.78	.....
20	81-38-3	1.79	.....
45	81-41-3	1.79	.....
41	80-40-3	1.80	.....
64	78-64	1.82	.....
01	78-180	1.83	.....
08	78-240	1.84	.....
17	80-38-1	1.84	.....
19	81-38-1	1.86	.....
18	80-38-3	1.89	.....
40	82-43-10	1.90	.....
24	82-38-7	1.91	.....
29	81-39-7	1.93	.....
07	78-246	1.98	.....
53	80-52-2	1.98	.....
33	80-43-3	2.04	.....
34	80-43-5	2.05	.....
49	81-42-5	2.08	.....
10	78-195	2.11	.....
42	81-40-9	2.12	.....
37	81-43-9	2.15	.....
15	82-3	2.16	.....
16	82-2	2.16	.....
63	78-63	2.17	.....
56	80-55-1	2.19	.....
47	80-42-1	2.21	.....
50	80-49-3	2.26	.....
51	81-49-2	2.27	.....
27	81-39-1	2.28	.....
39	82-43-2	2.31	.....
54	81-52-1	2.33	.....
09	78-164	2.37	.....
62	78-35	2.53	.....
06	MALMAISON	2.72	.....
05	78-32-III	2.73	+.....
13	78-187	2.73	+.....
57	80-56-1	2.73	+.....
48	81-42-1	2.84	+++++++.....
14	82-1	2.89	***++++++++.....

Tukey test of differences between variety means at the first scoring at Wisley in 1982 including neighbouring plots as covariates in the ANOVA.

+ significant at 5% level, * significant at 1% level.

43	81-40-10	1.49	
38	82-43-9	1.50	.
31	82-39-3	1.51	..
21	81-38-7	1.54	...
22	82-38-1	1.54	....
25	80-39-1	1.56	.....
36	81-43-3	1.58	.....
11	78-178	1.62	.....
30	82-39-1	1.62	.....
26	80-39-4	1.63	.....
35	81-43-1	1.63	.....
44	80-41-2	1.65	.....
46	81-41-5	1.68	.....
12	78-183	1.69	.....
04	78-88	1.70	.....
65	82-6	1.70	.....
61	78-38	1.73	.....
28	81-39-4	1.74	.....
52	81-49-6	1.78	.....
55		1.78	.....
20	81-38-3	1.79	.....
45	81-41-3	1.79	.....
41	80-40-3	1.80	.....
64	78-64	1.82	.....
01	78-180	1.83	.....
08	78-240	1.84	.....
17	80-38-1	1.84	.....
19	81-38-1	1.86	.....
18	80-38-3	1.89	.....
40	82-43-10	1.90	.....
24	82-38-7	1.91	.....
29	81-39-7	1.93	.....
07	78-246	1.98	.....
53	80-52-2	1.98	.....
33	80-43-3	2.04	.....
34	80-43-5	2.05	.....
49	81-42-5	2.08	++.....
10	78-195	2.11	+++.....
42	81-40-9	2.12	+++++.....
37	81-43-9	2.15	++++++.....
15	82-3	2.16	+++++++.....
16	82-2	2.16	+++++++.....
63	78-63	2.17	+++++++.....
56	80-55-1	2.19	+++++++.....
47	80-42-1	2.21	++++++++++.....
50	80-49-3	2.26	++++++++++.....
51	81-49-2	2.27	*++++++++++.....
27	81-39-1	2.28	***++++++++++.....
39	82-43-2	2.31	***++++++++++.....
54	81-52-1	2.33	******++++++++++.....
09	78-164	2.37	*******++++++++++.....
62	78-35	2.53	**********++++++++++.....
06	MALMAISON	2.72	**********++++++.....
05	78-32-III	2.73	**********+++++++.....
13	78-187	2.73	**********+++++++.....
57	80-56-1	2.73	**********+++++++.....
48	81-42-1	2.84	**********+++++++.....
14	82-1	2.89	**********++++++.....

t test of differences between variety means at the first scoring at Wisley in 1982 including neighbouring plots as covariates in the ANOVA.

+ significant at 5% level, * significant at 1% level.

16	82-2	2.88	
43	81-40-10	3.38	.
11	78-178	3.54	..
21	81-38-7	3.65	...
14	82-1	4.03	....
04	78-88	4.06	.....
08	78-240	4.13	.....
34	80-43-5	4.16	.....
45	81-41-3	4.18	.....
36	81-43-3	4.21	.....
41	80-40-3	4.24	.....
44	80-41-2	4.24	.....
54	81-52-1	4.26	.....
22	82-38-1	4.39	.....
55		4.39	.....
46	81-41-5	4.46	.....
24	82-38-7	4.47	.....
40	82-43-10	4.51	.....
33	80-43-3	4.53	.....
12	78-183	4.54	.....
65	82-6	4.54	.....
28	81-39-4	4.56	.....
64	78-64	4.60	.....
17	80-38-1	4.62	.....
61	78-38	4.68	.....
05	78-32-III	4.69	.....
39	82-43-2	4.69	.....
63	78-63	4.69	.....
18	80-38-3	4.70	.....
31	82-39-3	4.70	.....
52	81-49-6	4.77	.....
15	82-3	4.78	+.....
35	81-43-1	4.80	+.....
47	80-42-1	4.81	+.....
50	80-49-3	4.86	+.....
20	81-38-3	4.89	+.....
01	78-180	4.90	+.....
37	81-43-9	4.95	+.....
38	82-43-9	4.95	+.....
42	81-40-9	4.95	+.....
29	81-39-7	4.98	*.....
26	80-39-4	4.99	*.....
10	78-195	5.01	*.....
49	81-42-5	5.01	*.....
51	81-49-2	5.03	*.....
07	78-246	5.04	*.....
48	81-42-1	5.06	*.....
13	78-187	5.10	*.....
19	81-38-1	5.12	*.....
25	80-39-1	5.18	*.....
56	80-55-1	5.40	*+.....
30	82-39-1	5.55	**++.....
27	81-39-1	5.67	***+.....
06	MALMAISON	5.71	***+.....
09	78-164	5.89	****.....
62	78-35	5.97	****++.....
53	80-52-2	5.99	****++.....
57	80-56-1	6.12	****++++++.....

Tukey test of differences between variety means at the second scoring at Wisley in 1982 including neighbouring plots as covariates in the ANOVA.

+ significant at 5% level, * significant at 1% level.

16	82-2	2.88	
43	81-40-10	3.38	.
11	78-178	3.54	..
21	81-38-7	3.65	...
14	82-1	4.03	+...
04	78-88	4.06	*....
08	78-240	4.13	*.....
34	80-43-5	4.16	*.....
45	81-41-3	4.18	*.....
36	81-43-3	4.21	*.....
41	80-40-3	4.24	*.....
44	80-41-2	4.24	*.....
54	81-52-1	4.26	*.....
22	82-38-1	4.39	*+.....
55		4.39	*+.....
46	81-41-5	4.46	*++.....
24	82-38-7	4.47	*++.....
40	82-43-10	4.51	*++.....
33	80-43-3	4.53	*++.....
12	78-183	4.54	*++.....
65	82-6	4.54	*++.....
28	81-39-4	4.56	**++.....
64	78-64	4.60	**++.....
17	80-38-1	4.62	**++.....
61	78-38	4.68	**++.....
05	78-32-III	4.69	**++.....
39	82-43-2	4.69	**++.....
63	78-63	4.69	**++.....
18	80-38-3	4.70	**++.....
31	82-39-3	4.70	**++.....
52	81-49-6	4.77	***+.....
15	82-3	4.78	***+.....
35	81-43-1	4.80	***+.....
47	80-42-1	4.81	***+.....
50	80-49-3	4.86	****.....
20	81-38-3	4.89	****.....
01	78-180	4.90	****.....
37	81-43-9	4.95	****+.....
38	82-43-9	4.95	****+.....
42	81-40-9	4.95	****+.....
29	81-39-7	4.98	****++.....
26	80-39-4	4.99	****++.....
10	78-195	5.01	****++.....
49	81-42-5	5.01	****++.....
51	81-49-2	5.03	****+++.....
07	78-246	5.04	****+++.....
48	81-42-1	5.06	****++++.....
13	78-187	5.10	****+++++.....
19	81-38-1	5.12	****++++++.....
25	80-39-1	5.18	****+++++++++.....
56	80-55-1	5.40	**********++++++++.....
30	82-39-1	5.55	************++++++++++.....
27	81-39-1	5.67	****************++++++++++++++.....
06	MALMAISON	5.71	****************++++++++++++++.....
09	78-164	5.89	****************************++++++++++.....
62	78-35	5.97	********************************++++++++++++++.....
53	80-52-2	5.99	********************************++++++++++++++.....
57	80-56-1	6.12	************************************++++++++++++.....

t test of differences between variety means at the second scoring at Wisley in 1982 including neighbouring plots as covariates in the ANOVA.

+ significant at 5% level, * significant at 1% level.

094	81-54-13	1.74	
076	82-46-2	1.75	.
086	82-54-4	1.82	..
134	81-52-4	1.94	...
074	82-45-7	1.96	....
100	81-32-9	1.97	.....
130	81-50-3	1.97	.....
137	81-53-3	1.97	.....
138	81-53-5	1.97	.....
113	81-41-5	1.98	.....
016	82-2	2.00	.....
087	82-54-5	2.01	.....
127	81-49-6	2.02	.....
078	82-46-4	2.06	.....
079	82-46-6	2.08	.....
117	81-42-10	2.08	.....
124	81-48-7	2.11	.....
075	82-46-1	2.12	.....
105	81-36-6	2.12	.....
066	82-39-7	2.13	.....
114	81-41-7	2.13	.....
115	81-42-8	2.13	.....
008	78-240	2.14	.....
077	82-46-3	2.15	.....
121	81-48-2	2.15	.....
031	82-39-3	2.16	.....
099	81-32-7	2.17	.....
136	81-53-1	2.17	.....
131	81-50-6	2.18	.....
133	81-52-1	2.18	.....
135	81-52-7	2.19	.....
109	81-37-3	2.23	.....
067	82-39-8	2.24	.....
101	81-34-4	2.24	.....
118	81-42-12	2.29	.....
128	81-49-1	2.31	.....
073	82-45-5	2.33	.....
091	82-54-9	2.34	.....
096	81-32-1	2.35	.....
112	81-37-6	2.35	.....
120	81-42-14	2.35	.....
119	81-42-13	2.36	.....
071	82-45-3	2.38	.....
097	81-32-3	2.38	.....
132	81-50-7	2.38	.....
080	82-46-7	2.39	.....
123	81-48-6	2.40	.....
069	82-43-6	2.41	.....
098	81-32-6	2.41	.....
009	78-164	2.42	.....
125	81-48-10	2.46	.....
022	82-38-1	2.52	.....
102	81-34-6	2.53	.....
081	81-54-1	2.54	.....
015	82-3	2.55	.....
107	81-36-12	2.58	.....
038	82-43-9	2.59	.....
072	82-45-4	2.59	.....
030	82-39-1	2.60	.....
108	81-37-1	2.60	.....
039	82-43-2	2.69	.....
070	82-45-2	2.69	.....
103	81-36-1	2.70	.....
111	81-37-5	2.70	.....
126	81-49-5	2.70	.....
110	81-37-4	2.73	.....
104	81-36-3	2.74	.....
106	81-36-10	2.78	.....
014	82-1	2.87	+++.....
129	81-49-2	2.91	**+.....
116	81-42-9	2.92	**+.....
002	78-92	3.00	***+.....
122	81-48-5	3.02	***++.....

Tukey test of differences between variety means at the first scoring at Egham in 1982 including neighbouring plots as covariates in the ANOVA.

```
094   81-54-13 1.74
076   82-46-2  1.75  .
086   82-54-4  1.82  ..
134   81-52-4  1.94  ...
074   82-45-7  1.96  ....
100   81-32-9  1.97  .....
130   81-50-3  1.97  ......
137   81-53-3  1.97  .......
138   81-53-5  1.97  ........
113   81-41-5  1.98  .........
016   82-2     2.00  ..........
087   82-54-5  2.01  ...........
127   81-49-6  2.02  ............
078   82-46-4  2.06  .............
079   82-46-6  2.08  ..............
117   81-42-10 2.08  ...............
124   81-48-7  2.11  ................
075   82-46-1  2.12  .................
105   81-36-6  2.12  .................
066   82-39-7  2.13  ..................
114   81-41-7  2.13  ..................
115   81-42-8  2.13  ..................
008   78-240   2.14  ...................
077   82-46-3  2.15  ...................
121   81-48-2  2.15  ...................
031   82-39-3  2.16  ....................
099   81-32-7  2.17  ....................
136   81-53-1  2.17  ....................
131   81-50-6  2.18  ....................
133   81-52-1  2.18  .....................
135   81-52-7  2.19  .....................
109   81-37-3  2.23  ++...................
067   82-39-8  2.24  ++...................
101   81-34-4  2.24  ++...................
118   81-42-12 2.29  ++...................
128   81-49-1  2.31  +++..................
073   82-45-5  2.33  +++..................
091   82-54-9  2.34  +++..................
096   81-32-1  2.35  +++..................
112   81-37-6  2.35  +++..................
120   81-42-14 2.35  +++..................
119   81-42-13 2.36  +++..................
071   82-45-3  2.38  +++..................
097   81-32-3  2.38  +++..................
132   81-50-7  2.38  +++..................
080   82-46-7  2.39  *++..................
123   81-48-6  2.40  **+..................
069   82-43-6  2.41  **+..................
098   81-32-6  2.41  **+..................
009   78-164   2.42  **++.................
125   81-48-10 2.46  ***+++++..............
022   82-38-1  2.52  ***++++++............
102   81-34-6  2.53  ***++++++............
081   81-54-1  2.54  ***++++++............
015   82-3     2.55  ***++++++............
107   81-36-12 2.58  ****+++++............
038   82-43-9  2.59  ****+++++............
072   82-45-4  2.59  ****+++++............
030   82-39-1  2.60  ****++++++...........
108   81-37-1  2.60  ****++++++...........
039   82-43-2  2.69  **********+++++.......
070   82-45-2  2.69  **********+++++.......
103   81-36-1  2.70  **********+++++.......
111   81-37-5  2.70  **********+++++.......
126   81-49-5  2.70  **********+++++.......
110   81-37-4  2.73  **********+++++.......
104   81-36-3  2.74  **********+++++.......
106   81-36-10 2.78  **********++++++......
014   82-1     2.87  ***********++++++.....
129   81-49-2  2.91  ***********++++++.....
116   81-42-9  2.92  ***********++++++.....
002   78-92    3.00  ***********++++++.....
122   81-48-5  3.02  ***********++++++.....
```

t test of differences between variety means at the first scoring at Egham in 1982 including neighbouring plots as covariates in the ANOVA.

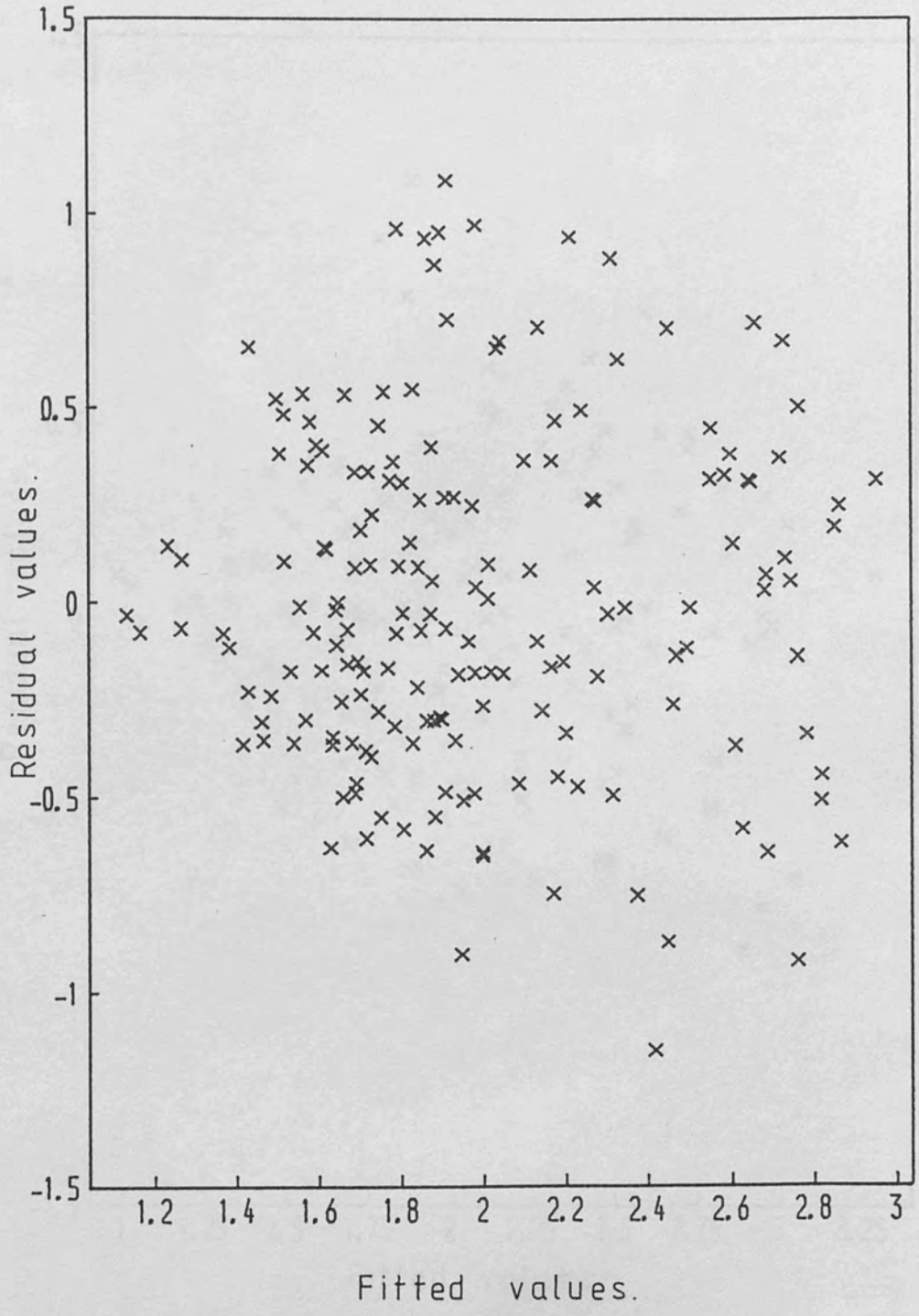
094	81-54-13	3.68	
087	82-54-5	3.69	.
086	82-54-4	4.04	..
105	81-36-6	4.13	...
137	81-53-3	4.40	....
101	81-34-4	4.42	.....
130	81-50-3	4.46	.....
121	81-48-2	4.53	.....
120	81-42-14	4.56	.....
115	81-42-8	4.58	.....
114	81-41-7	4.61	.....
127	81-49-6	4.63	.....
113	81-41-5	4.64	.....
128	81-49-1	4.64	.....
136	81-53-1	4.70	.....
119	81-42-13	4.72	.....
133	81-52-1	4.72	.....
124	81-48-7	4.73	.....
103	81-36-1	4.80	.....
132	81-50-7	4.81	.....
100	81-32-9	4.82	.....
122	81-48-5	4.82	.....
118	81-42-12	4.83	.....
022	82-38-1	4.84	.....
131	81-50-6	4.88	.....
117	81-42-10	4.89	.....
009	78-164	4.90	.....
031	82-39-3	4.90	.....
067	82-39-8	4.90	.....
069	82-43-6	4.95	.....
123	81-48-6	4.96	.....
112	81-37-6	4.98	.....
008	78-240	5.02	.....
126	81-49-5	5.05	.....
075	82-46-1	5.06	.....
099	81-32-7	5.07	.....
135	81-52-7	5.07	.....
109	81-37-3	5.12	.....
015	82-3	5.14	.....
081	81-54-1	5.14	.....
107	81-36-12	5.14	.....
016	82-2	5.16	.....
074	82-45-7	5.16	.....
080	82-46-7	5.17	.....
134	81-52-4	5.17	.....
129	81-49-2	5.21	.....
138	81-53-5	5.22	.....
079	82-46-6	5.24	.....
076	82-46-2	5.25	.....
038	82-43-9	5.28	.....
091	82-54-9	5.28	.....
106	81-36-10	5.28	.....
111	81-37-5	5.31	++.....
030	82-39-1	5.33	++.....
078	82-46-4	5.33	++.....
066	82-39-7	5.38	++.....
104	81-36-3	5.41	++.....
097	81-32-3	5.42	++.....
098	81-32-6	5.43	++.....
073	82-45-5	5.45	++.....
110	81-37-4	5.47	**.....
071	82-45-3	5.49	**.....
077	82-46-3	5.50	**.....
072	82-45-4	5.52	**.....
116	81-42-9	5.53	**.....
125	81-48-10	5.55	**.....
102	81-34-6	5.65	**+.....
039	82-43-2	5.79	**++.....
096	81-32-1	5.80	**++.....
014	82-1	5.86	***+.....
108	81-37-1	5.93	****.....
002	78-92	5.95	****.....
070	82-45-2	5.97	****.....

Tukey test of differences between variety means at the second scoring at Egham in 1982 including neighbouring plots as covariates in the ANOVA.

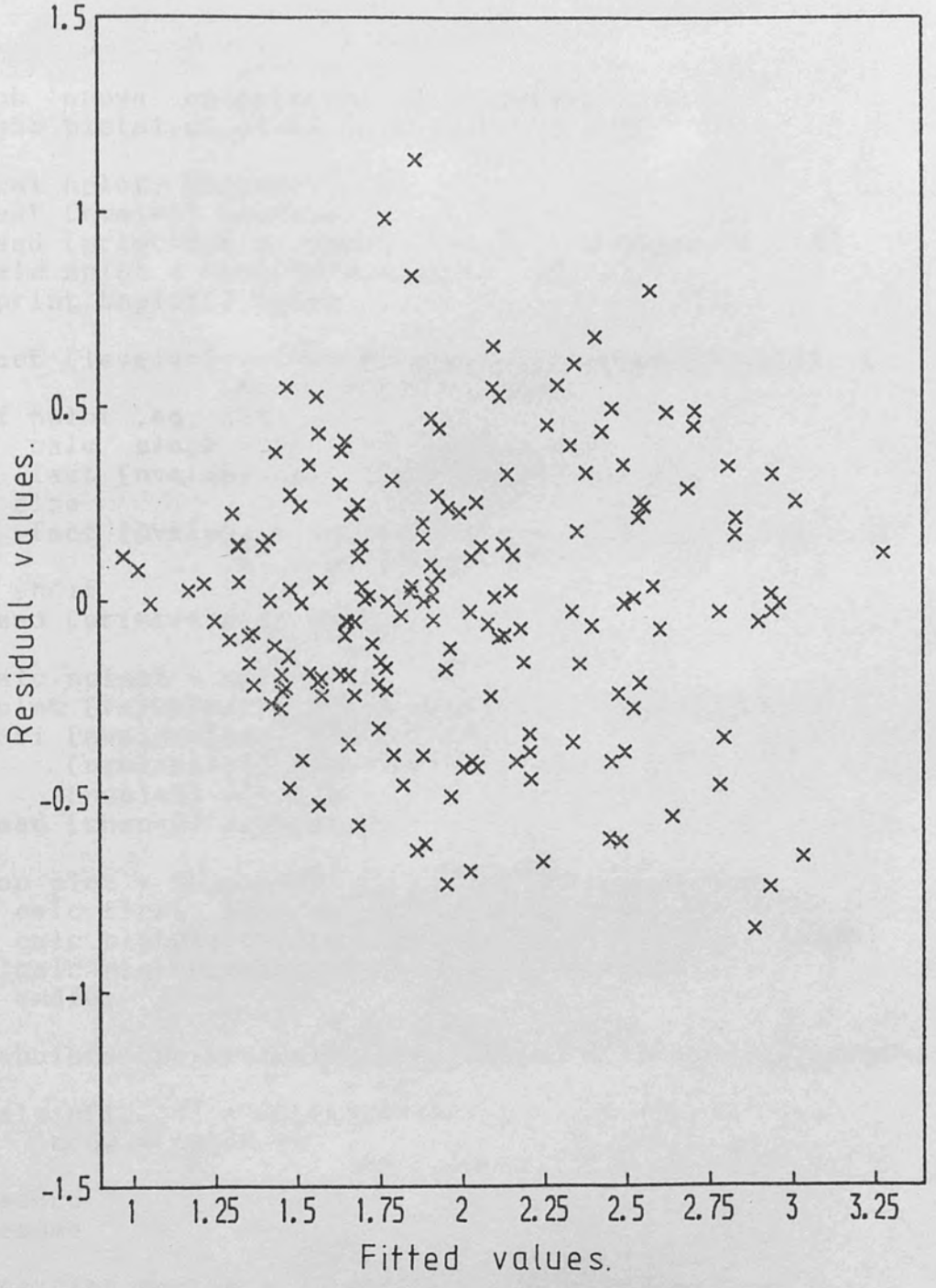
```
094   81-54-13 3.68
087   82-54-5  3.69  .
086   82-54-4  4.04  ..
105   81-36-6  4.13  ...
137   81-53-3  4.40  ....
101   81-34-4  4.42  +....
130   81-50-3  4.46  ++....
121   81-48-2  4.53  ++.....
120   81-42-14 4.56  ++......
115   81-42-8  4.58  ++.......
114   81-41-7  4.61  ++........
127   81-49-6  4.63  ++.........
113   81-41-5  4.64  ++..........
128   81-49-1  4.64  ++..........
136   81-53-1  4.70  **..........
119   81-42-13 4.72  **..........
133   81-52-1  4.72  **..........
124   81-48-7  4.73  **..........
103   81-36-1  4.80  **+.........
132   81-50-7  4.81  **+.........
100   81-32-9  4.82  **+.........
122   81-48-5  4.82  **+.........
118   81-42-12 4.83  **+.........
022   82-38-1  4.84  **+.........
131   81-50-6  4.88  **++........
117   81-42-10 4.89  **++........
009   78-164   4.90  **++........
031   82-39-3  4.90  **++........
067   82-39-8  4.90  **++........
069   82-43-6  4.95  **++........
123   81-48-6  4.96  **++........
112   81-37-6  4.98  **++........
008   78-240   5.02  ***+........
126   81-49-5  5.05  ***+........
075   82-46-1  5.06  ***+........
099   81-32-7  5.07  ***+........
135   81-52-7  5.07  ***+........
109   81-37-3  5.12  ****........
015   82-3     5.14  ****+.......
081   81-54-1  5.14  ****+.......
107   81-36-12 5.14  ****+.......
016   82-2     5.16  ****++......
074   82-45-7  5.16  ****++......
080   82-46-7  5.17  ****++......
134   81-52-4  5.17  ****++......
129   81-49-2  5.21  ****+++.....
138   81-53-5  5.22  ****+++.....
079   82-46-6  5.24  ****+++.....
076   82-46-2  5.25  ****+++.....
038   82-43-9  5.28  ****++++....
091   82-54-9  5.28  ****++++....
106   81-36-10 5.28  ****++++....
111   81-37-5  5.31  ****+++++...
030   82-39-1  5.33  ****++++++..
078   82-46-4  5.33  ****++++++..
066   82-39-7  5.38  *****++++++++
104   81-36-3  5.41  ******++++++++
097   81-32-3  5.42  ******++++++++
098   81-32-6  5.43  ******++++++++
073   82-45-5  5.45  *******+++++++
110   81-37-4  5.47  *******++++++++++
071   82-45-3  5.49  *******++++++++++
077   82-46-3  5.50  *******++++++++++
072   82-45-4  5.52  ********+++++++++
116   81-42-9  5.53  ********+++++++++
125   81-48-10 5.55  *********++++++++++
102   81-34-6  5.65  ***********++++++++++++
039   82-43-2  5.79  ******************++++++++++++
096   81-32-1  5.80  ********************++++++++++++
014   82-1     5.86  ************************++++++++++++
108   81-37-1  5.93  ****************************++++++++++++++
002   78-92    5.95  ******************************++++++++++++++
070   82-45-2  5.97  ********************************++++++++++++++
```

t test of differences between variety means at the second scoring at Egham in 1982 including neighbouring plots as covariates in the ANOVA.

APPENDIX 6



Scatter diagram of plot residuals against fitted values for the first scoring at Wisley in 1982 using the randomised complete block model.



Scatter diagram of plot residuals against fitted values for the first scoring of the trial at Wisley in 1982 using the randomised complete block with neighbouring plots as covariates model.

Appendix 7. Genstat 5 program written to analyse data from the trials in 1982.

```
job 'anova  on antirrhinum plotmeans'
"g5b plota1.g5,plota1.out,in2=xxxx.pdat"

scal nplot, bnplot[1...3]
text [nval=1] heading
read [print=d,e,s; chan=2] heading & bnplot[1...3]
calc nplot = vsum(!p(bnplot[1...3]))
 print bnplot[],nplot

fact [levels=3;value= #bnplot[1](1),#bnplot[2](2), \
                #bnplot[3](3)] block
if nplot .eq. 228
   calc  block = !(77(1),73(2),3,5(2),72(3))
   fact [nvalues=nplot;levels=140] variety
  else
   fact [nvalues = nplot;levels=!(1,(4...22),(24...31),(33...57),\
            (61...65))] variety
  endif
read [print=d,e,s; chan=2] variety

calc nplant = nplot * 9
point [value=north,south,east,west] neighbours
vari [nval=nplant] plantscore
&    [nval=nplot] plotscore, neighbours[]
&    [nval=9] plotplant
read [chan=2] plantscore

for plot = 1...nplot
  calc first, last = 1,9 +(plot-1) * 9
  calc plotplant$[1...9] = plantscore$[first...last]
  calc plotscore$[plot] = mean(plotplant)
  endfor
"
tabulate [print=counts,nobs;class=variety,block;margins=yes] plots
"
calc n[1...4] = shift(plotscore;+1,-1,+6,-6)
&   nrow = nplot /6
"
record
resume
"
restrict vector = neighbours[1,2]; condition =  \
    !((0,5(1))#nrow), !((5(1),0)#nrow)
calc neighbours[] =  n[]
restrict neighbours[1,2] ; !(#nplot(1))
```

```
calc neighbours[] = mvreplace(neighbours[];plotscore)
"
print  plotscore,n[], neighbours[], variety,block \
                          ; field = 5 ; dec=2
"
"
if nplot .eq. 228
restrict plotscore,block,variety,neighbours[];     \
 variety .ne. 7 .and. variety .ne. 23\
.and. variety .ne. 40 .and. variety .ne. 89
else
restrict plotscore,block,variety,neighbours[];variety .ne. 100
endif
"
block block
treat variety
anova [fprob=yes] plotscore; resid = nresid; fit=nfitval
covariate neighbours[]
anova [fprob=yes] plotscore; resid =covresid; fit=covfitval
record "[2]
resume

graph [multi=1,1] nresid,covresid;nfitval,covfitval

&    [multi=1,1,1,1] neighbours[];covresid
graph [multi=1,1,1,1]  neighbours[];plotscore
"
stop
```

Appendix 8. Program listing of the Fortran 77 program EPIGAR.

```
      C
      C   EPIDEMIC SIMULATION EPIGAR
      C
      C
      C   Disease increase within gardens and spread between
      C   gardens are simulated by a deterministic model, specified
      C   by FUNCTIONS and SUBROUTINES.
      C   The gardens are arranged in a 11 * 11 grid.
      C   Disease levels at time t(T), t-1(TLAT), t-2(TLATA) are used
      C   to calculate spore production (ARRAY SPORES).
      C   Spread between gardens is a function (DISPERSAL) of DISTance
      C   and the SCALEing factor.
      C   The mode of increase is defined by the SUBROUTINE INCREASE
      C   for exponential growth and LOGISTIC for a growth model
      C   including a maximum disease level DISMAX
      C   The PROGeny/PARent ratio (r) is a parameter of increase.

      C
      C   MANY IMPLIED TYPES ARE USED
      C
            INTEGER T, TLAT, TLATA, TIME
            LOGICAL OVER, EXFLAG
            EXTERNAL DISPERSAL

      C          DISEASE ARRAY, DISEASE(TIME, EAST-WEST, N-S)
            COMMON DISEASE(3,11,11),SPORES(11,11),TIME,T,TLAT,TLATA

      C  OPEN FILE FOR GRAPH OUTPUT DATA
            OPEN (UNIT = 8, STATUS = 'NEW', FILE = 'EPIGAR.OUT')

      C     SET MODEL PARAMETERS
      10    WRITE (6,30)
      30    FORMAT (/'    INPUT DATA'//)
            READ (5,*)  (SCALE, PROGPAR, DISMAX)
      C     SENTINAL PROGPAR LESS THAN 1
            IF (PROGPAR .LT. 0)  STOP

            IF (DISMAX .LT. 0) THEN
              WRITE(6,40)  (SCALE, PROGPAR)
              WRITE(8,40)  (SCALE, PROGPAR)
              EXFLAG = .TRUE.
            ELSE
              WRITE(6,50) (SCALE, PROGPAR, DISMAX)
              WRITE(8,50) (SCALE, PROGPAR, DISMAX)
              EXFLAG = .FALSE.
            ENDIF

      40    FORMAT (' S=', F6.1, '    R=', F6.1, ' EXPONENTIAL MODEL')
      50    FORMAT (' S=', F6.1, '    R=', F6.1, 'MAX=', F6.0)

```

```
C          CLEAR DISEASE ARRAY
C                AND SPORE ARRAY
      DO 100 J = 1, 11
        DO 100 K = 1, 11
          SPORES(J,K) = 0
          DO 100 I = 1, 3
            DISEASE(I,J,K)= 0
100   CONTINUE

C          SET INITIAL DISEASE FOCI
C               AT TIME = 0
      DISEASE(2,6,6) = 1

C          SET UP LOOP OVER TIME
      DO 190, TIME = 1, 10

C          FIND THE TIME INDEX VALUES.
C          THESE ARE RECYCLED IN 3 TIME UNITS
        CALL TIMEFIND

C          CALCULATE SPORE PRODUCTION IN EACH GARDEN
        CALL SPORE

C          CALC SPORES IN EACH GARDEN
        CALL GARDENS(SCALE, PROGPAR, DISMAX, EXFLAG, OVER)

C          Abandon if disease levels are very high
        IF (OVER) THEN
          WRITE(6,*)' Disease levels are very high.  Simulation
stoped'
          GOTO 10
        END IF

        CALL PRINTOUT
        CALL GRAPHDATA( DISEASE, T, SPORES, TIME, 11)

190   CONTINUE
      GOTO 10
      END

      SUBROUTINE TIMEFIND
      INTEGER TIME,T,TLAT,TLATA
      COMMON DISEASE(3,11,11),SPORES(11,11),TIME,T,TLAT,TLATA
C        CALCULATES THE INDEX VALUES TO USE WITH DISEASE ARRAY
      X = TIME
      X = X/3
      X = 3*(X-INT(X))-1

      IF  (X .LT. -0.01) THEN
C       REMAINDER IS 0
        T    = 2
        TLAT  = 1
        TLATA = 3
```

```
104               ELSE IF (X .GT. 0.01) THEN
105           C     REMAINDER IS 2
106                 T = 1
107                 TLAT  = 3
108                 TLATA = 2
109
110               ELSE
111           C     REMAINDER IS 1
112                 T    = 3
113                 TLAT  = 2
114                 TLATA = 1
115               END IF
116               RETURN
117               END
118
119               SUBROUTINE SPORE
120           C         CALCULATES SPORE PRODUCTION ARRAY
121               INTEGER TIME,T,TLAT,TLATA
122               COMMON DISEASE(3,11,11),SPORES(11,11),TIME,T,TLAT,TLATA
123               DO 200 I = 1, 11
124                 DO 200 J = 1, 11
125           C       Calculate active disease as disease one time unit ago
-
126           C       disease two time units ago.
127                   SPORES(I,J) = DISEASE(TLAT,I,J) - DISEASE(TLATA,I,J)
128           200   CONTINUE
129               RETURN
130               END
131
132               SUBROUTINE GARDENS(SCALE,PROGPAR,DISMAX, EXFLAG, OVER)
133           C       CALCULATES DISEASE INCREASE IN EACH GARDEN
134               INTEGER TIME,T,TLAT,TLATA
135               LOGICAL OVER, EXFLAG
136               COMMON DISEASE(3,11,11),SPORES(11,11),TIME,T,TLAT,TLATA
137
138           C   SET OVERFLOW FLAG
139               OVER = .FALSE.
140
141           C   FOR EACH GARDEN...
142               DO 110 J = 1, 11
143                 DO 110 I = 1, 11
144
145           C       SET NEW DISEASE TO 0
146                   DISEASE(T,I,J) = 0
147
148           C       CALCULATE THE DISEASE ARRIVAL FORM ALL GARDENS
149                   DO  90 K = 1, 11
150                     DO  90 L = 1, 11
151
152           C           DISTANCE BETWEEN PLOTS
153                       IF ((I .EQ. K) .AND. (J .EQ. L)) THEN
154
155           C             WITHIN GARDEN INCREASE
156                         DISEASE(T,I,J)=DISEASE(T,I,J)+SPORES(I,J)
```

```
                    ELSE
C                     CALCULATE DISTANCE BY PYTHAG.
                      IDEW = I - K
                      IDNS = J - L
                      DIST = SQRT(FLOAT(IDEW*IDEW+IDNS*IDNS))

C                     USE DISPERSAL FUNCTION TO FIND SPORE TRANSFER
C                     FROM (K,L) TO (I,J)
                      DISEASE(T,I,J)=DISEASE(T,I,J)+DISPERSAL(DIST,SCA
LE)*
     1                                SPORES(K,L)
                    END IF

 90               CONTINUE

C                 CALCULATE THE INCREASE

                  IF (EXFLAG) THEN
C                   USE EXPONENTIAL INCREASE SUBROUTINE
                    CALL INCREASE(DISEASE(T,I,J), DISEASE(TLAT,I,J), PRO
GPAR)

                  ELSE
C                   USE LOGISTIC INCREASE SUBROUTINE
                    CALL LOGISTIC(DISEASE(T,I,J), DISEASE(TLAT,I,J),
     1                PROGPAR, DISMAX)
                  ENDIF

C                   CHECK FOR OVERFLOW
                  IF (DISEASE(T,I,J) .GT. 999999) THEN
                    OVER = .TRUE.
                    GOTO 111
                  END IF

110           CONTINUE
111           RETURN
              END

              SUBROUTINE PRINTOUT
C             PRINT ACTIVE AND TOTAL DISEASE MAPS
              INTEGER T, TLAT, TLATA, TIME
              COMMON DISEASE(3,11,11),SPORES(11,11),TIME,T,TLAT,TLATA
                WRITE (6,900) TIME
                WRITE (6,890) (SPORES)
                WRITE (6,911) ((DISEASE(T,IP,JP),JP=1,11),IP=1,11)
890           FORMAT (1X'   ACTIVE DISEASE'/11(1X,11(F10.3)/))
900           FORMAT  ( 1X//'  MAPS AT TIME = ', I6)
911           FORMAT  ( 1X  ' TOTAL DISEASE' / 11(1X,11(F10.3)/))
              RETURN
              END

C             DISPERSAL DISTANCE SCALING FUNCTION
C             Calculates the proportional transfer of spores between
C             two gardens
              FUNCTION DISPERSAL(D,S)
              DISPERSAL = 1 / (S * (D ** 3))
              END
```

```
      C     INCREASE SUBROUTINE DEFINITION
            SUBROUTINE INCREASE(DIS, OLD, PROGPAR)
      C     DIS(in)       == inoculum arrivins
      C     DIS(out)      == new infection level
      C     OLD           == old infection level
      C     PROGPAR       == rate constant
      C     For exponential increase:
            DIS = OLD + (DIS * PROGPAR)
            RETURN
            END

      C    LOGISTIC INCREASE SUBROUTINE DEFINITION
            SUBROUTINE LOGISTIC(DIS, OLD, PROGPAR, DISMAX)
      C     DIS(in)       == inoculum arrivins
      C     DIS(out)      == new infection level
      C     OLD           == old infection level
      C     PROGPAR       == rate constant
      C     DISMAX        == maximum disease level
      C     For losistic increase:
            DIS = OLD+((DISMAX-OLD) * (DIS*PROGPAR) / DISMAX)

      C     This can overshoot DISMAX, especialy if PROGPAR or the
      C     influx of spores from other units is hish.
      C     If this happens, set DIS=DISMAX, the host can then be cons
idered dead

            IF (DIS .GT. DISMAX) THEN
              DIS = DISMAX
            ENDIF

            RETURN
            END

            SUBROUTINE GRAPHDATA( DISEASE, T, SPORES, TIME, SIZE)
      C     SELECTS DATA FOR PLOTING AND WRITES IT TO A FILE, UNIT 8

      C     NSECT   Number of sardens used to plot asainst time
      C     NTIMES  Number of times that a transect is output to
      C                       plot asainst distance
            INTEGER NSECT, NTIMES, TIME, TMAX, T, SIZE
            PARAMETER  (NSECT = 5,     NTIMES = 5,     TMAX  = 10)

            INTEGER SECT(NSECT), TIMES(NTIMES)
            REAL ATPLOT(TMAX,NSECT), DTPLOT(TMAX,NSECT),
           1        DISEASE(3,SIZE,SIZE), SPORES(SIZE,SIZE)

      C  ATPLOT and DTPLOT hold Active and total Disease over Time
            SAVE ATPLOT, DTPLOT

            DATA  SECT / 1,3,4,5,6 /
           1       TIMES   / 2,4,6,8,10 /

      C  CALCULATE MIDDLE OF DISEASE ARRAY
            MID = (SIZE+1) / 2
```

```
C  STORE THE VALUES NEEDED AT EVERY TIME INTERVAL
      DO 11 I = 1, NSECT
        ATPLOT(TIME,I) = SPORES(SECT(I),MID)
        DTPLOT(TIME,I) = DISEASE(T,SECT(I),MID)
11    CONTINUE

C  FIND OUT IF A DATA FOR SPREAD IS NEEDED THIS TIME

      DO 20 I = 1,NTIMES
        IF (TIME .EQ. TIMES(I)) THEN

C           OUTPUT THE VALUES NEEDED
          WRITE (8, 110) (SPORES (   MID, K), K=1,SIZE)
          WRITE (8, 110) (DISEASE(T, MID, K), K=1,SIZE)
110       FORMAT (1X, 11 F10.3)
        END IF
20    CONTINUE


C   ON THE LAST TIME, OUTPUT THE ACCUMULATED DATA OVER TIME

      IF (TIME .EQ. TMAX) THEN
        WRITE (8, 111) (' ACTIVE DISEASE OVER TIME ',ATPLOT)
        WRITE (8, 111) (' TOTAL  DISEASE OVER TIME ',DTPLOT)
111     FORMAT (1X, A40,/,5(1X, 10(F10.3)/))

      END IF

      END
```

Appendix 9. Program listing of the Fortran 77 program EPIPLOT.

```
      C   Tom Stoney.    14/9/83
            PROGRAM EPIPLOT
      C
      C   Reads data output by EPIGAR from a file EPIGAR.OUT
      C   and plots graphs using CALCOMP SUBROUTINES.
      C
      C   Graphs each have a several lines, using different plotting sy
     mbols.
      C   They are :
      C     1)  Active disease / distance at NDLINE different times
      C     1)  Total  disease / distance at NDLINE different times
      C     1)  Active disease / time     at NTLINE different position
     s
      C     1)  Total  disease / time     at NTLINE different position
     s
      C     NRUN  = number of different sets of conditions
      C     NGARD = number of gardens each way
      C     ITMAX = number of generations simulated
      C     XLEN, YLEN  = length of axis in inches
      C     XS(4) YS(4) = origin of each graph relative to previous or
     igin
      C     XH    YH    = ORIGIN OF PAGE HEADING
      C     XD(NGARD+2) = values and limits for X when X = Distance
      C     XT(ITMAX+2) = values and limits for X when X = Time
      C
            PARAMETER (NTLINE = 5,
           1           NDLINE = 5,
           1           NGARD  = 11,
           1           ITMAX  = 10,
           1           XLEN   = 2.7,
           1           YLEN   = 3.5,
           1           XH     = -3.5,
           I           YH     =  7.0)

            DIMENSION XS(4), YS(4),
           +          XD(NGARD+2), XT(ITMAX+2)

            DATA (XS(I),YS(I),I=1,4)/
           1    2.0, 3.5,
           1    3.2, 0.0,
           1   -3.2, 4.0,
           1    3.2, 0.0
           1    /

      C  Data values for X axis
            DATA XD /
           + 1.0,2.0,3.0,4.0,5.0,6.0,7.0,8.0,9.0,10.0,11.0,0.0,5.0/
           +     XT /
           + 1.0,2.0,3.0,4.0,5.0,6.0,7.0,8.0,9.0,10.0,0.0,5.0/

      C    structures to hold data and limits for ACTive and TOTal dis
     ease
```

```
      C    for graphs against TIMe and DIStance
           DIMENSION ACTTIM((NTLINE * ITMAX) + 2),
          +          TOTTIM((NTLINE * ITMAX) + 2),
          +          ACTDIS((NDLINE * NGARD) +2),
          +          TOTDIS((NDLINE * NGARD) +2)

           CHARACTER TEXT*40, HEADING*40

      C    NUMBER OF POINTS IN DISTANCE GRAPHS
           NPNTD = NDLINE * NGARD
      C    NUMBER OF POINTS IN TIME GRAPHS
           NPNTT = NTLINE * ITMAX

      C       SET PLOTTING AREA
      C      SCREEN
      C    CALL START(3.0,7.5)
      C      PLOTER
           CALL START(25.5,22.0)

      C    open data file
           OPEN (UNIT=9, STATUS='OLD', FILE='EPIGAR.OUT')

           READ (9, *) (NRUN)
      C    for each set of conditions
           DO 10 IRUN = 1, NRUN

      C    read in data
           READ (9,110) (HEADING)
           CALL READY(ACTDIS, TOTDIS, NGARD, NDLINE)
           READ (9,110) (TEXT)
           READ (9,*)   (ACTTIM(I),I=1, (NTLINE*ITMAX))
           READ (9,110) (TEXT)
           READ (9,110) (TEXT)
           READ (9,*)   (TOTTIM(I),I=1, (NTLINE*ITMAX))
           READ (9,110) (TEXT)
      110  FORMAT (A40)


      C    4 CALLs to GRAPH
      C    for testing put C infront of 3 of them

           CALL GRAPH(XS(1), YS(1), ACTDIS, NPNTD, XD, NGARD, NDLINE,
          +      'POSITION', 8, 'ACTIVE PATHOGEN', 15, XLEN, YLEN)

           CALL GRAPH(XS(2), YS(2), ACTTIM, NPNTT, XT, ITMAX, NTLINE,
          +      'TIME', 4, 'ACTIVE PATHOGEN', 15, XLEN, YLEN)

           CALL GRAPH(XS(3), YS(3), TOTDIS, NPNTD, XD, NGARD, NDLINE,
          +      'POSITION', 8, ' TOTAL PATHOGEN', 15, XLEN, YLEN)

           CALL GRAPH(XS(4), YS(4), TOTTIM, NPNTT, XT, ITMAX, NTLINE,
          +      'TIME', 4, ' TOTAL PATHOGEN', 15, XLEN, YLEN)

```

```
C     PAGE HEADING
      CALL SYMBOL(XH, YH, 0.14, HEADING, 0.0, 40)

C     For all but the first, must move to a new page
      XS(1) = 5.3
10    YS(1) = -3.5

      CALL ENPLOT
      END


C   PLOT 1 GRAPH:
      SUBROUTINE GRAPH(XSTART, YSTART, YDATA, NDATA, XDATA, NPNT
,
     +           NLINE, XLABLE, NCX, YLABLE, NCY, XLEN, YLEN)
C      NUMBER OF POINTS PERLINE +2
      PARAMETER (MAXPNT = 13)

C     NDAT    = length of input array
C     NPNT    = no. points on each line
C     MAXPNT  = 2 +  max value of NPNT allowed (PARAMETER)
C     NCX NCY     no. characters per lable
C     XLEN YLEN   axis length (inches)
C
C     Y data is input as one long array, with scaling info on th
e end
C     split this data into lines

      DIMENSION YLINE(MAXPNT),
     + YDATA(*), XDATA(*)

      CHARACTER XLABLE*(*), YLABLE*(*)

C     CALCULATE Y SCALE
      CALL SCALE(YDATA, YLEN, NDATA, 1)

C    SET ORIGIN
      CALL PLOT(XSTART, YSTART, -3)

      CALL AXIS(0.0, 0.0, YLABLE,  NCY, YLEN, 90.0,
     +          YDATA(NDATA+1), YDATA(NDATA+2))

      CALL AXIS(0.0, 0.0, XLABLE, -NCX, XLEN, 0.0,
     +          XDATA(NPNT+1), XDATA(NPNT+2))
```

```
144           C     SET SCALING FACTORS FOR ALL LINES
145                 YLINE(NPNT+1) = YDATA(NDATA+1)
146                 YLINE(NPNT+2) = YDATA(NDATA+2)
147
148                 DO 20 I = 0, ((NLINE-1)*NPNT), NPNT
149                   DO 30 J = 1, NPNT
150           30        YLINE(J) = YDATA(I+J)
151
152                   CALL LINE(XDATA, YLINE, NPNT, 1, 1, I)
153           20    CONTINUE
154
155                 END
156
157           C   READ in data for Y variate
158                 SUBROUTINE READY(FIRST, SECOND, NPNTS, NLINE)
159           C     READs alternate lines of NPNTS values into arrays, FIRST a
nd SECOND
160           C     for NLINE lines of each array.
161                 DIMENSION FIRST(*), SECOND(*)
162
163                 LASTIS = (NLINE-1)*NPNTS+1
164                 DO 10 ISTART = 1, LASTIS, NPNTS
165                   IFIN  = ISTART + NPNTS - 1
166           C          READ one line
167                   READ (9, *) (FIRST(I), I = ISTART, IFIN)
168                   READ (9, *) (SECOND (I), I = ISTART, IFIN)
169           10    CONTINUE
170           100   FORMAT (11(F10.3))
171                 END
-----
```

Appendix 10 Plotter driving subrountines called from the Calcomp graphics subroutine library.

Calcomp subroutines:

START(X,Y)
Plotter initialisation.

SCALE(ARRAY,AXLEN,NPTS,INC)
Examines data in ARRAY and returns scaling information for the AXIS subroutine.

PLOT(XPAGE,YPAGE,IPEN)
With IPEN=-3 sets the origin of each graph.

AXIS(X,Y,STRING,NCHAR,AXLEN,ANGLE,FIRSTV,DELTAV)
Draws and anotates a graph axis.

LINE(XARRAY,YARRAY,NPTS,INC,LINTYPE,INTEQ)
Plots one line on the graph, with the points marked.

SYMBOL(X,Y,HEIGHT,STRING,ANGLE,NCHAR)
Writes text.

ENPLOT
Ends plotting.

Selected variables from EPIGAR

Name:	Type:	Meaning:
TIME	INTEGER	time from start of simulation
T	INTEGER	Indecies for the array
TLAT	INTEGER	DISEASE(time,X,Y) to select values
TLATA	INTEGER	at time TIME,TIME-1 and TIME-2
DISEASE(3,11,11)	REAL	Total disease levels in current and latest two time units.
SPORES(11,11)	REAL	Active disease calculated as the increase during the last time unit.