

HOST DENSITY EFFECTS ON
FUNGAL DISEASES OF PLANTS

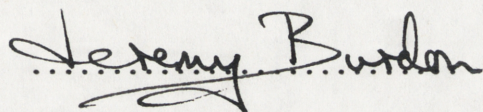
by

Jeremy James Burdon

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With the exception of the assistance acknowledged on
the following page, all work reported in this thesis was carried
out by myself.

A handwritten signature in cursive script that reads "Jeremy Burdon". The signature is written in dark ink and has a decorative flourish at the end of the word "Burdon".

J.J. BURDON

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TABLE OF CONTENTS

	Page
Summary	i
SECTION A: GENERAL INTRODUCTION - DENSITY-DEPENDENT EFFECTS IN PLANT POPULATIONS	1
I. PLANT-ENVIRONMENT INTERACTIONS	1
II. PLANT-PLANT INTERACTIONS	4
1. <i>The control of recruitment into a population</i>	4
a) <i>The availability of seed</i>	5
b) <i>The suitability of micro-sites</i>	6
2. <i>Mortality in a population</i>	7
a) <i>The 'thinning' process in single species stands</i>	7
b) <i>The thinning process in mixtures</i>	9
3. <i>The causes of mortality in a population</i>	10
a) <i>The causes of mortality in pure stands</i>	10
b) <i>The causes of mortality in mixtures</i>	11
III. PLANT-PARASITE INTERACTIONS	13
1. <i>The effect of parasites on single species populations</i>	13
a) <i>Recruitment</i>	13
b) <i>Mortality of plants</i>	14
2. <i>The effect of parasites in mixtures</i>	15
a) <i>The problem of co-existence of species</i>	15
b) <i>The role of parasites in niche separation</i>	16

	Page
IV. THE OBJECTIVES OF THE PRESENT PROJECT	22
V. THE ORGANIZATION OF THIS THESIS	24
SECTION B: CONTROLLED ENVIRONMENT EXPERIMENTS ON EPIDEMICS CAUSED BY A SOIL-BORNE PLANT PATHOGEN	26
CHAPTER ONE: GENERAL EXPERIMENTAL APPROACHES	26
I. INTRODUCTION	26
a) <i>The relevant biology of Pythium irregulare Buisman</i>	26
b) <i>Host plant situations studied</i>	28
c) <i>Disease parameters studied</i>	28
II. GENERAL METHODS	29
1. <i>Physical environmental conditions</i>	29
2. <i>Establishment of host plant arrays and initiation of disease</i>	29
a) <i>Stocks of host plant and pathogen</i>	29
b) <i>Setting up and planting of seed boxes</i>	30
c) <i>Preparation of inoculum and initiation of disease</i>	30
3. <i>Collection and analysis of data</i>	32
a) <i>Collecting data on the different disease parameters</i>	32
b) <i>Analysis of data</i>	33
CHAPTER TWO: THE EFFECT OF HOST PLANT DENSITY ON EPIDEMICS OF DAMPING-OFF	34
I. RESULTS	34
1. <i>The occurrence of primary infection foci</i>	34

	Page
a) <i>The effect of varying host density</i>	34
b) <i>The effect of varying inoculum density</i>	35
2. <i>Multiplication of disease in randomly inoculated seedling stands</i>	37
3. <i>Rate of advance of the disease front</i>	39
4. <i>The contribution of falling seedlings to disease increase and spread</i>	40
a) <i>The rate of multiplication of disease in supported stands</i>	41
b) <i>The rate of spread of disease radially outwards from a point source</i>	42
 CHAPTER THREE: <i>EPIDEMICS OF DAMPING-OFF IN MIXTURES OF TWO PLANT SPECIES</i>	 44
I. METHODS	44
II. RESULTS	46
1. <i>The occurrence of primary infection foci</i>	46
2. <i>Multiplication of disease in randomly inoculated seedling stands</i>	47
a) <i>Comparison between 50:50 mixtures and monocultures</i>	47
b) <i>The effect of varying the proportion of resistant and susceptible plants</i>	47
3. <i>Rate of advance of the disease front</i>	48
a) <i>Comparison between 50:50 mixtures and monocultures</i>	48

	Page
b) <i>The effect of varying the proportion of resistant and susceptible plants</i>	49
CHAPTER FOUR: THE EFFECT OF PLANTING PATTERN ON EPIDEMICS OF DAMPING-OFF	50
I. METHODS	50
1. <i>Planting patterns used</i>	51
2. <i>Inoculation of seed boxes</i>	52
3. <i>Assessment</i>	52
II. RESULTS	54
1. <i>Varying the number of clumps while the proportion of the total area occupied by clumps was held constant</i>	54
a) <i>The occurrence of primary infection foci</i>	54
b) <i>The rate of multiplication of disease in randomly inoculated seedling stands</i>	55
c) <i>The rate of advance of disease</i>	57
2. <i>Varying the proportion of the total area occupied by clumps while the number of clumps was held constant</i>	57
a) <i>The occurrence of primary infection foci</i>	57
b) <i>The rate of multiplication of disease in randomly inoculated seedling stands</i>	59
c) <i>The rate of advance of disease</i>	60
CHAPTER FIVE: DISCUSSION OF EXPERIMENTS INVOLVING EPIDEMICS OF DAMPING-OFF	61

	Page
1. <i>Host plant density per se</i>	62
a) <i>The occurrence of primary infection foci</i>	62
b) <i>Proportion of damping-off at a particular time</i>	63
c) <i>Multiplication and advance of damping-off with time</i>	64
2. <i>Mixtures</i>	69
3. <i>Pattern</i>	74
a) <i>Varying the number of clumps while the proportion of the total area occupied by clumps was held constant</i>	74
b) <i>Varying the proportion of the total area occupied by clumps while the number of clumps was held constant</i>	76
 SECTION C: CONTROLLED ENVIRONMENT EXPERIMENTS ON EPIDEMICS CAUSED BY AN AIR-BORNE PLANT PATHOGEN	 79
CHAPTER ONE: GENERAL EXPERIMENTAL METHODS	79
I. INTRODUCTION	79
a) <i>General considerations</i>	79
b) <i>The relevant biology of Erysiphe graminis DC. f.sp. hordei Marschal</i>	80
II. GENERAL METHODS	82
1. <i>Physical environmental conditions</i>	82
a) <i>Light, humidity and temperature</i>	82
b) <i>The pattern of air flow in LBH cabinets</i>	83
2. <i>Establishment of host plant arrays and initiation of disease</i>	86

	Page
a) <i>Stocks of host plant and pathogen</i>	86
b) <i>Setting up the experimental system</i>	87
c) <i>Initiation of disease</i>	90
3. <i>Monitoring and assessment of disease</i>	90
a) <i>Overall rate of increase of disease</i>	90
b) <i>Rate of increase of disease due to between-plant transmissions of inoculum</i>	92
c) <i>Rate of increase of disease due to within-plant transmissions of inoculum</i>	93
 CHAPTER TWO: <i>THE EFFECT OF PLANT DENSITY PER SE ON EPIDEMICS OF POWDERY MILDEW</i>	 95
I. METHODS	95
II. RESULTS	96
a) <i>Overall rate of increase of disease</i>	96
b) <i>Rate of increase of disease due to between-plant transmissions of inoculum</i>	97
c) <i>Rate of increase of disease due to within-plant transmissions of inoculum</i>	101
 CHAPTER THREE: <i>EPIDEMICS OF POWDERY MILDEW IN MIXTURES OF SUSCEPTIBLE AND IMMUNE PLANTS</i>	 102
I. METHODS	102
1. <i>Plant varieties used</i>	102
2. <i>Planting patterns used</i>	103

	Page
a) <i>Random placement</i>	103
b) <i>Placement in rows</i>	105
II. RESULTS	106
a) <i>Overall rate of increase of disease</i>	106
b) <i>Rate of increase of disease due to between-plant transmissions of inoculum</i>	107
c) <i>The interception of inoculum by wheat and barley plant barrier rows</i>	108
CHAPTER FOUR: <i>DISCUSSION OF EXPERIMENTS INVOLVING EPIDEMICS OF POWDERY MILDEW</i>	110
1. <i>Epidemic rates</i>	110
2. <i>Host plant density per se</i>	111
3. <i>Mixtures</i>	115
a) <i>Mixtures in general</i>	115
b) <i>The effect of patterning in mixtures</i>	121
c) <i>Disease dispersal gradients</i>	121
SECTION D: <i>THE EFFECT OF DISEASE ON COMPETING MIXTURES OF HOST AND NON-HOST SPECIES</i>	126
I. INTRODUCTION	126
II. METHODS	127
1. <i>Experimental procedures</i>	127
a) <i>Setting up and planting of pots</i>	128
b) <i>Inoculation of pots</i>	129
c) <i>Harvesting</i>	130

	Page
2. <i>Theoretical considerations</i>	130
a) <i>Relative yield</i>	131
b) <i>Relative yield total</i>	131
c) <i>Relative replacement rate</i>	132
III. RESULTS	134
a) <i>The effect of host plant density on the incidence and impact of disease</i>	134
b) <i>The effect of disease on the relative competitive abilities of host and non-host plants during the early vegetative growth phase</i>	135
c) <i>The effect of disease on the relative competitive abilities of host and non-host up to the time of grain filling</i>	138
IV. DISCUSSION	140
SECTION E: GENERAL DISCUSSION	144
I. MAIN FINDINGS OF THIS STUDY	145
1. <i>Host density per se</i>	145
2. <i>Pattern in single species stands</i>	149
3. <i>Mixtures</i>	152
4. <i>Competition in mixtures</i>	155
II. SIGNIFICANCE FOR AGRICULTURAL SYSTEMS	158
1. <i>Monocultures</i>	158
2. <i>Mixtures</i>	159
III. SIGNIFICANCE FOR NATURAL ECOSYSTEMS	164
1. <i>Host density per se</i>	164
2. <i>Mixtures</i>	165

	Page
a) <i>Mixtures in general</i>	165
b) <i>Pattern of host and non-host placement in mixtures</i>	168
IV. PROSPECTS FOR FUTURE WORK	170
SECTION F: BIBLIOGRAPHY	173
SECTION G: APPENDIX I - SOME PUBLISHED PAPERS RELEVANT TO THIS THESIS	

Summary

Recently it has been suggested that host-specific parasites may play a significant role in the creation and maintenance of diversity in plant communities by differentiating the ecological niches of competing plant species. Such suggestions make the following three important assumptions: that parasites are capable of causing ecologically significant levels of damage, that a significant proportion of these parasites are host-specific or show considerable host preference and finally that host and parasite numbers are related in a density-dependent manner. While the first two of these assumptions are quite strongly attested by the literature, little information is available concerning the effect of host plant density on parasites, particularly fungal pathogens.

This thesis describes how environmentally controlled experiments were used to investigate the epidemiological response of a soil-borne (*Pythium irregulare* Buisman) and an air-borne (*Erysiphe graminis* D C. f.sp. *hordei* Marschal) fungal pathogen to changes in the density of host plants (*Lepidium sativum* L. and *Hordeum vulgare* L. respectively).

In both host-parasite systems, it was found that increases in plant density increased, and decreases in plant density decreased, epidemic rates. The precise relationship differed, however, in the two systems. Measurements of rates of increase and rates of advance of *P. irregulare*-induced damping-off in seedlings stands showed that these rate parameters were related to increases in plant density in

a curvi-linear manner. Rates of increase of powdery mildew caused by *E. graminis* f.sp. *hordei* showed, by contrast, an approximately linear relationship between host plant density and the rate of increase of disease over the range investigated.

Host density also proved to be the main factor affecting epidemics of the two diseases when susceptible plants were grown together in mixtures with resistant plants (e.g. *L. sativum* with *Lolium rigidum* Gaud. and *H. vulgare* with *Triticum aestivum* L.). In both soil and air-borne parasite systems, it was found that the epidemic rates in mixtures could be predicted from a knowledge of the net density of susceptible plants present. The density, or even presence, of resistant plants *per se*, appeared to have only a minor influence.

In the powdery mildew system, various arrangements of the immune and susceptible plant species appeared to produce little change in epidemic rates, provided that the net density of host plants remained constant. However, in the damping-off system, various clumped planting patterns of the host plants were shown to support lower epidemic rates than evenly seeded stands of the same overall density. The large distances between clumps of plants in this case acted as a barrier to spread of the disease.

Some exploratory experiments were also carried out to determine how a host-specific pathogen may alter the relative competitive abilities of competing host and non-host species.

The significance of these experimental findings are discussed in relation to both agricultural and natural ecosystems, with special reference to 'multilines' and the diversity of natural plant communities.

SECTION A

GENERAL INTRODUCTION -

DENSITY-DEPENDENT EFFECTS IN PLANT POPULATIONS

DENSITY-DEPENDENT EFFECTS IN PLANT POPULATIONS

I. PLANT-ENVIRONMENT INTERACTIONS

Until relatively recently, the main impetus in plant ecological studies centred around 'biogeographical' problems concerning vegetation and vegetational analyses (Greig-Smith, 1964). Such studies have demonstrated clear correlations between the comparative physiology of plant species and the climatic and edaphic conditions of their natural habitat (Harper, 1967). As a result of this preoccupation with these vegetational studies, there has been, as Huffaker (1959) states 'a consequent neglect of biotic forces of many and varied sorts which may drastically alter utilization of such resources as are otherwise available to given plants'.

Although Darwin (1859) in his work '*The Origin of Species*' gave a solid grounding in the field of population dynamics to the fledgling science of plant ecology, it is only in the last fifteen years or so that such studies have gained prominence in ecological literature with two main experimental approaches being used (Harper, 1970; Putwain and Harper, 1970). The first of these, the *Synthetic* approach, involving the construction of very simple one or two species communities, has been successful in showing the response of plants to increases in population density (for example, Harper and Gajic, 1961; Harper and McNaughton, 1962; Marshall and Jain, 1969). In contrast to this, the *Analytic* approach, whereby naturally occurring populations of plants are modified experimentally (for example, by the removal of one of the component species of a natural community), provides valuable clues concerning the mechanisms of population regulation in natural ecosystems (for example, Foster, 1964; Cavers and Harper, 1967; Cantlon, 1969). More recently still, an expanding interest in demographic census studies of plants in their natural habitats (Sagar, 1959; Sarukhan, 1971; Sarukhan and Harper, 1973), is providing further insight into the effect of competitive interactions on plant populations.

Plants, like all other living organisms, are capable of geometric increases in population size. In natural situations, however, rapid increases in plant populations are generally followed by periods of stability or decline as individuals become increasingly crowded for environmental resources and intra-specific competition increases. Paradoxically, some of the best examples of

the stability of plant populations are seen in species which have posed biological control problems. Comparisons between the number of individuals of such species in their natural habitat with the number attained in new areas, successfully invaded, provide clear evidence of the relative stability of populations of such species in their natural habitats (for example, *Hypericum perforatum* in Europe compared with that in California in the early 1950's or *Opuntia* spp. in South America compared with that in Queensland in 1925).

All plant-plant interactions are characterized by competition which has been defined by Crombie (1947) as 'the demand, at the same time, by more than one organism for the same resources of the environment in excess of the immediate supply'. Definitions of this type and others (Clements, Weaver and Hanson, 1929; Williamson, 1957; Harper, 1961; Donald, 1963; Odum, 1971) obviously imply an interaction between organisms, or plants, which will of geometric necessity intensify as plant density increases and lessen as density decreases. Such mechanisms, because of their negative feedback nature, counter any tendency of the total population of plants to move away from an optimum utilization of the available environmental resources. In this way then, the physical environment provides a primary control on the size of plant populations, in that the total size of a population or populations cannot exceed the available water, nutrients and other requirements supplied by the environment.

II. PLANT-PLANT INTERACTIONS

Within the constraint that plant productivity cannot exceed the available environmental resources, individuals of the same or different species will compete to monopolize these limiting resources and thus gain advantage over less successful individuals. The problem of the control of population size can thus be viewed in light of the between-plant (both intra- and inter-specific) interactions required to maximize the efficiency of resource utilization.

Regulation of plant populations occurs by alteration of either recruitment (birth) factors or mortality (death) factors.

1. *The control of recruitment into a population*

The number of new recruits entering a population is determined by an interaction between the availability of seed and the availability of 'micro-sites' suitable for germination (Harper, Willaims and Sagar, 1965; Harper, 1970). While such recruitment 'controls' place an absolute or maximum ceiling on the numbers of inputs or births into a population, in themselves they appear to show little potential as population regulatory mechanisms. Despite this, however, they may reflect the activity of two plant-plant interactive processes which do give some possibility of density-dependent control on seedling recruitment. The first of these, phenotypic plasticity

of adult plants, may affect the availability of seed in the following generation, while the second plant-plant interaction, allelopathy, may affect the suitability of the available, physically appropriate micro-sites for germination.

a) *The availability of seed*

In competitive situations, plants commonly react to the presence of neighbours in a plastic manner. Such plastic responses or phenotypic plasticity has been shown for a wide range of plant species with reductions in individual plant size and reproductive output being correlated with the intensity of competition for the limited environmental resources (for example, Harper and McNaughton, 1962). Under competitive conditions then, individuals in high density stands suffer, on average, greater reductions in overall dry weight and reproductive output than do individuals occurring in low density stands. At high enough densities such intensifications of competitive effects may result in yield (measured as either total dry weight or total seed number) per unit area becoming a constant independent of population density (the law of constant final yield: Kira, Ogawa and Sakazaki, 1953; Hozumi *et al.*, 1956). Thus Harper and Gajic (1961) found that despite a ten-fold increase in the planting density of *Agrostemma githago*, seed production was stable at about 32,000 seeds per m². Although such phenotypic plasticity-induced variations in seed production provide no immediate control on plant numbers, they may play a very significant role in determining population sizes the following year. The size of *A. githago* populations is dependent on seed input from the previous year's

population plus seed introduced with the crop (Harper and Gajic, 1961).

b) *The suitability of micro-sites*

From the work of Harper, Williams and Sagar (1965) and Harper and Benton (1966) it is apparent that soil surfaces do not provide a homogeneous continuum of suitable conditions for successful seedling germination and establishment. Rather, a variable number of safe 'micro-sites' (zones little bigger than a seed in which the environment is favourable for germination: Harper, 1970) are dotted randomly over the soil surface. These workers, in considerations of what constitutes a 'safe' micro-site, concentrated their studies on the texture and degree of soil surface compaction, water and humidity levels. While these are all valid factors influencing the occurrence of safe germination sites, they provide a maximum ceiling on recruitment levels rather than a regulation.

A further factor which may influence micro-site suitability and at the same time have some density-dependent link with plant population levels is the occurrence of compounds toxic to seed germination and establishment which are produced by plants already present in the area. Allelopathic interactions between plants have attracted a considerable amount of interest and, over the years, a large body of data has been accumulated (Grummer, 1961; Muller, 1969; Whittaker, 1970; Rice, 1974) which supports the contention that such substances play a significant role in at least some plant communities. Many plants have been shown to produce substances

inhibitory to seed germination (Evenari, 1949) and while such substances are commonly directed at other plant species (Garb, 1961) there are a number of cases of specific auto-toxicity. Thus Webb, Tracey and Haydock (1967) found that *Grevillea robusta* seedlings were prevented from successfully germinating and establishing close to parent trees due to toxic compounds produced by the parents. Similarly, McNaughton (1968) has found that *Typha latifolia* is unable to regenerate from seed in an existing stand, despite favourable physical conditions, due to the accumulation of toxic residues from the parent plants. As such toxic compounds must act by affecting the quality or suitability of a 'micro-site' for germination, it is possible to see how at high adult plant stand densities most, if not all, sites may be unfavourably affected while at low stand densities few if any are affected. This then provides the possibility for a degree of density-dependent regulation of seedling recruitment.

2. Mortality in a population

a) The 'thinning' process in single species stands

Density-induced checks on recruitment affect dense seedling stands to a greater extent than sparse ones and thus act to reduce the range of densities that may have been potentially possible. Despite these checks, however, seedling populations are generally so dense (particularly weedy species) that as plants grow they

compete with one another for the limited resources available. This competition results in differential growth rates between members of the stand and thus a 'hierarchy of resource exploitation' (White and Harper, 1970), with a large proportion of the available resources being monopolized by a relatively few individuals. While some plant species are able to absorb such competitive stress almost exclusively through plastic phenotypic responses (for example, *Agrostemma githago*: Harper and Gajic, 1961) most species ultimately respond through reductions in stand density.

This mortality response of pure species stands to highly competitive conditions was first analysed by Yoda *et al* (1963). From studies of thinning in a range of over-crowded pure stands they found that the mean weight and number of surviving plants could be related by the equation $w = Cp^{-3/2}$, where w is the mean weight, p is survivor density and C is a constant. Yoda *et al* called this equation the 3/2 power law of self-thinning. The general applicability of this 'law' has recently been confirmed by White and Harper (1970) who extended its application to several species not previously examined. Ford (1975), on the other hand, feels that the law need not necessarily apply universally.

The 'law' of self-thinning is important in any consideration of population regulatory mechanisms as it predicts a maximum possible density for an actively competing population based on the mean average weight of the surviving plants. Thus the rate of elimination or mortality of individuals is directly related to the growth rate of the surviving members of the population, with

mortality increasing as growth rates increase. Moreover, as White and Harper (1970) have shown, the mortality risk tends to remain constant with time so long as the members of the stand continue to compete.

Self-thinning occurs when the total resource requirements of a plant stand exceeds those available. The self-thinning mechanism thus operates to maintain resource demand and resource availability in equilibrium, and to achieve this, the size of the population is regulated by mortality of the 'weaker' individuals. Self-thinning processes thus confer potentially self-regulatory properties on single species populations.

b) *The thinning process in mixtures*

In mixed populations, thinning phenomena are complicated by the occurrence of both self- and alien-thinning (Harper and McNaughton, 1962), the two processes being 'subtly different' (Harper, 1967). Although, taken as a total stand (all species together), mixtures thin in response to extreme competitive stress in much the same way as pure stands (for example, mixtures of three species of *Betula*, Yoda *et al.*, 1963; mixtures of *Raphanus sativus* and *Brassica napus*, White and Harper, 1970), one or both components of such mixtures may suffer disproportionate levels of mortality.

3. *The causes of mortality in a population*

It is known that some plants in dense actively competing stands die. From studies like those of Koyama and Kira (1956) and of Obeid, Machin and Harper (1967), it has been shown that the frequency distribution of plant weights become extremely skewed in response to competitive stress, the most abundant class being that with the lowest plant weights. Additionally, from the work of Black (1958) and White and Harper (1970) there is evidence to permit the tentative suggestion that this mortality is concentrated in the lowest weight (size) classes. However, despite this knowledge of the physical changes that occur in plant populations little is known about the actual causes of death.

a) *The causes of mortality in pure stands*

The actual causes of death in suppressed and weakened plants in pure stands have not been studied. Certainly, as Harper and White (1974) state, the ultimate cause of a major proportion of deaths can be attributed to the presence of too many neighbours needing the same resources at the same time. Of greater interest, however, are the factors which determine the partitioning of resources in a stand and thus the relative success of individual plants. In a few cases these have been correlated with obvious morphological factors like seed size (Black, 1958) or with the order of seedling establishment (for example, Ross and Harper, 1972). The possible role of 'external' biotic factors like parasites and

herbivores appears to have been largely ignored.

If mortality exceeds the level necessary to equilibrate resource demand with resource supply, the causes of plant death may be extremely important from an ecological point of view. If such conditions persist, 'partially vacated niches' (Harper, 1969) will occur, into which further plant species may enter. Simple competitive interactions between plants in adult stands are not capable of producing density reductions of such magnitude (allelopathic effects may prevent further recruitment), but it is possible that herbivores and parasites^{*}, responding to the presence of abundant food, may reduce densities sufficiently.

b) *The causes of mortality in mixtures*

As in pure stands, the major ultimate cause of death in mixtures can be attributed to simple resource starvation - too many neighbours needing the same resources at the same time. The distribution of these deaths between different components of the mixtures in respect to their original relative frequencies is far more interesting, however, as this may provide clues concerning the mechanism of mixture stability. The regulation of population size in mixtures is thus a question of how a balance is achieved

* The term parasite is used throughout this discussion in a broad sense, intended to include phytophagous and seed-eating insects with those organisms, such as fungi, to which this term is usually applied.

between the relative competitive abilities of the component species.

Mixtures of two or more species may be either stable or unstable. In unstable mixtures, the more aggressive (competitive) species dominates, suppresses and eventually eliminates the less aggressive ones. This success may result simply from a faster growth rate *per se* (in which case, death in the unsuccessful species will be caused by resource starvation) or from an ability to gain competitive advantage through the production of phyto-toxic substances. Thus in mixed plantings of walnut and black locust, Baxter (1967) found that the black locust component of the mixture was strongly suppressed by allelopathic substances produced by the walnut component.

In stable mixtures composed of species having different ecological requirements, differences in competitive action within and between species (self- and alien-thinning) may be sufficient to permit stable co-existence (Harper and McNaughton, 1962). The death of individuals in mixtures of this type is probably caused by resource starvation, resulting from unsuccessful competition with other individuals of the same species. Other stable mixtures appear to be composed of species having more or less identical ecological requirements and in these, simple plant-plant interactions are inadequate to explain the stability of the association. It is quite possible that the neglected and under-estimated action of parasites may be extremely important in at least some of these cases.

III. PLANT - PARASITE INTERACTIONS

The role of plant consumers in influencing the structure or size of plant populations has been little explored. In animal ecology, the concept of individual species being limited and controlled by predation or parasitism has been frequently considered theoretically (for example; Gause, 1934; Slobodkin, 1961; Slatkin, 1974) and is well based on experimental observations (Slobodkin, 1964; Paine, 1966; Porter, 1972). By way of contrast, plant ecologists have generally ignored the possible influences of parasites on individual populations or communities of plants, and in fact, it has been left to entomologists in the field of biological control to produce some of the most clear-cut evidence for such effects (for example, Huffaker, 1959; 1970).

1. *The effect of parasites on single species populations*

a) *Recruitment*

As noted previously one major factor influencing the size of seedling populations is the availability of seed (Harper, 1970), and there is considerable evidence to suggest that many plants suffer very heavy pre- and or post-dispersal seed predation by parasites or herbivores (for example; Shaw, 1968a; 1968b; Janzen, 1969; Smythe, 1970). Pre-dispersal seed predation may alter a plant's seed

shadow and thus lower the probability that any particular safe 'micro-site' (Harper, Williams and Sagar, 1965) is occupied (Janzen, 1971), while post-dispersal predation may reduce the number of safe sites in the habitat (Janzen, 1971).

b) *Mortality of plants*

In naturally occurring pure stands of plants the development of a hierarchy of dominance and suppression may be greatly assisted by the effects of differential impact of parasites on individuals within the stand. Thus in stands of wild lupins (*Lupinus amplus*), differential flower predation, and thus success in reproduction, has been associated with variability in alkaloids (Breedlove and Ehrlich, 1972; Dolinger *et al*, 1973). Similarly, in populations of *Trifolium repens*, in which the cyanogenic polymorphism is present, individuals of the environmentally weaker cyanogenic form are assisted in competitive interactions with the more aggressive acyanogenic form by the selective grazing of two species of slug (Angseesing, 1974). It can be seen then, that even in situations where the total size of a population is determined by the 'carrying capacity' of the physical environment, and deaths are ultimately caused by resource starvation, parasites may act as potent selective agents determining which individuals will die.

In many circumstances the impact of parasites may exceed the compensatory growth capacity of the population as a whole. Obviously, such occurrences are not easily studied as this failure to fully utilize all available resources leads to 'partially vacated

niches' (Harper, 1969) which new species may rapidly fill. That parasites may act more intensely in dense one-species stands than in mixtures has been hypothesized for some time (for example; Ridley, 1930) and in this respect some of the best examples of the potential 'power' of parasites are found in the changes that occur in the population size of species successfully limited by biological control. The massive reductions in the density of *Opuntia* spp. in Australia (Dodd, 1940) and *Hypericum perforatum* in California (Huffaker and Kennett, 1959; Huffaker, 1970) as a result of parasites introduced from the plants' natural habitats are classic examples, while other examples are in the control of species of *Ulex*, *Senecio*, *Emex* and *Acaena* (DeBach, 1964; Huffaker, 1971).

2. *The effect of parasites in mixtures*

a) *The problems of co-existence of species*

The ecological problems which arise when similar species are found living together in the same area are expressed in the competitive exclusion principle. This principle, defined by Gause (1934) as 'two similar species scarcely ever occupy similar niches' has since been restated on numerous occasions (Lack, 1947; Allee *et al.*, 1949; Green, 1951; DeBach and Sundby, 1963). Although the universality of this principle has been challenged (Ayala, 1969; 1971; 1972) it expresses a generally held ecological belief that 'complete competitors cannot co-exist' (Hardin, 1960).

In some cases, superficially similar species may, on closer examination, be found to minimize competitive interactions by exploiting different parts of the environment (for example, Carnahan; cited by Chilvers, 1972; found that three co-existing species of grass exploited different zones in the soil), or by showing differences in their requirement and tolerance of certain environmental factors (for example, the differences in water requirements and tolerances of *Ranunculus* species described by Harper and Sagar, 1953). There are instances, however, where two or more structurally and physiologically similar species appear to be able to co-exist in stable equilibrium despite the fact that their resource requirements appear to be essentially identical, for example, eucalypts (Pryor, 1959; Burdon and Pryor, 1975), rainforest trees (Janzen, 1970) and black and white oaks (Whittaker, 1969). Such species appear to defy the principle of competitive exclusion, and recently it has been suggested that this dilemma may be resolved if proper account is taken of the power of host-specific parasites to differentiate the ecological niches of the plant species concerned (Janzen, 1970; Chilvers and Brittain, 1972).

b) *The role of parasites in niche separation*

While a few workers (Darwin, 1859; Ridley, 1930; Harper *et al.*, 1960; Gillett, 1962; Bullock, 1967) have suggested that parasites may play a role in plant communities, it is only relatively recently that a number of models have been developed which explicitly show how they may influence plant community diversity and stability. Thus Harper (1969) described how a reduction in the density of a

plant species, as a result of host-specific parasite activity, could provide room in the same niche for further species to become established. Following this, Janzen (1970) used static graphical models to explain how the high species diversity and the long distance between conspecific adult trees in lowland tropical forests might result from the activity of host-specific seed predators which prevent seedlings from becoming established near a pre-existing tree. More recently still, Chilvers and Brittain (1972) have devised a simple continuous systems model to illustrate how two competing plant species could be maintained in dynamic equilibrium with one another as a result of density-dependent feedback from their respective host-specific parasites.

Such host-parasite models are based on three important assumptions (Chilvers, 1972). The first of these is that parasites are capable of causing ecologically significant levels of damage. Secondly a significant proportion of the parasites present in such a system must be host-specific, while finally there must be a density-dependent relationship between host and parasite numbers.

(i) *The occurrence of ecologically significant levels of damage* - For parasites to have an ecologically significant effect (in terms of community diversity and stability) they must cause at least enough damage to redress the balance between the relative competitive abilities of the competing species. Ecologically

significant levels of damage may, therefore, be very low* (for example; Bray, 1964) or very high. Obviously, if parasites can be shown to cause high levels of damage in natural or semi-natural ecosystems they will tend to fulfil this criterion.

Damage may be measured in a variety of different ways. Thus numerous instances of significant losses (>15%) of merchantable timber have been detailed in forestry literature (for example, Davidson and Buchanan, 1964; Readshaw and Mazanec, 1969; Rafes, 1970) while many examples are also available concerning changes in stand composition as a result of insect or fungal damage. Kegg (1971) found that two years heavy defoliation of *Quercus* species by *Porthetria dispar* caused 28 percent of the affected oaks to die; McCambridge and Knight (1972) found that a spruce beetle outbreak reduced the percentage of Engelmann spruce in a mixed stand from 82 to 57 percent; and Day and Monk (1974) found that *Endothia parasitica* reduced the percentage of American chestnut in a mixed stand from 31.1 to 0.1 percent. Accurate measurements of defoliation levels are not so common but Burdon and Chilvers (1974a; 1974b) recorded average losses of up to 46 percent in some sapling eucalypt stands. Kulman (1971) in a review of 174 cases of natural and artificial defoliation concluded that all but seven showed a direct correlation between the severity of defoliation and the consequent reduction in growth.

* This may partly explain why the significance of parasites has been generally overlooked by plant ecologists.

(ii) *Host-parasite specificity* - It can be logically deduced (also van Valen, 1974) that equivalent or random non-specific predation cannot increase the number of species in a natural ecosystem (except in a few very specialized cases, van Valen, 1974). Thus specificity is an important requirement in any consideration of parasites influencing community diversity. Even large herbivores, although commonly feeding on a range of plant species readily show preference and their selective grazing may significantly alter the botanical composition of communities (e.g. voles - Summerhayes, 1941; sheep and cattle - Beadle, 1948; Biddiscombe, 1953; Moore, 1959; rabbits - Harper, 1969). Similarly, the degree of specialization of parasites varies tremendously. Insects show a range of broad to narrow dietary habits (Norris, 1970), while fungi vary in their parasitic relationships with plants from unspecialized (for example, some *Pythium* species) to highly specialized (for example, races of *Puccinia graminis* f.sp. *tritici*). The majority of fungi and insects are probably restricted in their attack to plants of the same genus or subgenus. Thus even in eucalypt forests where both host species are members of the same genus, Burdon and Chilvers (1974a) were able to show a considerable degree of host-specificity within both insect and fungal parasites.

(iii) *Density-dependent interactions between host and parasite*

- If parasites are to provide effective control of host numbers, then a given increase in host density must produce a disproportionate increase in parasite numbers, which in turn, causes a disproportionate increase in the amount of damage. The third assumption of models like those advanced by Janzen (1970)

and Chilvers and Brittain (1972) may thus be viewed from two different angles - the effect of parasite density on hosts and conversely, the effect of host density on parasites.

α) *The effect of parasite density on hosts* - The effect of different levels of disease or pathogen damage on the ultimate yield of host plants is reasonably well documented in plant pathological literature. In work on stem and leaf rusts of wheat there have been numerous attempts to establish relationships between disease levels or ratings and grain yield reductions (Large, 1966). Thus Kirby and Archer (1927), Greaney (1933; 1936), Chester (1946) and Batts and Elliott (1952) have employed a variety of methods to produce tables which relate disease ratings to losses in grain yield, with increasing disease levels resulting in declining grain yields. More recently, such results have been presented in simpler mathematical forms by a number of workers. For example, Scott (1973) showed a direct correlation between yield loss in a range of wheat cultivars and the degree of infection by *Septoria nodorum*; Slope and Etheridge (1971) found an inverse linear relationship between grain yield of winter wheat and the incidence of *Ophiobolus graminis*; and Large and Doling (1962) recorded direct relationships between grain yield losses of both barley and oats and the severity of attack by mildew, measured at a particular time prior to harvesting.

β) *The effect of host density on parasites* - While many instances of the effect of parasite density on hosts can be culled from the pertinent literature, there is not a great deal of information available concerning the effect of host density on parasites.

Although there has been considerable interest in factors affecting insect populations, studies of the response of insect populations to increasing host plant density are relatively limited. Moreover, the results available provide a rather confused picture. For wheat stem sawfly (Luginbill and McNeil, 1958), aphids (A'Brook, 1964; 1968; 1973; Blencowe and Tinsley, 1951; Hull, 1964), and Miridae attacking cacao (Williams, 1953) increasing plant density has been associated with *decreasing* levels of infestation, while in the case of mealy bugs (Cornwall, 1958), tomato fruit-worm (Fery and Cuthbert, 1974), carrot fly (Hardman and Wheatley, 1971) and aphids (Way and Heathcote, 1966) increasing plant density has been associated with *increasing* levels of infestation. In addition, Pimentel (1961) showed that increasing plant density also affected the total number of animal taxa present.

The available evidence for density-dependence in fungal pathogen - host plant interactions is even more limited. Other than a few general, unsubstantiated comments (Gaumann, 1950; Baxter, 1952; van der Plank, 1963), three studies with *Pythium* or *Rhizoctonia* species (Johnson, 1914; Hartley, 1921; Gibson, 1956) and one with *Sclerotium cepivorum* (Scott, 1956) have shown a general positive correlation between disease incidence and plant density, while one study with *Fusarium* (Bloomberg, 1973) found no correlation. There appears to be no specific data available concerning air-borne fungal parasites, although the effect of mixtures on disease rates has been examined by Leonard (1969). These studies (with the exception of Scott, 1956, and Leonard, 1969) measured disease incidence at a single arbitrary time after

commencement of the experiment. Such data is not very useful, however, as the outcome of dynamic interactions like disease epidemics (and thus the role of disease in natural ecosystems) is governed mainly by the rate processes within such interactions and is relatively independent of the states of the variable components (van der Plank, 1963; 1968; Chilvers, Brittain and Burdon, 1973; Slatkin, 1974). Ultimately then, a total comprehension of how parasites may influence community diversity and stability will be dependent on measurement of the rates of change of disease parameters in response to changes in host plant density. Obviously there is very little data available in this area.

IV. THE OBJECTIVES OF THE PRESENT PROJECT

Motivated by the above considerations, the main objective of the present project was to collect precise quantitative data (especially rate data) on the response of fungal pathogens to changes in host plant density.

Fungal pathogens can be broadly divided into two main groups - air-borne and soil-borne, according to their mode of dispersal and the portion of the host plant that they attack. The spread and increase of diseases caused by soil-borne pathogens is often achieved by the growth of hyphae through the soil. In air-borne

pathogens, disease spreads between and within plants by means of air-borne propagules (spores or conidia). Because the mode of dispersal is so different in these two systems, results obtained from one system are not necessarily applicable to the other. Consequently, two different fungal pathogens were selected for experimentation - one, *Erysiphe graminis* DC. f.sp. *hordei* Marschal, the cause of a typical air-borne disease, powdery mildew of *Hordeum vulgare*, and the other, *Pythium irregulare* Buisman, the cause of a typical soil-borne disease of a number of plant species.

The main aim in each of these experimental systems was to measure the response of the parasite to changes in host plant density. The basic experimental designs used to measure the response of parasites to changes in host density were also elaborated to study mixtures of host and non-host plants. In the soil-borne system the response of the pathogen to changes in the pattern of placement of hosts was also investigated.

In both the soil and the air-borne pathogen systems, the response of the parasites was measured in terms of the rate of multiplication of disease. In addition, in the soil-borne system the number of primary infection foci and the rate of advance of the disease front were also recorded. In both of these systems plant-plant competition was prevented.

All these experiments were carried out under controlled environmental conditions. This was necessary since, although

field studies in which certain environmental variables are measured in different climatic areas or at different times have provided important information on factors influencing disease development (Dimock and Baker, 1951), unavoidable environmental 'noise' often makes the assessment of the role of individual factors extremely difficult (Cohen and Rotem, 1971). The use of controlled environment conditions avoids this and enables precise studies to be made on the effect of individual variables on the rate of disease development.

In addition to the controlled environment experiments, glasshouse experiments were carried out in an attempt to determine whether an air-borne parasite could mediate the outcome of competition in mixtures of host and non-host plants. Parasite impact was evaluated in terms of the effect it had on yield.

V. THE ORGANIZATION OF THIS THESIS

The experimental work is organized into three sections (B, C, and D) which deal respectively with controlled environment experiments on epidemics of a soil-borne pathogen, controlled environment experiments on epidemics of an air-borne pathogen and the effect of disease on competing mixtures of host and non-host species. Experimental results are interpreted and

discussed at the end of each section. The findings are reviewed and their significance to agricultural and natural ecosystems is discussed in the final section (E).

* * * * *

Copies of three published papers are bound into the back of the thesis. Papers 1 and 2 relate to the general problem of co-existence of similar species, Paper 3 relates to some of the material in Section B. Figures, plates and tables are inserted in the text close to where they are first mentioned.

SECTION B

**CONTROLLED ENVIRONMENT EXPERIMENTS ON EPIDEMICS
CAUSED BY A SOIL-BORNE PLANT PATHOGEN**

CHAPTER ONE

GENERAL EXPERIMENTAL APPROACHES

I. INTRODUCTION

Damping-off diseases provide particularly suitable experimental systems for the study of the effects of host-pathogen interactions because, as Garrett (1970) so aptly puts it, they represent 'true epidemics in microcosm'. One such system which can be examined on a laboratory scale is *Pythium*-induced post-emergence damping-off of seedlings.

a) *The relevant biology of Pythium irregulare Buisson*

Pythium irregulare-induced post-emergence damping-off disease is characterized by the collapse and death of seedlings resulting

from the maceration and death of host tissue in the hypocotyl region as fungal hyphae invade. After the seedling topples over, the remainder of the shoot is then killed and radiating hyphae grow out horizontally at or just below ground level. In a stand of seedlings these hyphae progressively infect, and cause the collapse of, adjacent plants thus resulting in a gradually enlarging disease focus (Plate 1). In this way the disease is perpetuated in an epidemic manner.

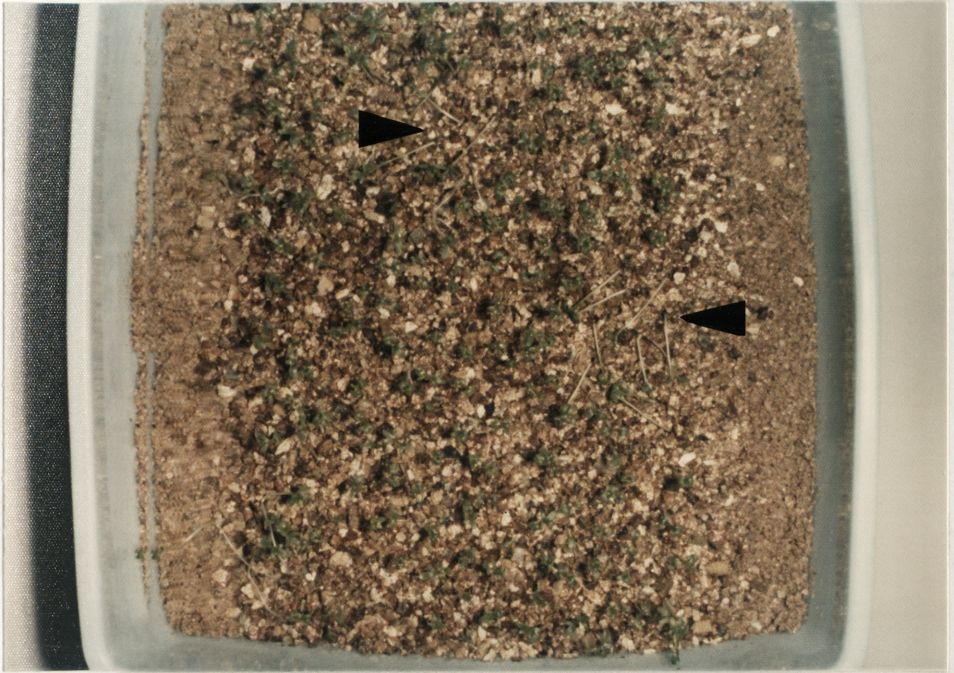
A very wide range of host, pathogen or environmental factors may affect the speed with which a pathogen is able to kill individual seedlings and thus the rate at which it increases in or advances through a seedling stand. One of the most important of these is the change that occurs in the susceptibility of seedling tissue with age. While the rate at which this occurs depends upon the species of plant involved and upon the environmental conditions (Garrett, 1970), disease incidence can generally be viewed as a 'race' between the rate of seedling development and the rate at which the pathogen mobilizes for the attack (Webster *et al.*, 1970). Leach (1947) showed that at any particular temperature the proportion of plants killed prior to emergence could be predicted from the ratio of the linear growth rate of the pathogen to the velocity of seedling emergence. Although these results relate specifically to a pre-emergence damping-off situation, a similar ratio linking the proportion of plants killed after emergence to the growth rate of the pathogen and the rate of tissue maturation (Garrett, 1970; Webster *et al.*, 1970) probably exists. Accordingly all experiments described in

PLATE 1.

Pythium irregulare-induced damping-off of *Lepidium sativum* seedling stands.

a) The general appearance of a stand of *Lepidium sativum* seedlings randomly inoculated with *Pythium irregulare* showing two developing disease foci (arrowed).

b) Close-up view of a disease focus showing symptoms characteristic of *Pythium irregulare*-induced damping-off disease with diseased seedlings toppled over as a result of fungal attack at or near ground level.



this section were carried out in warm humid conditions with subdued lighting in order to prolong the susceptible state in the host plant and thereby minimize the effect of increased resistance with time.

b) *Host plant situations studied*

Damping-off is one of the very few disease syndromes for which actual data has been collected concerning the effect of varying host density on disease incidence (Johnson, 1914, Hartley, 1921; Gibson, 1956). However, all of this work concentrated on measuring disease levels at a single arbitrary time after setting up the experiment so that no detailed analysis of the epidemic is available. Moreover, the conclusions drawn by Gibson are somewhat contradictory.

In the work described in this section, a basic *Pythium irregulare* - *Lepidium sativum* parasite-host experimental system (elaborated where necessary) was used to study the effect of changes in host plant density *per se*, the effect of changes in mixtures of host and non-host plants and the effect of different degrees of host clumping on three different disease parameters.

c) *Disease parameters studied*

In all three types of plant stand, measurements were made of the occurrence of primary infection foci, the rate of multiplication of disease and the rate of advance of disease.

II. GENERAL METHODS

1. *Physical environmental conditions*

All experiments investigating epidemics of damping-off caused by *Pythium irregulare* were carried out under controlled environmental conditions in LB phytotron cabinets (Morse and Evans, 1962). After preparation, experimental containers were sealed with thin plastic film (sandwich wrapping) to maintain a high humidity and then incubated at a constant temperature of 21°C using a 14.5 hour day with a light intensity of 48 lumens per m².

2. *Establishment of host plant arrays and initiation of disease*

a) *Stocks of host plant and pathogen*

Lepidium sativum L. was chosen as the host plant for use throughout these experiments because of its rapid germination rate and known susceptibility to damping-off diseases. Seed was obtained in 500g lots from a commercial seed source (Rumseys Seeds Pty Ltd) and was stored in dry conditions at room temperature until required. All seed used showed extremely high germination (98.0%).

Pythium irregulare Buisman was isolated from a moist loamy soil by baiting out with young cress seedlings, and after tests to

determine its pathogenicity towards *L. sativum*, was used as the fungal pathogen throughout these experiments. The identity of this fungus was confirmed by Dr D. J. Stamps of the Commonwealth Mycological Institute and a sub-culture is deposited in that collection (IMI 183522). This isolate of *P. irregulare* is highly pathogenic and showed no apparent decline in virulence over the 27 month experimental program. The fungus, maintained in culture on potato-carrot agar (Dade and Gunnell, 1966), was transferred to fresh medium once every three weeks.

b) *Setting up and planting of seed boxes*

All experiments were carried out in plastic food containers measuring 25cm by 25cm and 7cm deep. 1000g of coarse washed river sand, with the water content adjusted to a constant 14% by weight, was distributed evenly over the bottom of each container. *L. sativum* seed calculated to represent appropriate densities, was then planted evenly in a hexagonal array over an area 21cm by 17cm in each container. After inoculation, containers were sealed and incubated in controlled environmental conditions.

c) *Preparation of inoculum and initiation of disease*

Two different types of inoculum were used according to the experiments being carried out. In experiments designed to measure rates of multiplication of disease or the occurrence of primary infection foci, random inoculation of host stands was achieved by using particulate inoculum. In experiments aimed at measuring

rates of advance of the disease front, diseased *L. sativum* seedlings were used to initiate the disease.

(i) *Particulate inoculum* - Particulate inoculum was prepared according to the method described by Chilvers (1962). 250ml conical flasks containing 100ml of sterile vermiculite (Grade 3; Neuchatel Asphalt Co.) moistened to maximum moisture holding capacity with 60ml of a nutrient solution containing 20g of dextrose per litre of carrot-potato extract (Dade and Gunnell, 1966), were inoculated with *Pythium irregulare* and incubated for eight to ten days at 25°C. The resulting concentrated inoculum was washed six times in 200ml lots of sterile distilled water to remove most of the residual nutrients and to separate the granules of vermiculite. The concentrated inoculum was then diluted to the required strength by mixing thoroughly with dry sterile vermiculite. Disease was then initiated by sprinkling 150ml of this dilute inoculum evenly over experimental plots. This was damped down with a fine spray of distilled water from a plastic wash bottle.

(ii) *Diseased plant inoculum* - Four to five day old, *Lepidium sativum* seedlings were cut off at ground level and laid upon the surface of two to three day old plates of *P. irregulare* mycelium growing on potato dextrose agar. The seedlings became rapidly infected and were used as inoculum on the following day. In experiments to measure the rate of linear advance of disease, seed in plots was covered with 150ml of sterile vermiculite and then inoculated by laying five diseased seedlings in a continuous line along one edge of each plot.

3. *Collection and analysis of data*

a) *Collecting data on the different disease parameters*

In all experiments, the numbers of seedlings appearing three days after sowing and inoculation were compared with the known seeding rates for evidence of pre-emergence damping-off. In all cases the majority of seedlings emerged successfully, suggesting that the initial germination and growth of the seedlings was faster than the mobilization of the fungus.

(i) *Recording the appearance of primary infection foci* - After three days incubation the plots were inspected and the number of separate disease foci appearing in each container was recorded. This inspection was repeated daily until no more disease foci appeared. Foci developing within 2cm of the edge of plots were ignored in order to minimize edge effects.

(ii) *Measuring the rate of advance of disease* - On the third day after inoculation and at 24 hour intervals thereafter until the ninth day after inoculation, five measurements were made in each container of the distance the disease front had advanced from the inoculated edge of the plot. The measurements were spaced evenly across the plot excluding the outermost 2cm on each side.

(iii) *Measuring the rate of multiplication of disease* - On the third day after inoculation, and at every subsequent 24 hour interval up to the ninth day, the number of diseased and healthy plants in each container was counted. Plants within 2cm of the edge of plots were ignored.

b) *Analysis of data*

Data collected in experiments on primary infection foci and experiments on the rate of advance of the disease front are readily comparable between respective treatments and require no further manipulation. Thus rates of advance on day n are obtained simply by subtracting the total distance travelled by the disease from day zero to day $n - 1$ from that travelled by day n .

In experiments designed to investigate the multiplication rate of disease in randomly inoculated stands, simple counts of the number or proportion of plants diseased (x), when added cumulatively through time, give characteristically sigmoid disease progress curves. Such distributions are difficult to compare with one another in the raw state but may be transformed to produce straight lines. One of the simplest but most effective of the transformations available is the logit transformation, $\log_e (x/1-x)$, used by van der Plank (1960; 1963). For a logarithmic disease epidemic, graphing this function against time produces a rectilinear plot of points, the gradient of which is a measure of r , the apparent infection rate (van der Plank, 1963). The experimental data obtained from investigating the multiplication of disease in randomly inoculated stands was transformed using this function to give the estimates of r presented in the results.

CHAPTER TWO

THE EFFECT OF HOST PLANT DENSITY ON EPIDEMICS OF DAMPING-OFF

I. RESULTS

1. The occurrence of primary infection foci

a) The effect of varying host density

Three separate experiments were carried out to investigate how the number of primary infection foci was influenced by increasing host density from 450 to 7200 plants per m² while inoculum was applied in a random fashion at a constant density of 0.5%. As can

be seen from the dosage-response curves of Figure 1a, there is good agreement between the results of the different experiments. Plots of the number of primary infections against host density (Figure 1a) show that a generally curvi-linear relationship exists, although there is clear evidence of a direct proportionality in the lower range of host densities. For example, when host density was doubled between 450 and 900 plants per m^2 and between 900 and 1800 plants per m^2 , the number of infection foci also doubled each time (average of all experiments: 1.4 to 2.8, then to 5.5 primary infection foci). Further evidence for this direct proportionality between inoculum density and the number of primary infection foci is provided by Figure 1b, in which data from the lower host density range is plotted on a log-log basis. Only the three lowest host density values were transformed in this way, as data from the plateau or even the transitional region of the curvi-linear relationship failed to respond significantly to the log-log transformation. (A similar transformation has been used by Baker (1971) and Benson and Baker (1974) in studies of the effect of varying inoculum density on disease incidence). In all experiments, slopes increased when the first three points were used in the analysis instead of four or all five. The average slope of the linear regression lines fitted to these three points is very close to unity (0.99).

b) *The effect of varying inoculum density*

Although, strictly speaking, only the effect of varying host density on the frequency of primary infection foci comes within the compass of the title of this chapter, the effect of varying

FIGURE 1.

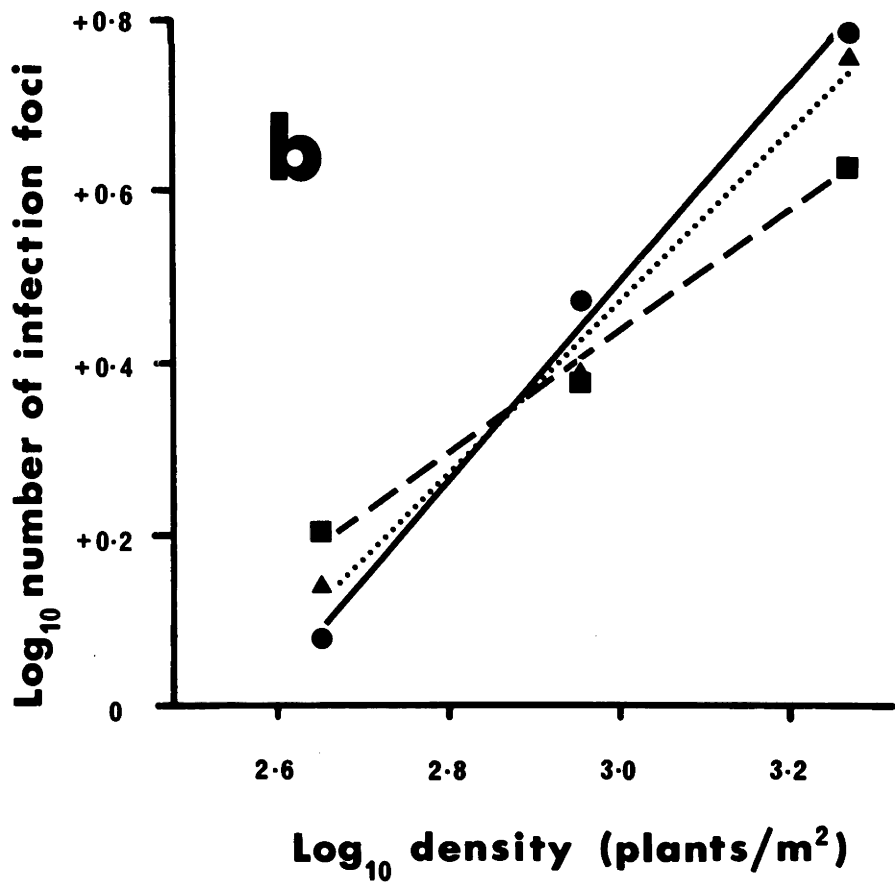
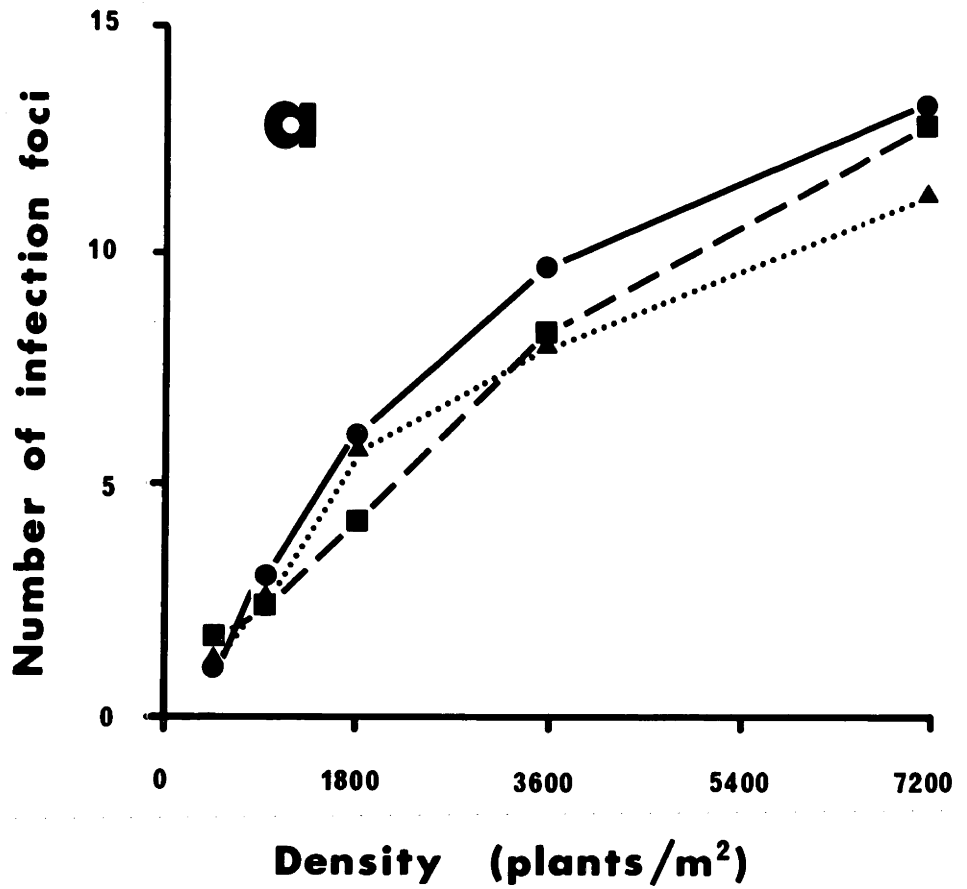
The effect of changing host plant density on
the number of primary infection foci per plot.

a) Arithmetic plot.

b) Log-log transformation of data from the lower
density range.

■, ▲, ●, three separate experimental runs.
(Gradients of the three linear regression lines
fitted to log data: 0.76, 1.02 and 1.18 respectively).

Each treatment was replicated four times.



inoculum levels was also measured since this aspect is reasonably well understood from the studies of Martinson (1963), Baker (1965; 1968; 1971), Baker, Maurer and Maurer (1967), Benson and Baker (1974) and others. The results obtained from the present experiments provide, therefore, a useful yardstick for the purposes of making comparisons.

Figure 2a shows the results of three separate experiments designed to investigate the effect of increasing inoculum density on the resulting number of infection foci. In all three experiments host plant density was held constant at 1800 plants per m^2 , while the inoculum density in the vermiculite layer was varied from 0.125% to 2% in one experiment and from 0.25% to 4% in the other two. Results obtained from the three experiments were all very similar. Once again, the number of primary infection foci was related to inoculum density in a curvi-linear manner (Figure 2a), although at the lower end of the inoculum density range there is evidence of a direct proportionality. Thus when inoculum density was doubled from 0.25% to 0.5%, the average number of infection foci also increased by a factor of about 2 (mean of all three experiments: 3.2 to 6.8 primary infection foci). When data from the lower end of the inoculum density range is plotted on a log-log scale (Figure 2b), the average slope of the linear regression lines fitted to these plots is again close to unity (0.97).

FIGURE 2.

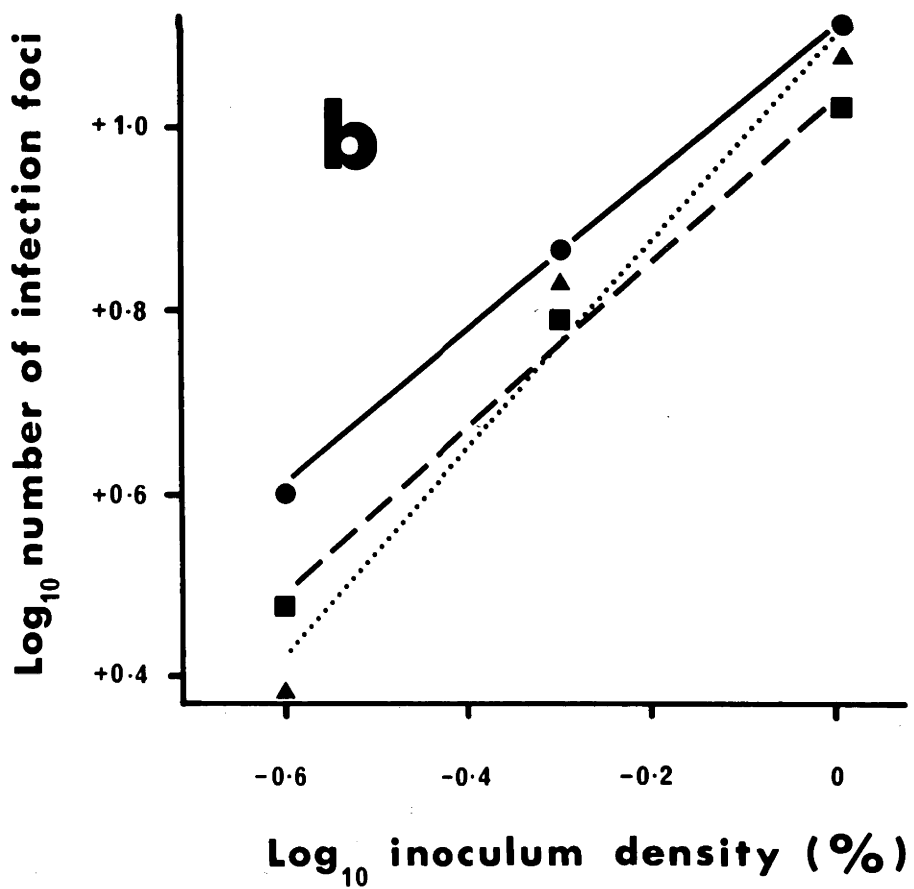
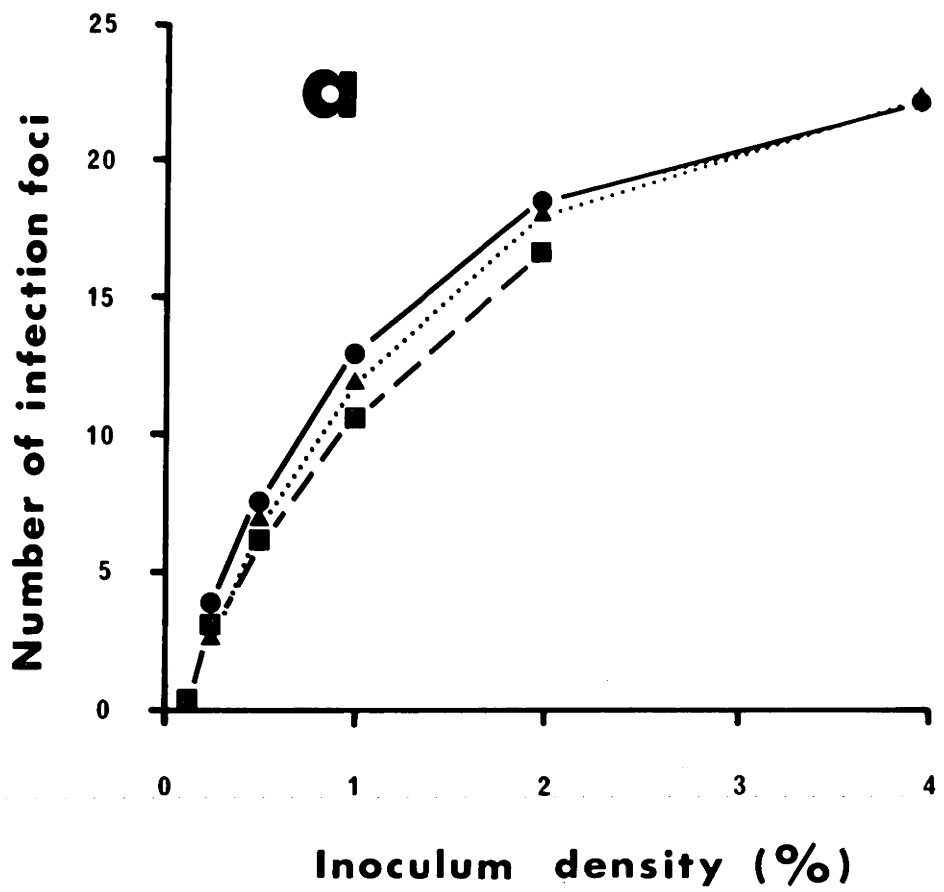
The effect of changing inoculum density on the number of primary foci per plot.

a) Arithmetic plot.

b) Log-log transformation of data from the lower density range.

■, ▲, ●, three separate experimental runs.
(Gradients of the three linear regression lines fitted to log data: 0.91, 1.16 and 0.85 respectively).

Each treatment was replicated four times.



*2. Multiplication of disease in
randomly inoculated seedling stands*

Five separate experiments were carried out to investigate the effect of a range of different host plant densities on the multiplication rate of disease in randomly inoculated seedling stands. In all experiments the concentrated inoculum was diluted by mixing with sterile vermiculite in a ratio of 1:400 before being spread randomly over each experimental plot. The inoculum density in the vermiculite layer was thus a constant 0.25%. This dilution factor was selected after a preliminary experiment involving three planting and three inoculum densities (Table 1).

Plant density (plants per m ²)	Inoculum density %		
	0.25	0.50	1.00
2880	8.0	36.3	57.3
9000	48.5	72.7	90.7
27000	71.2	94.1	99.6

Table 1 The interaction of inoculum density and host density expressed as the percentage of seedlings killed 6 days after sowing and inoculation. All values are the mean of four replicates.

The results of a single experimental run investigating the rate of multiplication of disease in randomly inoculated seedling

stands are shown in Figure 3. In Figure 3a the results are expressed as the proportion of seedlings damped-off against host density, to permit comparisons to be made with earlier work of Hartley (1921) and Gibson (1956) who expressed their findings in this manner (c.f. Chapter Five of this Section). It can be seen that early in the epidemic there is a more or less linear relationship between the proportion of seedlings damped-off and host density, but that as time progresses this relationship is lost and a more and more curvilinear interaction becomes apparent.

Disease progress curves may be plotted as either frequency or cumulative curves, each curve being deducible from the other. The results obtained here are presented in these two different ways in Figures 3b and 3c respectively. In Figure 3b the amount of disease, expressed as the proportion of seedlings newly diseased, is plotted against time (mean of five replicates). From the two curves that go close to completion (plots with 7200 and 9000 plants per m^2) it can be seen that the epidemic, measured in terms of the rate of appearance of new 'cases' follows the expected bell-shaped normal distribution. At first the number of new cases occurring is low, this rises rapidly to a maximum, and finally declines away again as most of the host population becomes saturated with infected plants. At the lowest density illustrated (1800 plants per m^2), the peak rate of appearance of new cases has not yet been reached.

In Figure 3c the results presented above in the form of a frequency curve are redrawn and presented in a cumulative form where they appear as sigmoid shaped epidemic growth curves (the

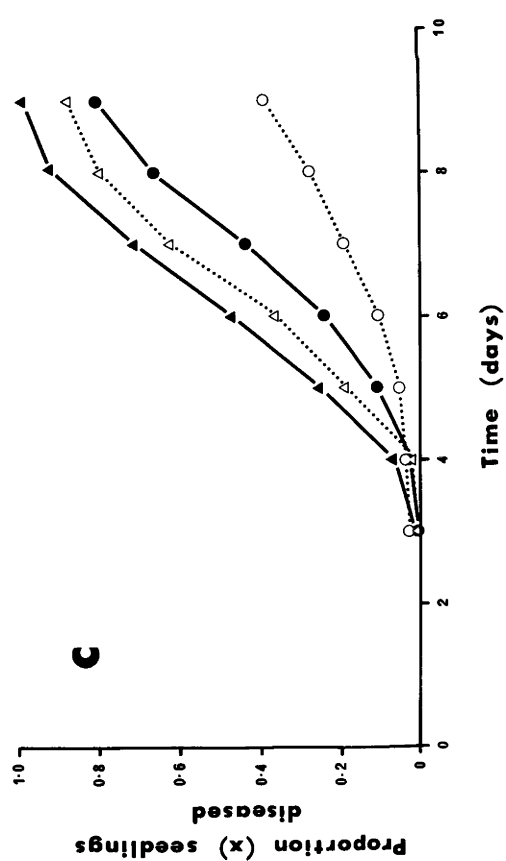
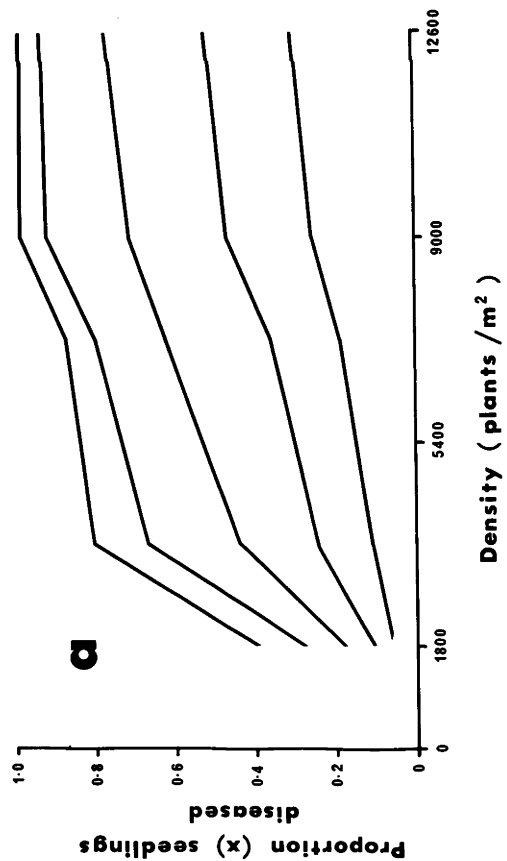
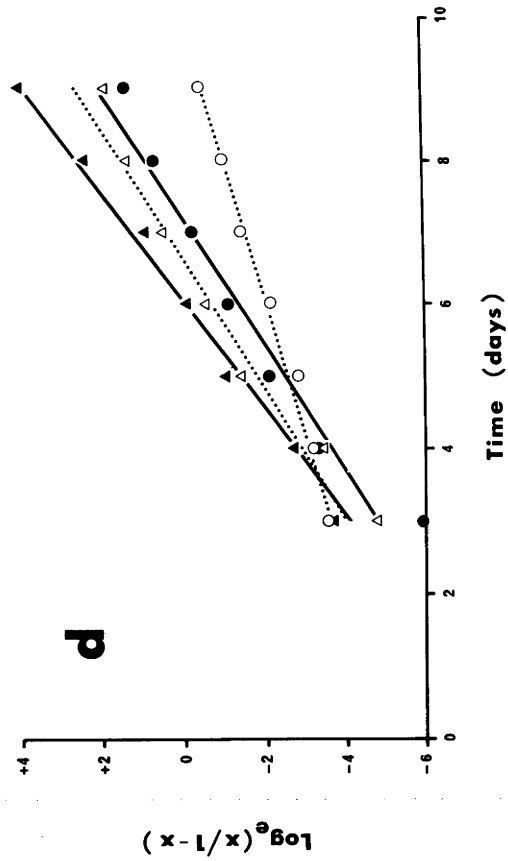
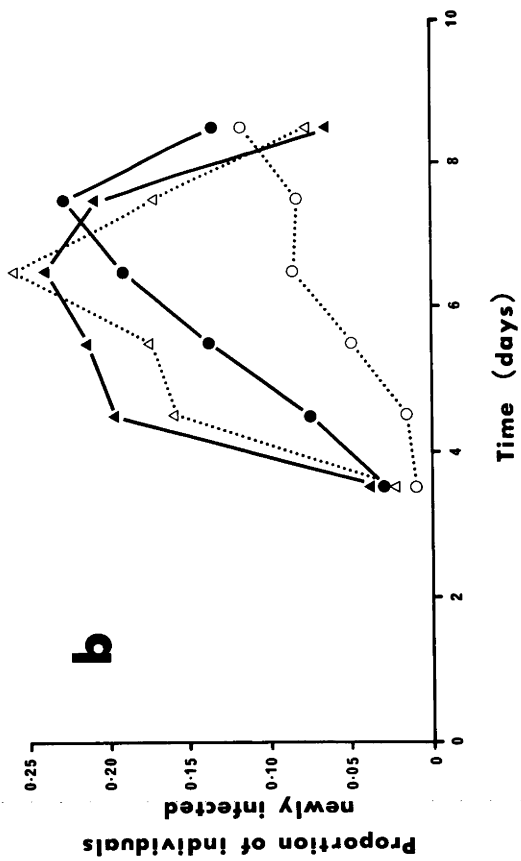
FIGURE 3.

Detailed results of a single experiment of damping-off in randomly inoculated plots.

- a) The proportion (x) of seedlings diseased in relation to planting density, plotted for five consecutive days.
- b) The proportion of seedlings newly diseased (within the previous 24 hour period) plotted against time.
- c) Changes in the proportion (x) of diseased seedlings with time.
- d) $\text{Log}_e (x/1-x)$ plotted against time and fitted by linear regression.

○, 1800 plants per m²; ●, 3600 plants per m²;
△, 7200 plants per m²; ▲, 9000 plants per m².

Each treatment was replicated five times.



proportion of seedlings damped-off is plotted against time). These curves were straightened by plotting $\log_e(x/1-x)$ against time (van der Plank, 1963). Figure 3d shows the previous values plotted in this manner together with their lines of best fit as determined by linear regression analysis. The gradients of these lines then provide the best estimate of the 'compound interest rate' or apparent infection rate (r) of increase of the disease for each host density. From both the sigmoid growth curves of Figure 3c and the straightened lines of Figure 3d, it can be seen that the fastest rates of increase occur in the denser host stands, while the slowest rates occur in the least dense stands.

In Figure 4, r values derived (in the above manner) from the results of four separate experimental runs are plotted against the density of seedlings in the stand. Each data point is the mean of a minimum of five replicates. Despite variation between separate experiments a general curvi-linear relationship is clearly discernable. Within the range 0 to 2000 seedlings per m^2 , increases in host plant density produce large increases in the apparent infection rate within the system. However, as further increases in host density above that range occur there are progressively smaller and smaller increments in r until a limiting rate is approached in the vicinity of 10,000 seedlings per m^2 .

3. *Rate of advance of the disease front*

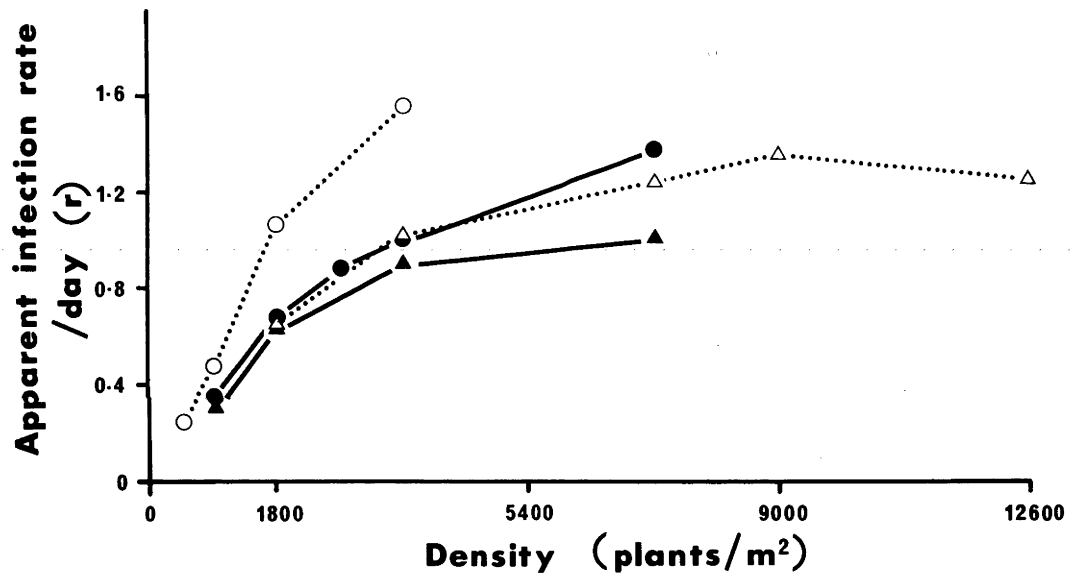
A series of four experiments were carried out to measure the

FIGURE 4.

The effect of changing host plant density on the apparent infection rate of damping-off in randomly inoculated seedling stands.

○ , ● , △ , ▲ , results of four separate experiments.

Each treatment was replicated five times.



rate of advance of damping-off disease through host plant stands of different density. Rates of disease advance were monitored daily by measuring the distance the disease front had advanced from the inoculated edge of each plot. Thus in each experiment, mean values for the daily advance of damping-off (a), were derived from more than a hundred individual measurements within each treatment (five measurements in each of four plots on all seven days).

In Figure 5 the mean values for the daily rate of advance of damping-off obtained in these experiments are plotted against seedling density. Where the resulting four graphs overlap within the same density range they show considerably better agreement than do those for rates of increase (Figure 4). This is no doubt due in large part to the greater number of measurements available in this case. The general form of the relationship between the rate of advance of the disease (a) and host density is evidently similar to that for the rate of increase of disease and host density. Rapid increases in a occur in response to increasing host plant density within the range of 0 to 2,000 seedlings per m^2 but asymptote thereafter towards a limiting rate of 1.45cm advance per day which is attained somewhere in the vicinity of 20,000 seedlings per m^2 .

4. The contribution of falling seedlings to disease increase and spread

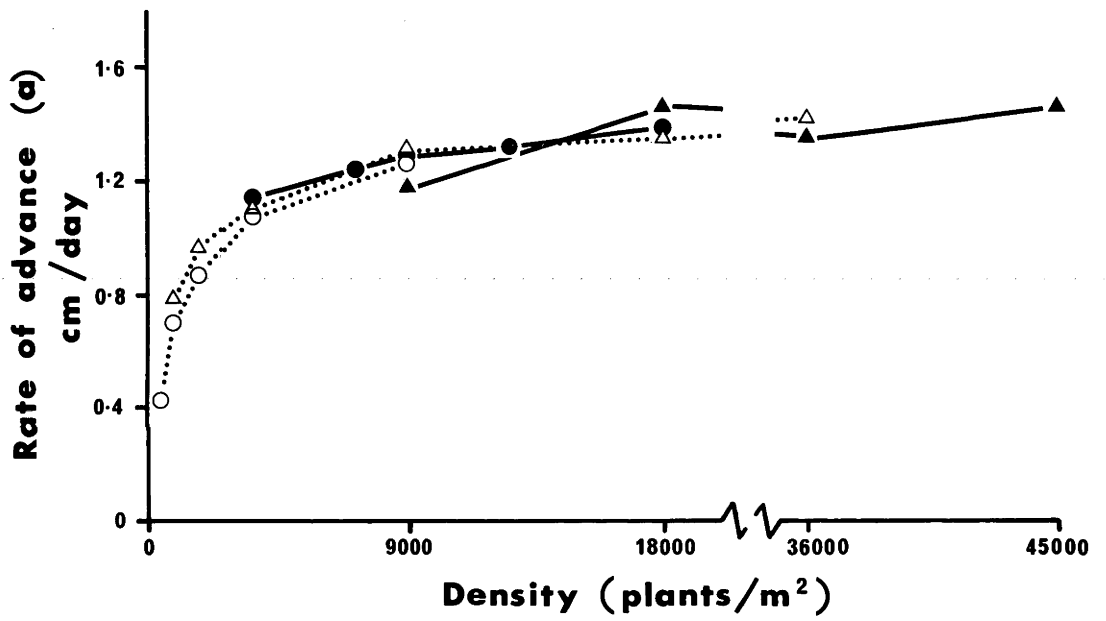
It was apparent during the course of these experiments on the

FIGURE 5.

The effect of changing host plant density on the rate of advance of damping-off in plots inoculated along one edge of the seedling stand.

○ , ● , △ , ▲ , results of four separate experiments.

Each treatment was replicated four times.



rate of increase and advance of damping-off that the 'behaviour' of infected seedlings differed somewhat between high and low density stands. In order to investigate the possible effect that this might have on the rate factors, two experiments were carried out to:

- a) Compare the rate of multiplication of disease between stands in which damped-off seedlings were allowed to collapse in the usual manner and stands in which seedlings were prevented from collapsing;
- b) Measure the rate of spread of disease radially outwards from a point source.

a) *The rate of multiplication of disease in supported stands*

Experimental plots were prepared, sown and inoculated in exactly the same manner as for experiments investigating the rate of multiplication of disease in randomly inoculated stands (Chapter One of this Section). Half the plots were left as controls, while over the other half, one centimetre square fine wire mesh supports were placed approximately 1.5cm above the surface of the vermiculite. Three different host densities were used (1020, 2040, and 3060 plants per m²) with four replicates of each treatment. Figure 6 shows the results of this experiment. At low densities, preventing the collapse of seedlings caused marked reductions in the apparent infection rate compared with those in control plots, while at higher densities (3060 plants per m²) there was little or no effect on the rate of disease increase.

FIGURE 6.

The results of a single experiment to measure the apparent infection rate of damping-off in supported and unsupported seedling stands of varying density (all plots were randomly inoculated).

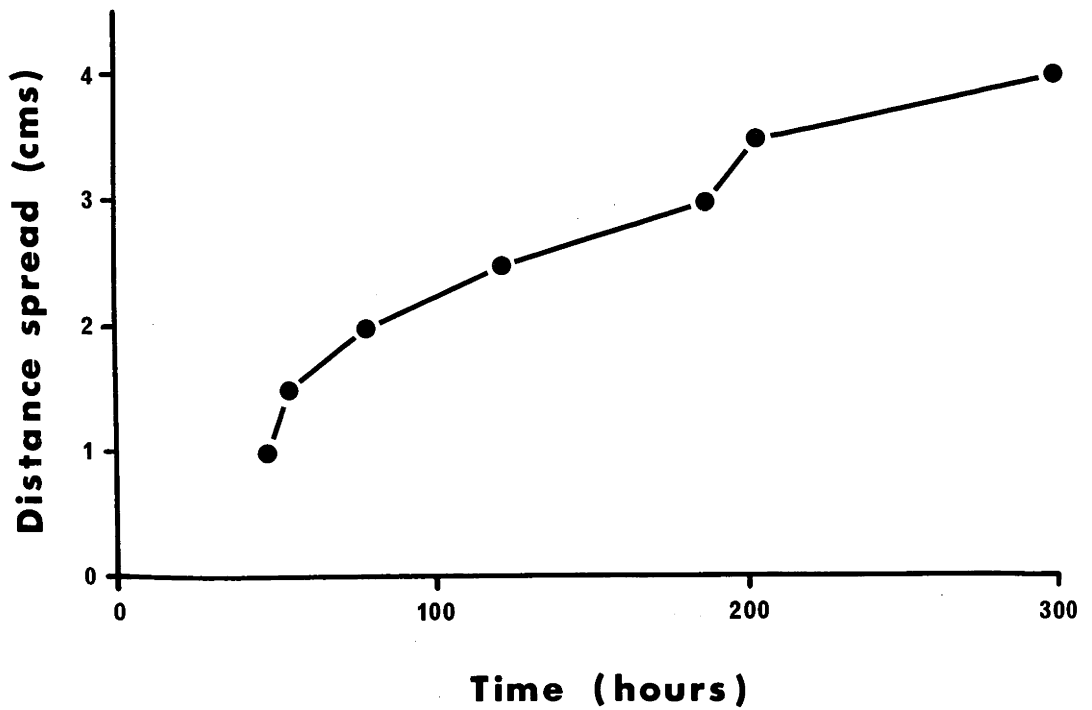
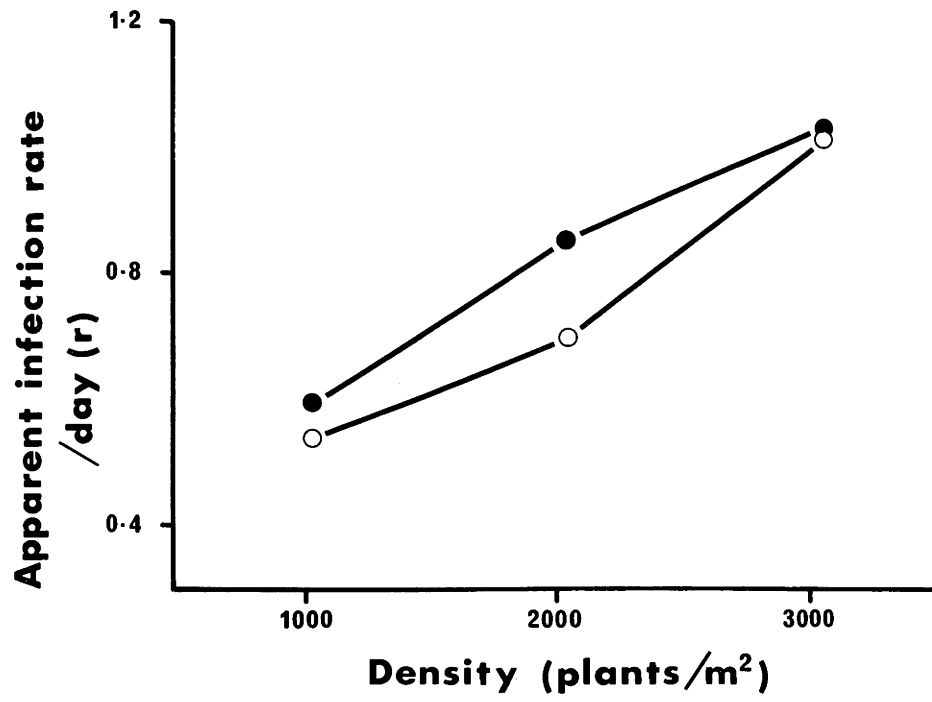
○ , seedling stand supported by wire mesh grid; ● , seedling stand unsupported (control).

Each treatment was replicated four times.

FIGURE 7.

The rate of spread of damping-off radially outwards from a point source as shown by the distance travelled by damping-off (cms) plotted against the time taken in hours.

Each treatment was replicated a minimum of eight times.



b) *The rate of spread of disease radially outwards from a point source*

A single experiment was conducted to measure the furthest limit of growth of *P. irregulare*-induced damping-off outwards from a diseased seedling. Experimental plots were prepared in the same way as for all other experiments. In each plot a circle was marked out, seed was placed at equidistant points around the circumference, and the centre of the circle was marked with a pin. The entire plot was then covered with sterile vermiculite and incubated for four days, after which time all seedlings had emerged. At this time each circle was inoculated at the centre with a single infected seedling produced in the manner described earlier. By using a range of circles (radii of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0 cms), each replicated a minimum of eight times, a good estimate of the rate of outward spread of the disease was obtained. The number of seedlings along the circumference varied between six (small radius circles) and twelve (large radius circles). Scoring was carried out at six hourly intervals for the first sixty hours and at twelve hourly intervals thereafter up to a total of 336 hours (i.e. 14 days). On each occasion, the number of successful transmissions was recorded, and when a third of the seedlings in any given circle had collapsed the disease was deemed to have spread that far. The results of this experiment are shown in Figure 7, where the distance travelled by the disease is plotted against the time taken in hours. As can be seen, a curvi-linear relationship appears to exist between the distance travelled and the time taken. Thus

while it took 10 hours for the disease to travel 0.5cms between 1.0 and 1.5cms from the focus, it required 95 hours to travel 0.5 cms between 3.5 and 4.0cms from the focus. From Figure 7 it would appear that the maximum distance that *P. irregulare*-induced damping-off can traverse across sterile vermiculite is about 4cm, and in this regard it was noted that 12cm diameter circles failed to become infected even after 504 hours (21 days). The declining rate at which the disease appears to travel may be attributed to two factors - a slower growth rate of the fungus the further it extends from the source, and a reduced capability of hyphae to infect plants as they become more distant from the original focus. The relative importance of these factors was not determined.

CHAPTER THREE

EPIDEMICS OF DAMPING-OFF IN MIXTURES OF TWO PLANT SPECIES

I. METHODS

The present Chapter investigates the effect of mixed species stands on the epidemiology of damping-off. The same basic *Lepidium sativum* - *Pythium irregulare* system as previously used was employed, but elaborated by the regular replacement of *Lepidium sativum* host plants with Wimmera ryegrass (*Lolium rigidum* Gaud.). This species was selected after preliminary trials showed it to be very resistant to *P. irregulare*-induced damping-off under the particular experimental conditions used and because it is reasonably comparable to *L. sativum* in terms of seed size and rate of seedling development.

The techniques used in these experiments were the same as those previously employed, except that, while the *L. sativum* was sown directly on to the washed river sand, the *L. rigidum* seed was pre-germinated on moist filter paper at 25°C for two days before planting. This ensured that the seedlings of both species were of comparable size during the course of the epidemic.

For experiments investigating the rate of multiplication of disease in randomly inoculated seedling stands, the analysis of sigmoid curves was somewhat more complicated than in the previous chapter. Sigmoid disease curves obtained for control plots of pure density *L. sativum* were transformed, as before, using the straightener $\log_e(x/1-x)$ plotted against time. The transformation of disease progress curves generated in mixed stands was however somewhat different with $\log_e(x/1-x)$ being changed to $\log_e(x/X-x)$, where x is the proportion of seedlings diseased and X is the proportion of susceptible individuals in the population.

Within the experimental arrangements used, the seedling populations could be varied in two ways. Firstly, by altering the proportion of *L. sativum* to *L. rigidum* seedlings, a replacement series (de Wit, 1960) could be established, permitting the effect of varying proportions of susceptible and resistant plants to be studied while overall plant density was maintained constant. Alternatively, the overall density of the stand could be varied whilst the proportion of *L. sativum* to *L. rigidum* seedlings was held constant (e.g. 50:50). In all experiments, results obtained for mixtures were compared with those obtained

from various monoculture populations of *L. sativum*. All treatments were replicated four times.

II. RESULTS

1. *The occurrence of primary infection foci*

Two separate experiments were initiated in order to compare the number of primary infection foci occurring in 50:50 mixtures of resistant and susceptible individuals with the number occurring in monocultures of the susceptible species. (No infection foci formed in monocultures of the resistant species). In each experiment, inoculum density in the vermiculite layer was held constant at 0.5% while the density of the mixed species stands was varied between 900 and 3600 plants per m². This allowed comparisons to be made at three different seedling densities (Figures 8a and 8b). The results of the two experiments are in reasonably close agreement and show a consistent trend, in that the number of primary infection foci occurring in mixtures containing 50% susceptible plants was always considerably less than the number occurring in monocultures of the same overall stand density. The response of the number of primary infection foci to changes in host plant density is in good agreement with the results obtained in the previous chapter (Figure 1a).

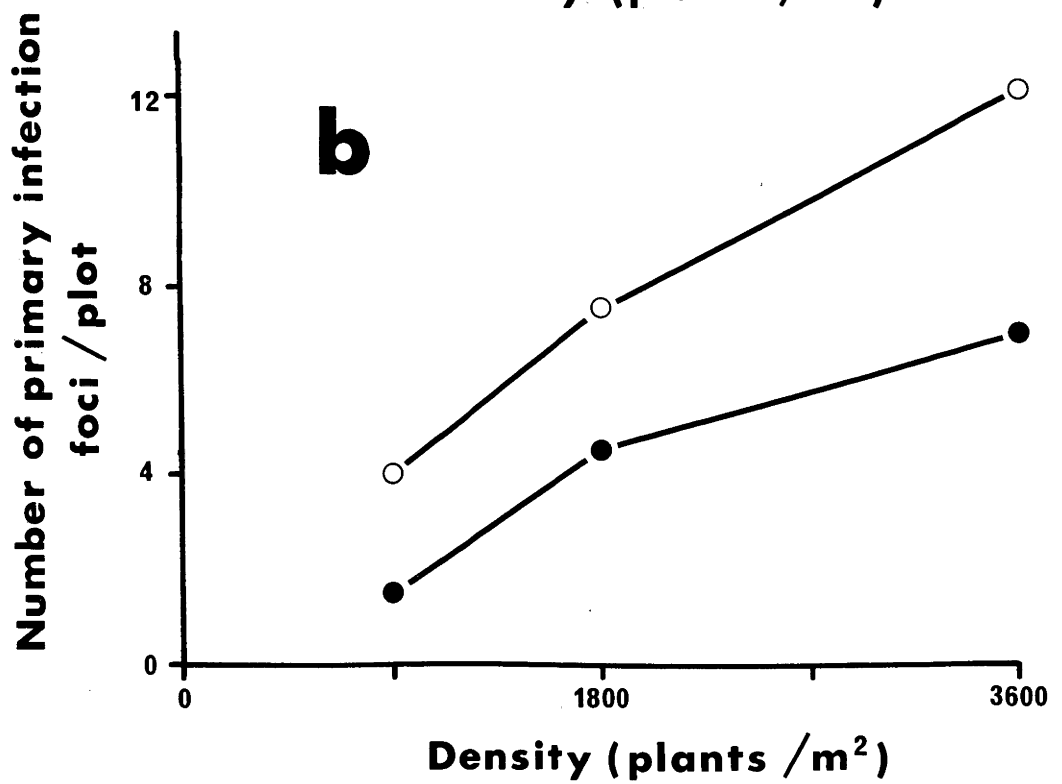
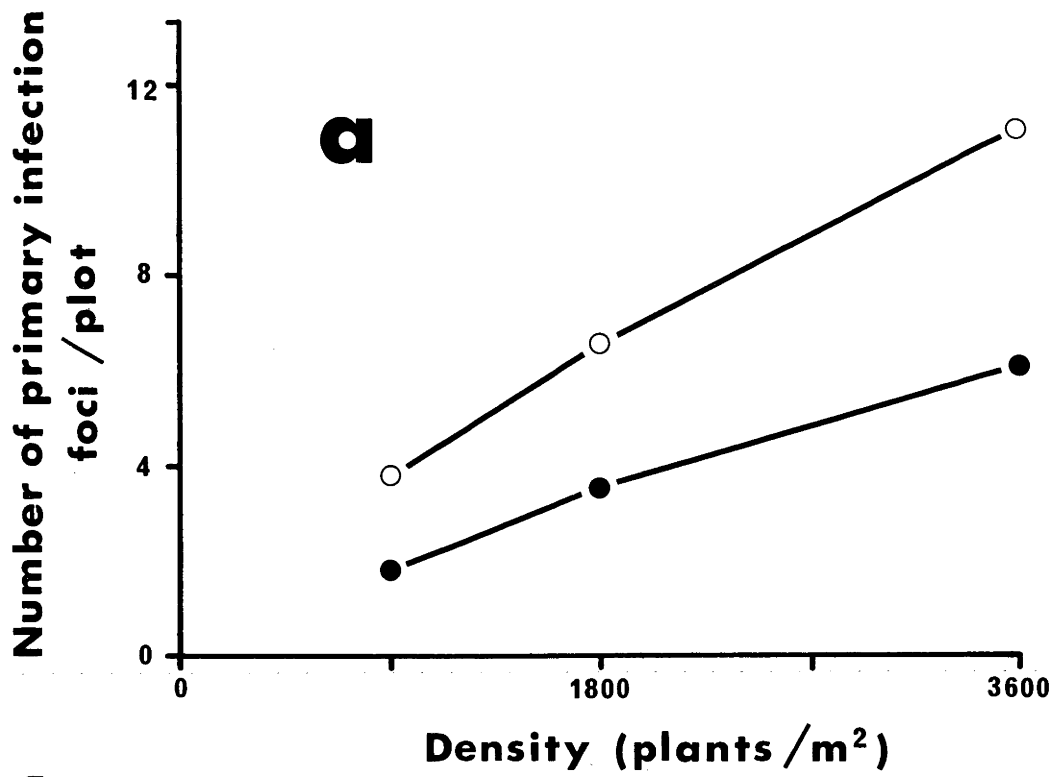
FIGURE 8.

The occurrence of primary infection foci in 50:50 mixtures of susceptible (*Lepidium sativum*) and resistant (*Lolium rigidum*) species in comparison with the occurrence of primary infection foci in monocultures of the susceptible species planted at the same overall densities.

a), b), two separate experimental runs.

○ , monoculture of the susceptible species;
● , 50:50 mixture of the susceptible and resistant species.

Each treatment was replicated four times.



2. *Multiplication of disease in randomly
inoculated seedlings stands*

a) *Comparison between 50:50 mixtures and monocultures*

Initially, three experiments were carried out to compare the rate of multiplication of damping-off in 50:50 mixtures of resistant and susceptible seedlings with those in monocultures of the susceptible species. Monocultures of the resistant species were unable to sustain epidemics of damping-off. The results of the three experiments, each of which provided for comparisons at three different seedling densities (1800, 3600, and 7200 plants per m^2 in one experiment; 900, 1800, and 3600 in the other two), are shown in Figure 9. Although agreement between experiments was not particularly good, results are consistent in showing that apparent infection rates in mixtures containing 50% resistant plants are substantially less than those in monocultures of the same overall stand densities. In general, the biggest effect appears to be at the higher densities even although at such densities (7200 plants per m^2) the susceptible proportion of the mixture (3600 plants per m^2) would on its own constitute a reasonably dense stand of plants.

b) *The effect of varying the proportion of resistant and
susceptible plants*

Experiments were then carried out to investigate the effect of varying the proportion of resistant and susceptible species within seedling stands having the same overall density. Figure 10a shows

FIGURE 9.

Apparent infection rates of damping-off in 50:50 mixtures of susceptible (*Lepidium sativum*) and resistant (*Lolium rigidum*) species compared with those in monocultures of susceptible species planted at the same overall densities.

a) Overall density of 3600 plants per m².

b) Overall density of 3600 plants per m².

c) Overall density of 7200 plants per m².

○ , monoculture of the susceptible species;

● , 50:50 mixture of the susceptible and resistant species.

Each treatment was replicated four times.

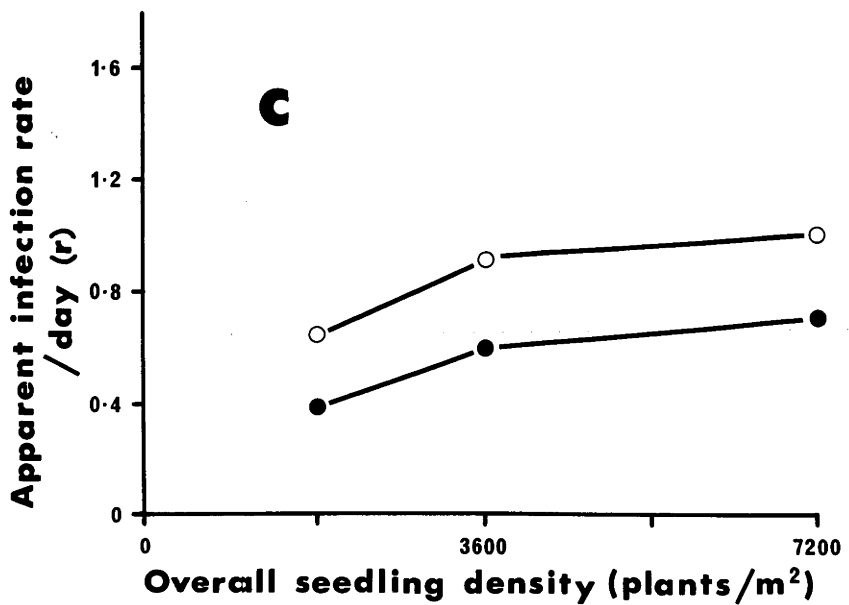
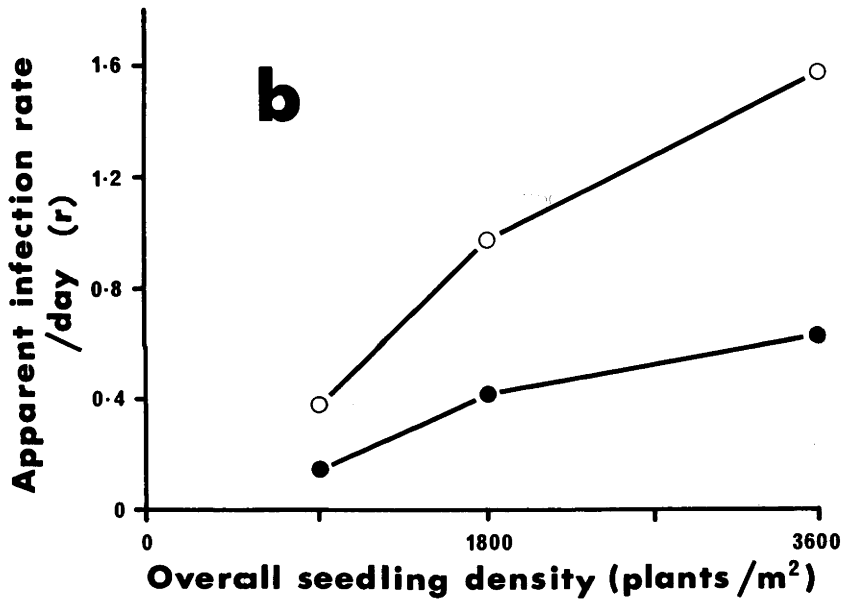
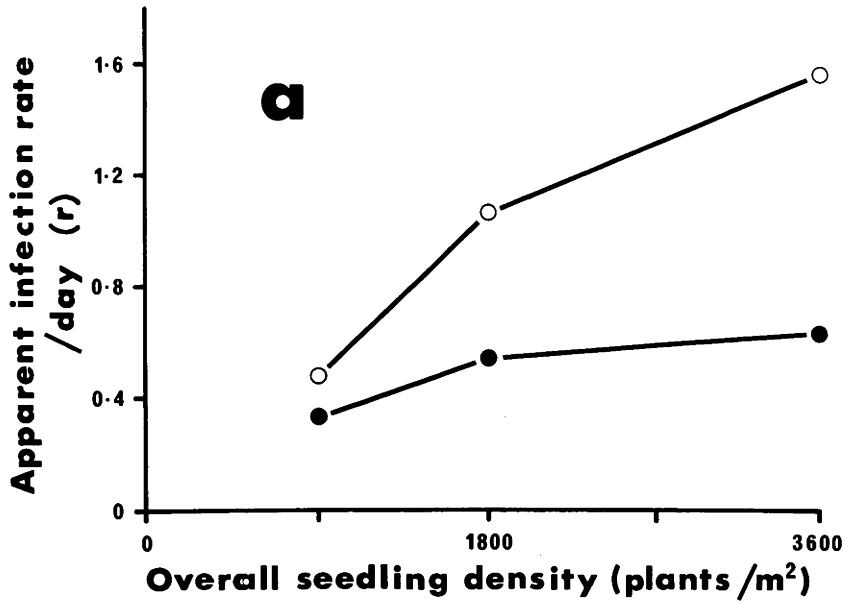


FIGURE 10.

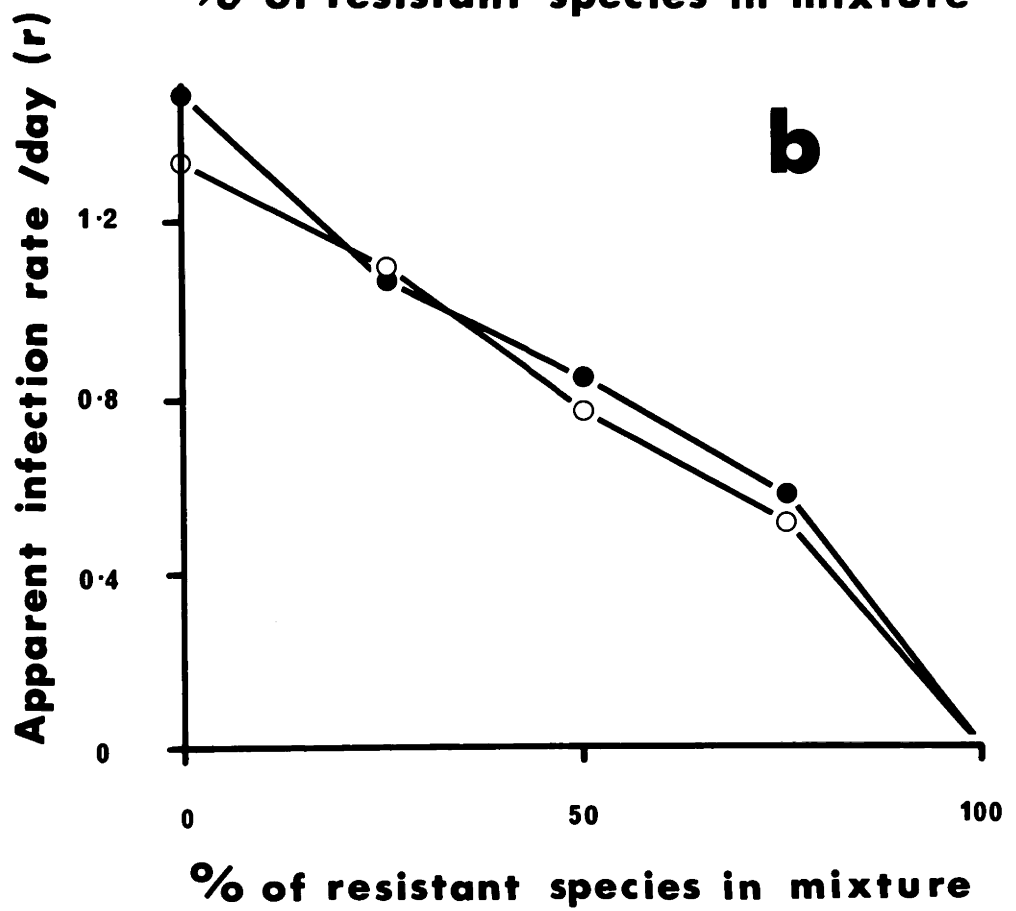
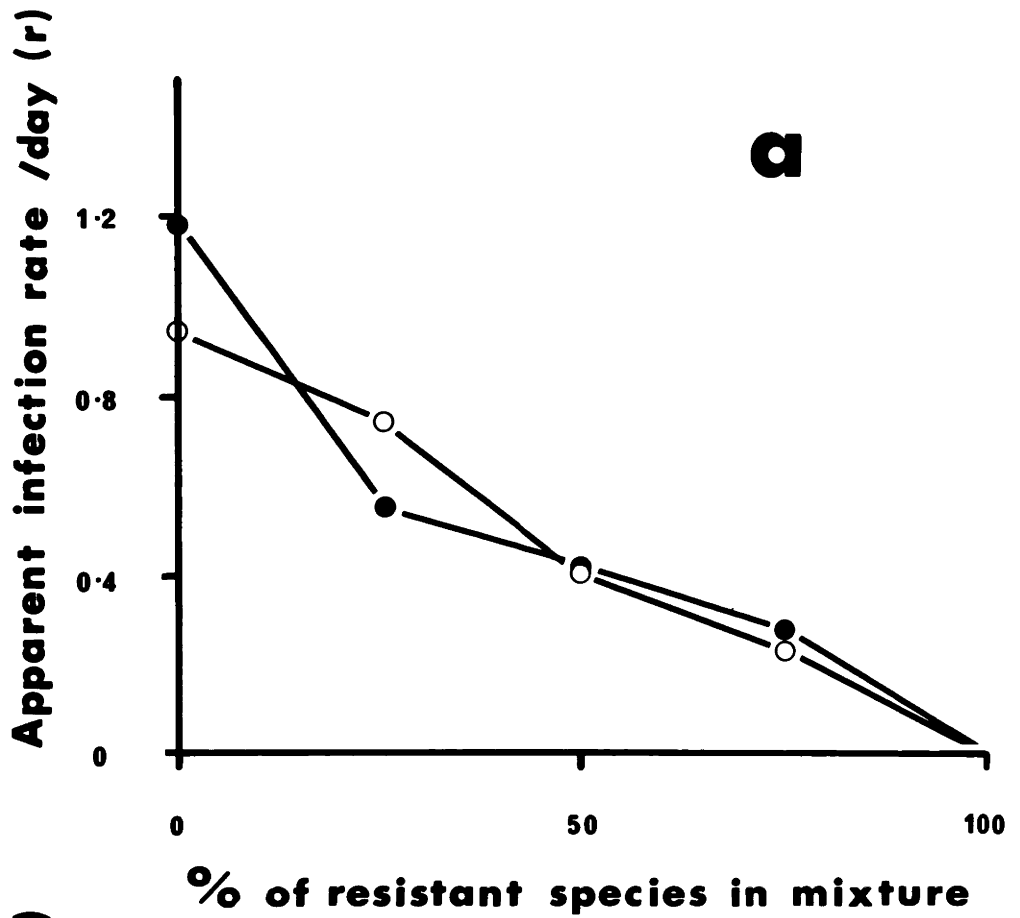
The effect of changing the proportion of susceptible (*Lepidium sativum*) and resistant (*Lolium rigidum*) species in a mixture on the apparent infection rate of damping-off.

a) Overall density of 1800 plants per m².

b) Overall density of 3600 plants per m².

○ , ● , separate experimental runs.

Each treatment was replicated four times.



the results of two experiments using an overall density of 1800 plants per m^2 , while Figure 10b shows the results of two other experiments using 3600 plants per m^2 . In this case, there is good agreement between the duplicate experiments. At both densities the apparent infection rate of disease within the seedling stands declined progressively as the proportion of resistant plants increased and the proportion of susceptible individuals decreased. In the two experiments conducted at the lower overall density this decline was very nearly linear, while in the other two experiments there is a suggestion of a convex slope (i.e. as the proportion of susceptibles in the mixture continues to fall the rate of decline of r becomes progressively greater).

3. *Rate of advance of the disease front*

a) *Comparison between 50:50 mixtures and monocultures*

Figures 11a and b show the results of two separate experiments which compared the rate of advance of damping-off in 50:50 mixtures of resistant and susceptible plants with those in monocultures of the susceptible species at the same overall seedling density. Each experiment covered a range of three densities. Although the two experiments explored different density ranges, reasonably good agreement was obtained between the results in the limited overlapping region. The results reflect those obtained for multiplication of disease in randomly inoculated stands, with the rate of advance in all 50:50 mixtures being substantially

FIGURE 11.

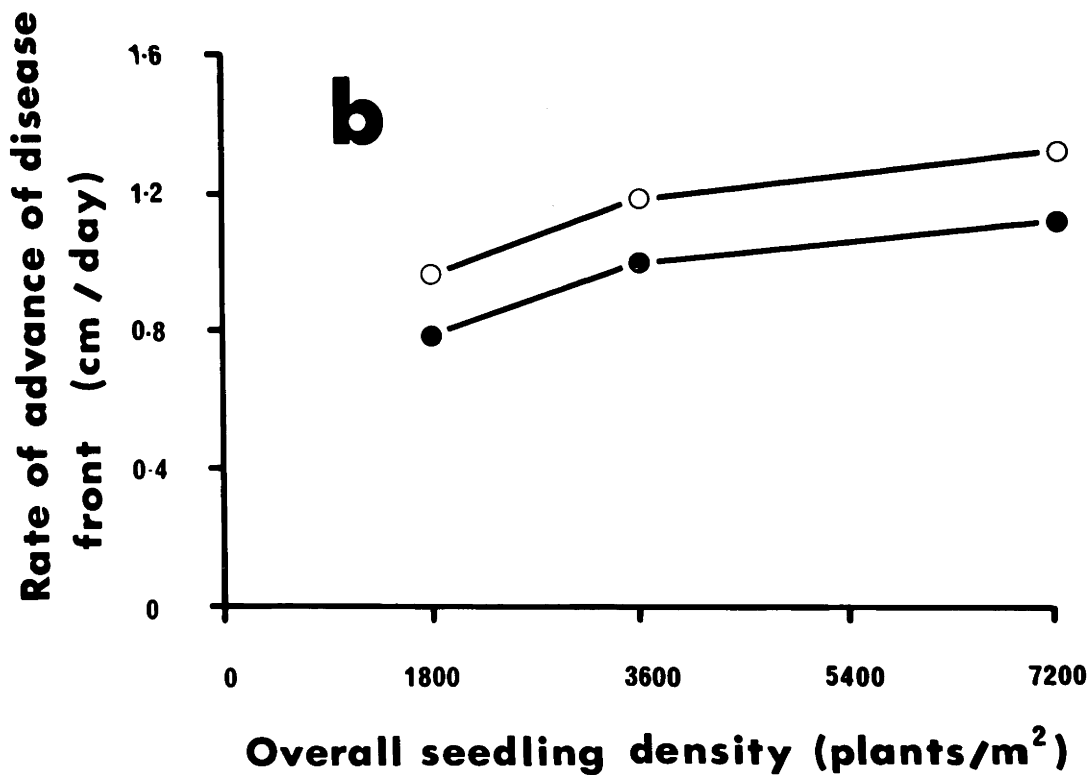
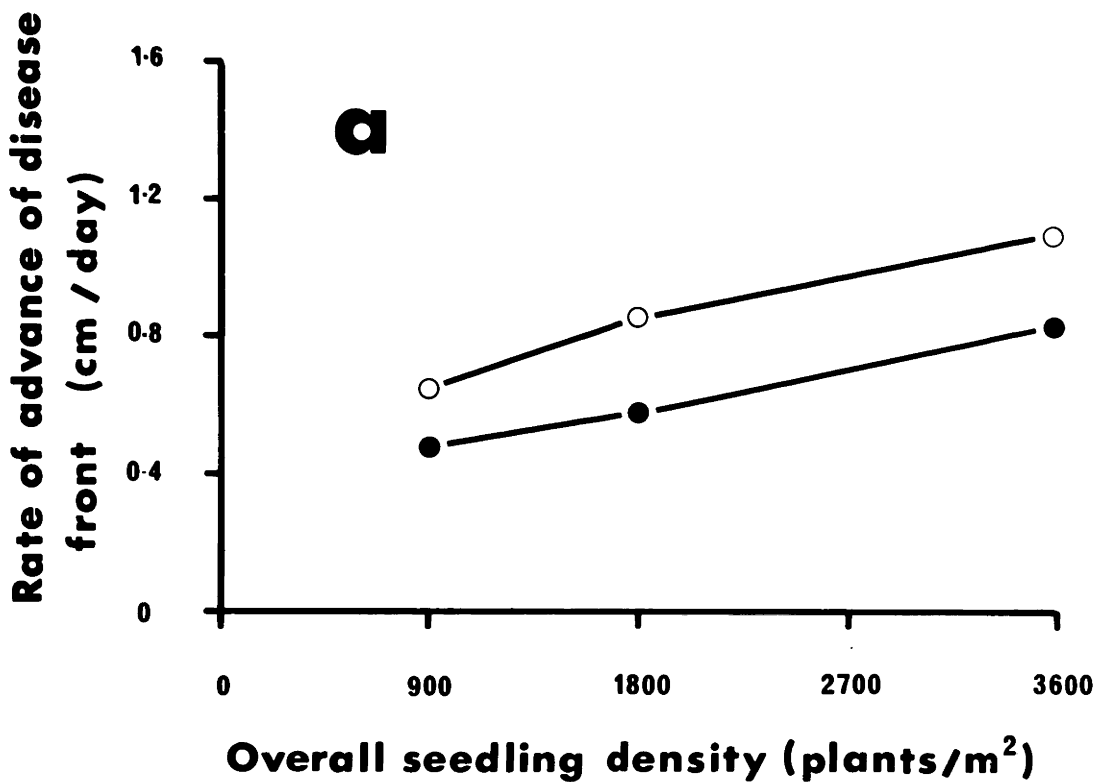
Rates of advance of damping-off in 50:50 mixtures of susceptible (*Lepidium sativum*) and resistant (*Lolium rigidum*) species compared with those in monocultures of the susceptible species planted at the same overall densities.

a), b), separate experimental runs.

○ , monoculture of the susceptible species;

● , 50:50 mixture of the susceptible and resistant species.

Each treatment was replicated four times.



less than that in the appropriate susceptible monoculture control.

b) *The effect of varying the proportion of resistant and susceptible plants*

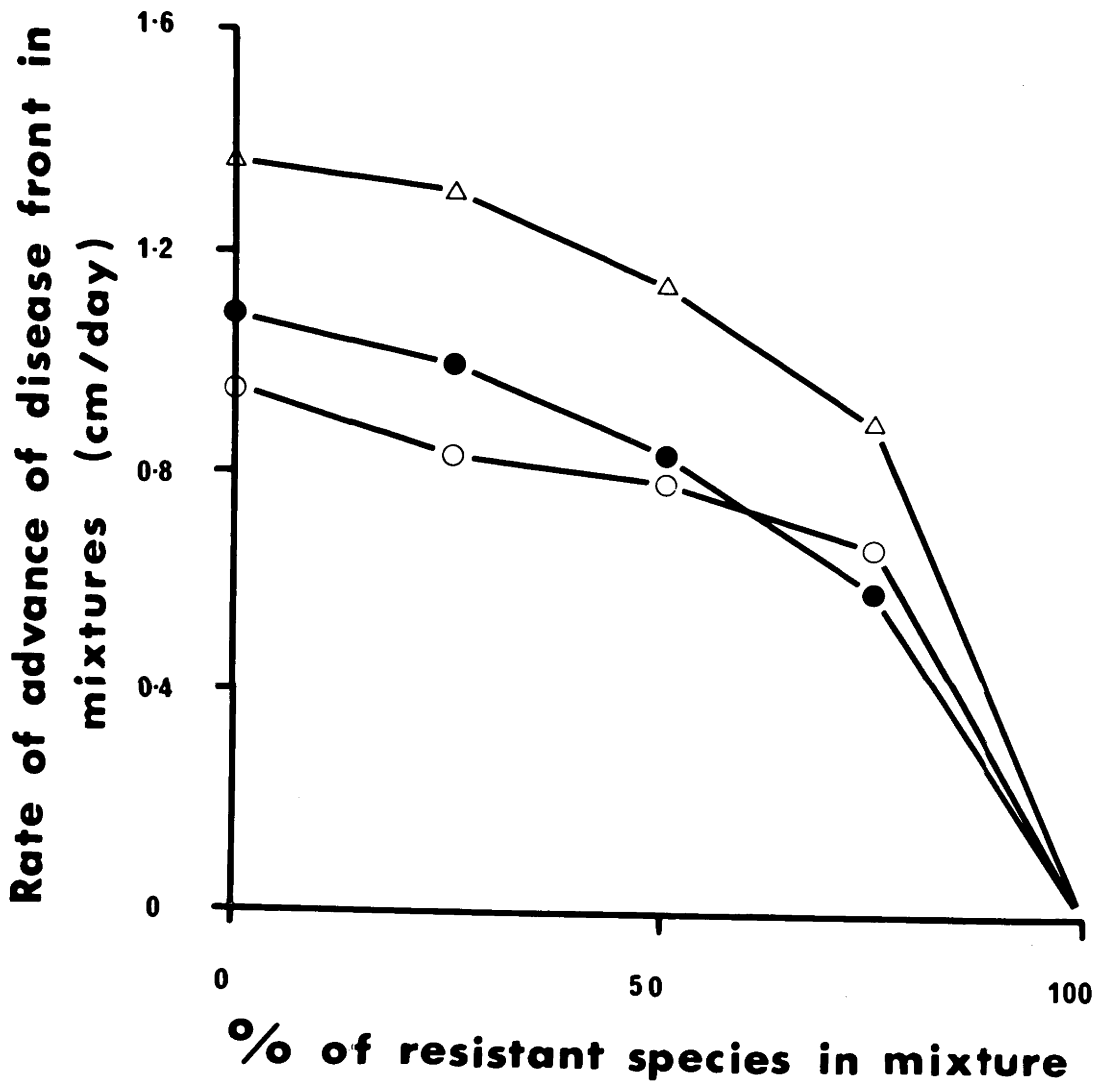
Figure 12 shows the results of three experiments which examined the effect of varying the proportion of resistant and susceptible species on the rate of advance of damping-off. The overall planting density was held constant within each experiment but varied between experiments such that three different densities were examined (1800, 3600 and 7200 plants per m²). In all three experiments, the rate of advance of disease declined as the proportion of resistant plants in the stands increased. The rate of advance fell off particularly rapidly as the proportion of susceptible plants approached zero. These results show no evidence of the simple linear relationship found for the multiplication of disease in low density randomly inoculated stands (Figure 10a).

FIGURE 12.

The effect of changing the proportion of susceptible (*Lepidium sativum*) and resistant (*Lolium rigidum*) species in a mixture on the rate of advance of damping-off. The three graph lines represent the results of three separate experiments carried out at three different overall planting densities.

○ , 1800 plants per m²; ● , 3600 plants per m²; Δ , 7200 plants per m².

Each treatment was replicated four times.



CHAPTER FOUR

THE EFFECT OF PLANTING PATTERN ON EPIDEMICS OF DAMPING-OFF

I. METHODS

In this chapter the *Pythium irregulare* - *Lepidium sativum* model epidemic system used in the previous studies was modified to investigate the effects of varying planting pattern upon the epidemiology of damping-off.

The preparation of inoculum and the setting up of the seed boxes, prior to sowing and inoculation, was exactly the same as that detailed in Chapter One of this Section. The placement of seed and in some cases the inoculation of the resultant experimental arrays were, however, more complicated.

1. *Planting patterns used*

For simplicity in setting up and interpreting experiments, planting patterns were formed of circular plant clumps placed in hexagonal arrays. Within the circular areas designated as clumps, individual plants were distributed evenly, while no plants at all were planted in the area between clumps.

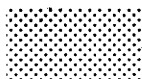
At constant overall stand density, such clumped arrays may then be varied in two separate ways. Firstly, the numbers of clumps per unit area could be varied while the proportion of the total area occupied remained constant. Such a pattern is shown in Figure 13 where the number of clumps varies while the proportion of the area occupied remains constant at 0.5. In this arrangement the density of host plants per unit area of clump is also constant. Alternatively, the proportion of the stand area occupied by clumps could be varied while the number of clumps per unit area remained constant. This is shown in Figure 14 where the number of clumps is held constant while the area occupied varies from 0.5 to 0.125. In such arrays the density of host plants in each clump also varies, although the overall stand density remains constant.

Experiments were carried out to investigate the effect of varying these two parameters separately. The number of clumps was varied between 100 and 1600 per m^2 in four geometric steps (100, 200, 400, 800, 1600), while the proportion of the total area occupied was varied between 0.5 and 0.031, also in four geometric steps (0.5, 0.25, 0.125, 0.063, 0.031). In both types of

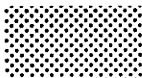
FIGURE 13.

Diagrammatic representation of the planting patterns used in experiments on the effect of pattern on disease rates. I. Varying the number of clumps while the proportion of the total area occupied by clumps was held constant.

- a) 0.5 of total area occupied by clumps.
- b) 0.5 of total area occupied; twice the number of clumps per unit area as in pattern (a).
- c) 0.5 of total area occupied; four times the number of clumps per unit area as in pattern (a).
- d) Unclumped, evenly planted seedling stand.

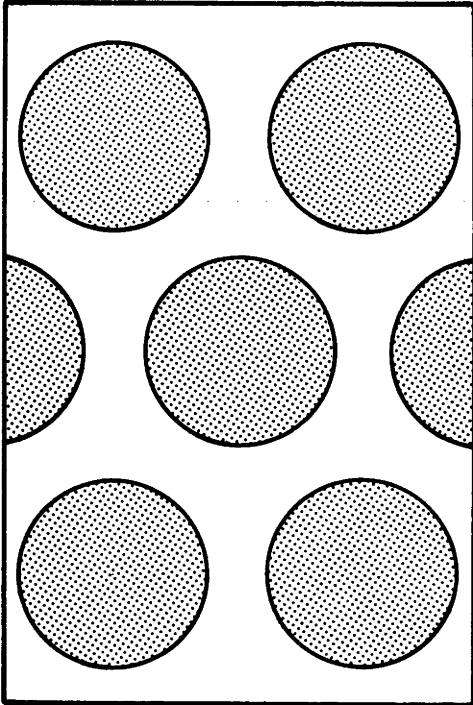


, standard seedling density;

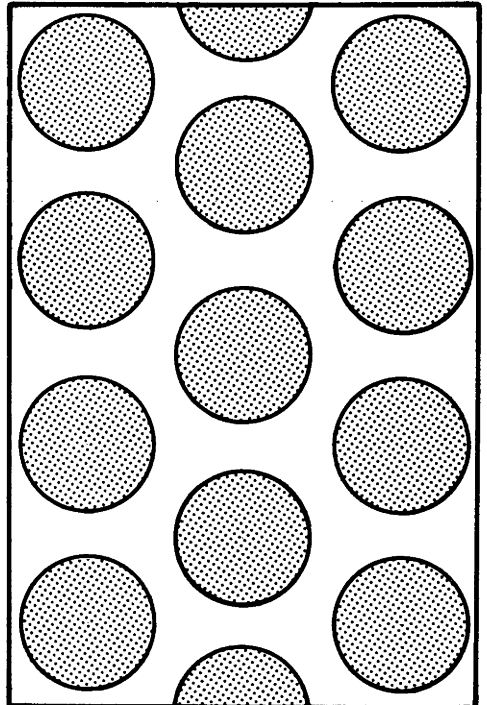


, twice the standard seedling density.

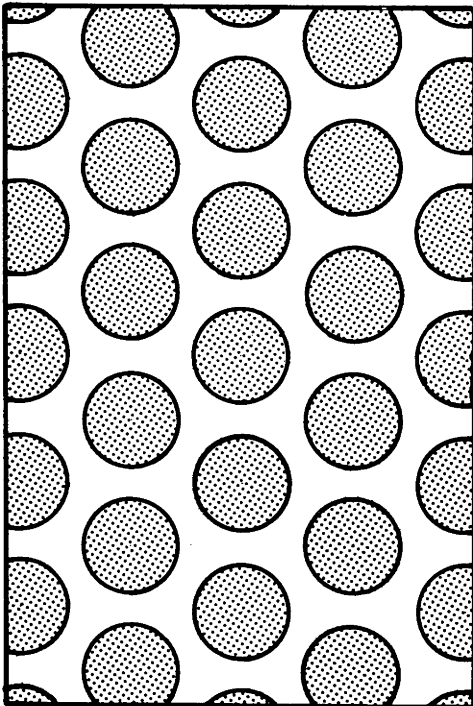
a



b



c



d

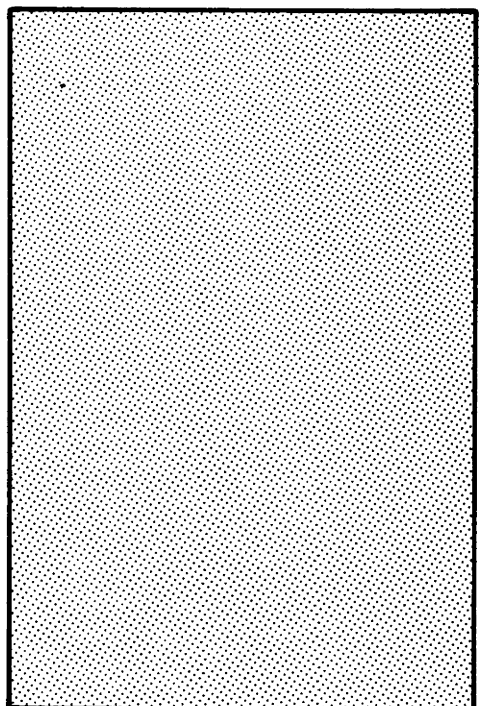
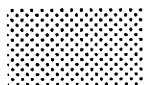


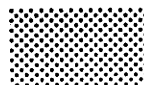
FIGURE 14.

Diagrammatic representation of the planting patterns used in experiments on the effect of pattern on disease rates. II. Varying the area occupied by clumps while the number of clumps was held constant.

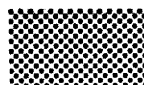
- a) 0.5 of total area occupied by clumps.
- b) 0.25 of total area occupied; same number of clumps per unit area as in pattern (a).
- c) 0.125 of total area occupied; same number of clumps per unit area as in pattern (a).
- d) Unclumped, evenly planted seedling stand.



, standard seedling density;



, twice the standard seedling density;

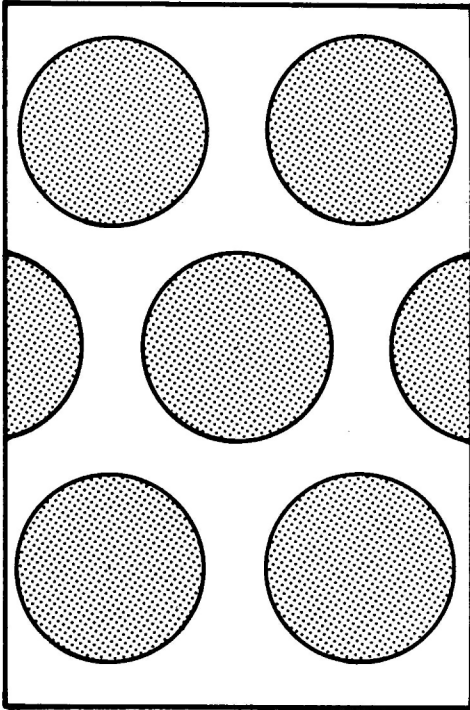


, four times the standard seedling density;

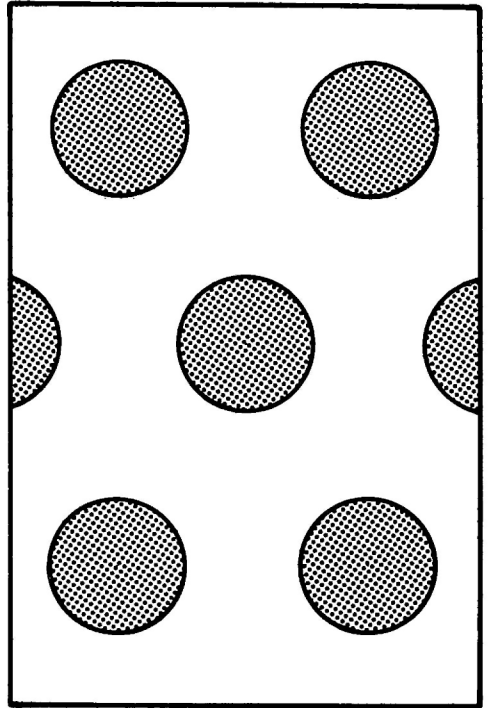


, eight times the standard seedling density.

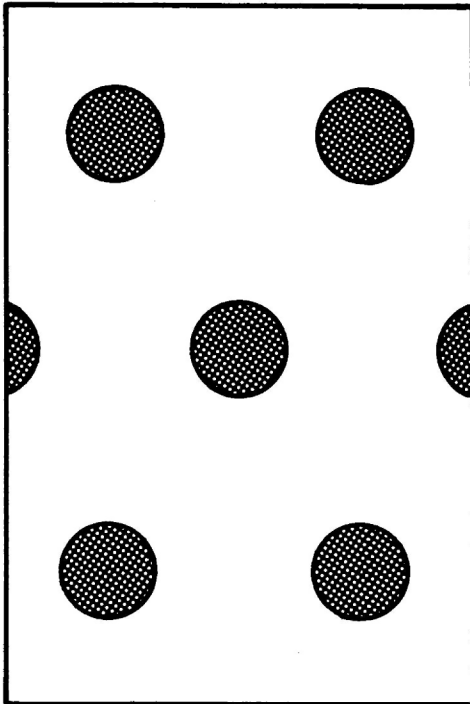
a



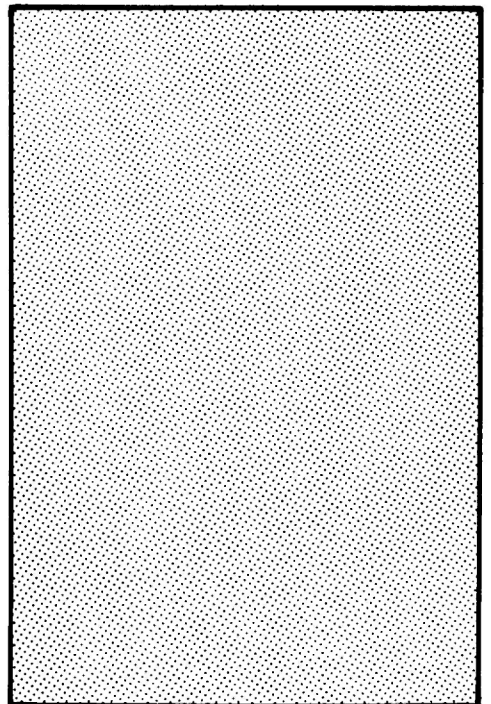
b



c



d



experiment, when the occurrence of primary infection foci or the rate of multiplication of disease were being examined, control plots were provided in which the disease was allowed to multiply within non-clumped evenly planted stands of the same overall density (Figures 13d and 14d).

2. *Inoculation of seed boxes*

In experiments investigating the effect of planting pattern on the occurrence of primary infection foci and on the rate of multiplication of disease, stand inoculation was carried out in the same manner as previously used for such experiments, with 150ml of 0.5% vermiculite inoculum being spread evenly over the stand. In experiments designed to monitor the rate of advance of damping-off in patterned stands, the array was organized so that one clump was as centrally placed as possible within the experimental plot. This clump was then surrounded by six equi-distant neighbouring clumps. The centrally placed clump was inoculated (centrally) with a single diseased *L. sativum* seedling (produced by laying healthy young seedlings on the surface of *P. irregulare* mycelium growing on potato dextrose agar).

3. *Assessment*

The monitoring of the occurrence of primary infection foci and

the rate of multiplication of damping-off was carried out as before.

Disease spread was monitored as it advanced outwards from the inoculated clump. Plots were examined at 12 hour intervals and the position of the disease front noted. Rates of advance were determined by recording the time taken by the disease to travel from the outer edge of the centrally placed clump to the outer edge of neighbouring clumps. Knowing the time taken and the distance travelled, it was then possible to obtain an estimate of the rate of advance which was an average rate for spread across gaps and clumps.

In most experiments in which the number of clumps per m^2 was varied, the area occupied was standardized at 0.125 of the total plot area. Similarly in most experiments where the proportion of the total area was varied, the number of clumps was standardized at 200 per m^2 . In both cases, the majority of these experiments were carried out at an overall stand density of 1840 plants per m^2 . A number of other experiments at different proportions of the total area occupied, numbers of clumps per m^2 and total stand density were carried out to provide comparisons between any observed relationships and results from a wider range of background parameters.

II. RESULTS

1. *Varying the number of clumps while the proportion of the total area occupied by clumps was held constant*

- a) *The occurrence of primary infection foci*

Figure 15 shows the results of an experiment to relate the occurrence of primary infection foci to the number of clumps per m^2 . In this particular experiment, overall host density was held constant at 1840 plants per m^2 while the proportion of the total area which was occupied by clumps was 0.125. As can be seen from Figure 15a, the number of primary infection foci, expressed in terms of the total number of foci appearing per square metre, fluctuated considerably between different clumping treatments with no consistent trend being evident. This is confirmed by an examination of the standard errors provided for each mean value, which indicate that there is no significant difference (at the 5% level) between the numbers of foci recorded in the various clumping treatments.

If, instead of considering the total number of foci per square metre, one looks at the proportion of clumps in each treatment which were infected by primary infection foci, the relationship shown in Figure 15b is obtained. Clearly a logarithmic type relationship exists, so that while a doubling in the number of clumps from 100 to 200 per m^2 caused the proportion of clumps infected to fall from

FIGURE 15.

The effect on the incidence of primary infection foci of varying the number of clumps per m^2 when a constant 0.125 of the total area is occupied by clumps.

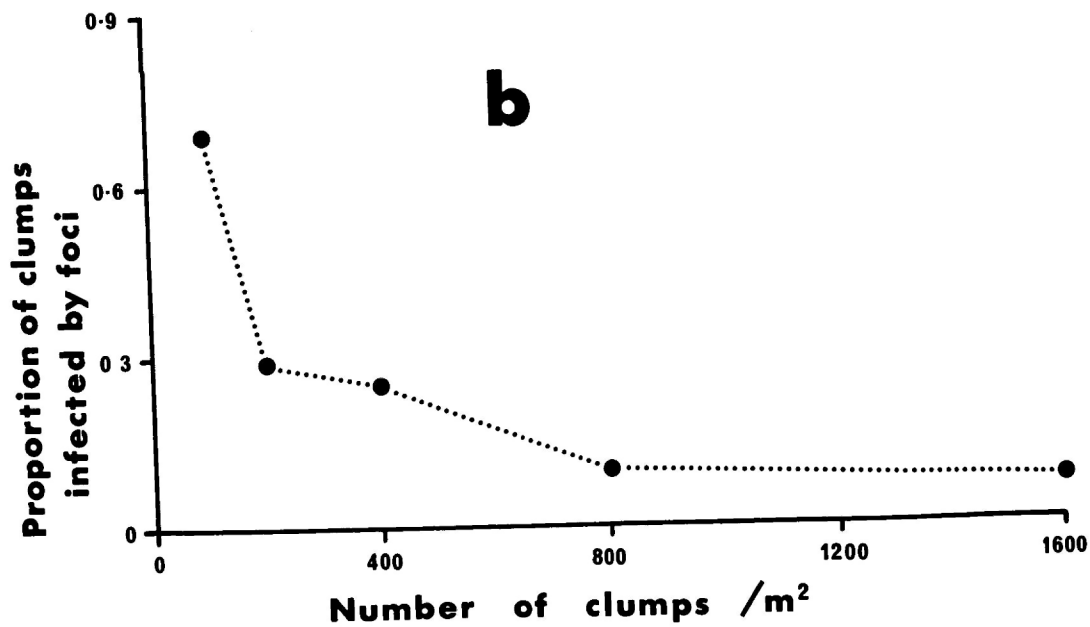
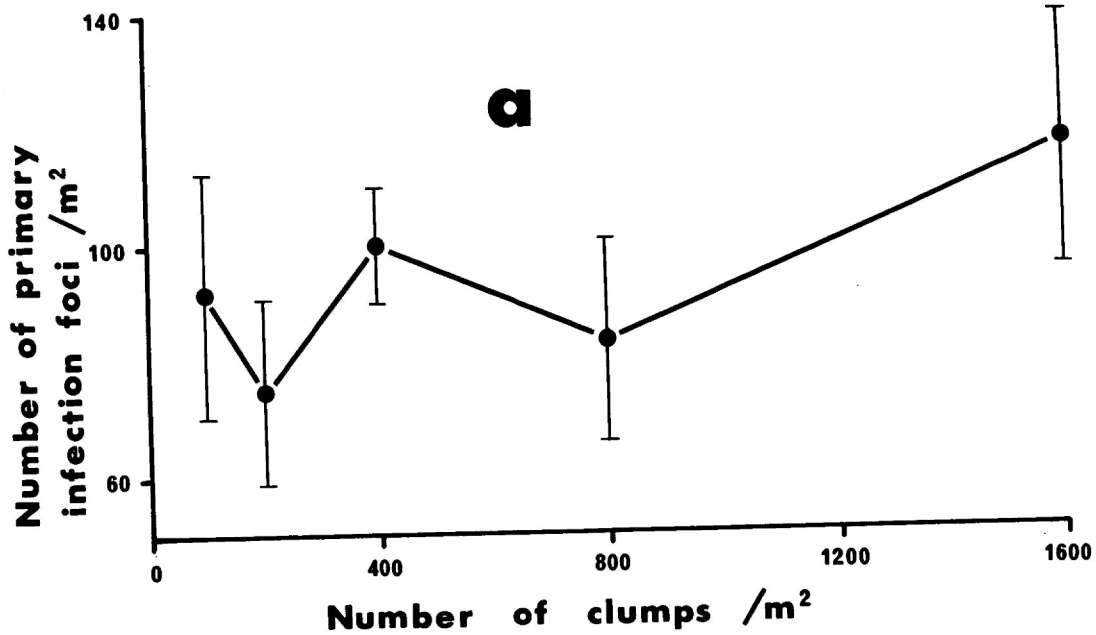
- a) The results of a single experiment showing the relationship between the number of clumps per m^2 and the number of primary infection foci per m^2 .

- b) The results of the same experiment as in (a) showing the relationship between the number of clumps per m^2 and the proportion of plant clumps which were infected at one or more foci.

Overall seedling density of 1840 plants per m^2 .

Each data point represents the mean of a minimum of five values.

Vertical bars indicate \pm standard error of mean.



0.69 to 0.29, a further eight fold increase in the number of clumps to 1600 per m^2 was necessary to produce a further decline to 0.08. Only mean values are shown, as standard errors cannot be determined for percentages or proportions.

b) *The rate of multiplication of disease in randomly inoculated seedling stands*

The results of part of one experimental run, expressed as the proportion of seedlings damped-off (x) plotted against time, are shown in Figure 16a. It can be seen that while the epidemic induced in the evenly planted control seedling stand follows a smooth sigmoidal type of growth curve, those induced in the clumped arrays are often characterized by a disjointed disease curve. Common features of such curves are regions of rapid disease increase caused by the disease spreading rapidly through clumps, and regions of slower increase resulting from disease spread across the gaps between clumps. These curves were 'straightened' by transforming x to $\log_e (x/1-x)$, and lines were fitted by regression analysis (Figure 16b). Although the irregular progress of the disease in the clumped arrays resulted in rather poorly fitting regression lines, the gradients of these lines were used to provide an estimate of the apparent infection rate (r) of the disease.

r values derived in this manner from the results of five separate experiments, are plotted against the number of seedling clumps per m^2 (Figure 17). Figure 17a shows the effect of varying the number of clumps,

FIGURE 16.

Detailed results from a single experiment on damping-off in differently patterned seedling arrays.

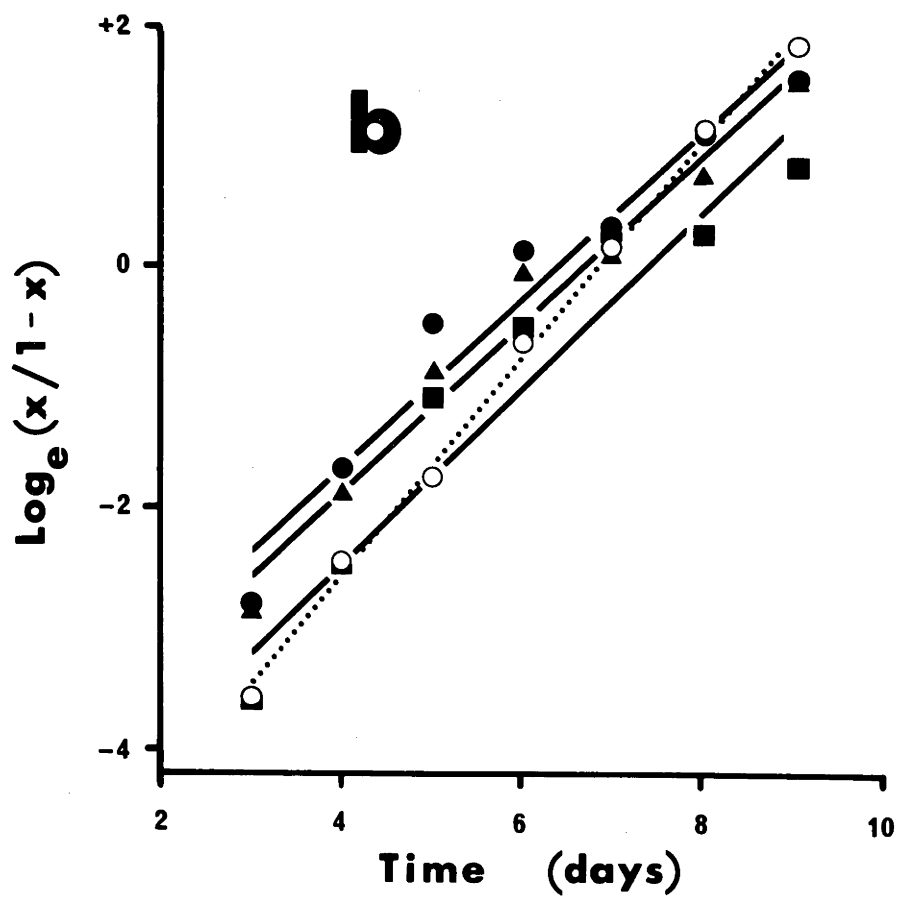
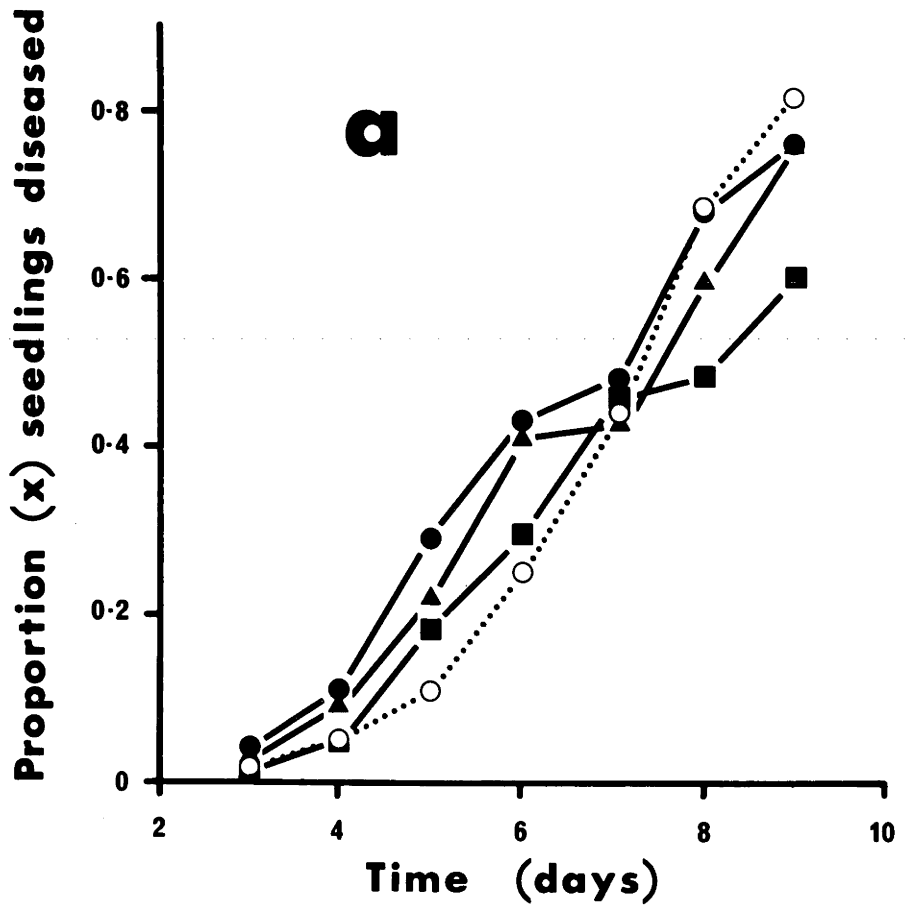
a) Changes in the proportion (x) of seedlings diseased with time.

b) $\log_e (x/1-x)$ plotted against time and fitted with linear regression lines.

(Constant 0.125 of total area occupied by clumps; overall density maintained at 1840 plants per m^2).

○ , even distribution; ● , 200 clumps per m^2 ; ▲ , 400 clumps per m^2 ; ■ , 1600 clumps per m^2 .

Each treatment was replicated four times.



while maintaining the area occupied by clumps at 0.125 of the total area available. In total, three experiments were carried out at this particular proportion of the total area occupied. One of these was conducted at an overall stand density of 1220 plants per m^2 while the other two were planted at an overall density of 1840 plants per m^2 . The results of these latter two experiments were amalgamated into the single line shown in Figure 17a. Standard errors were determined for all mean values shown. As expected, r values obtained for the different clumped arrays at 1840 plants per m^2 were consistently greater than those recorded in arrays with an overall density of 1220 plants per m^2 . In neither density was there any consistent trend in apparent infection rates with respect to the degree of clumping except in the case of plots patterned at 100 clumps per m^2 . Plots patterned at 100 clumps per m^2 gave consistently greater r values than those obtained in all other arrays at the same overall seedling density. In fact, in the experiment at 1220 plants per m^2 , this difference was significant (at the 5% level) when compared with all other results obtained during the same experiment. Rates recorded for all other clumped arrays were extremely close to those obtained for unclumped controls.

In a parallel experiment, varying the number of clumps present against a constant background of 0.5 of the total plot area occupied (Figure 17b), had similar effects, in that there was little consistent variation in apparent infection rates from those obtained in regular arrays. At 100 clumps per m^2 , a higher apparent infection rate was observed than in more densely clumped arrays in the experiment conducted at 1840 plants per m^2 , while in the single

FIGURE 17.

The effect on the apparent infection rate of damping-off of varying the number of clumps per m^2 while maintaining constant the area occupied by clumps.

a) The apparent infection rate of damping-off in relation to the number of clumps per m^2 when a constant 0.125 of the total area is occupied by clumps.

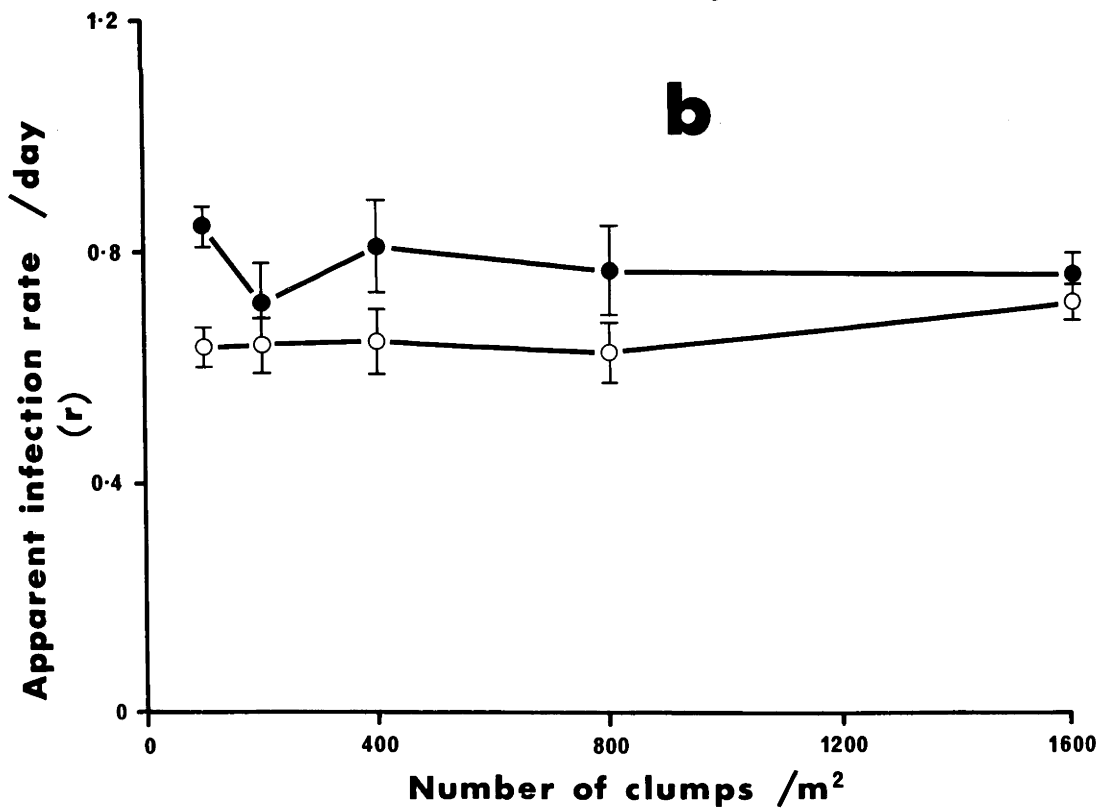
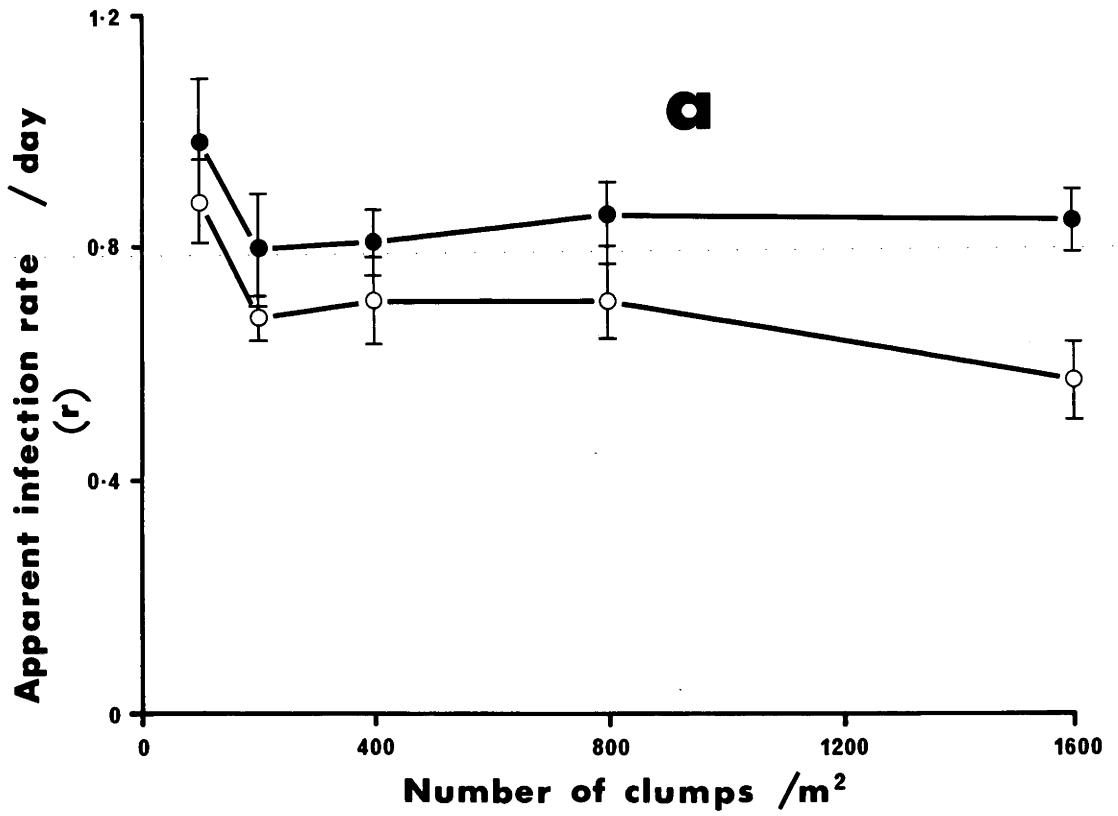
● , combined results of two experiments at an overall density of 1840 plants per m^2 ; ○ , overall density of 1220 plants per m^2 .

b) The apparent infection rate of damping-off in relation to the number of clumps per m^2 when a constant 0.5 of the total area is occupied by clumps.

● , overall density of 1840 plants per m^2 ;
○ , overall density of 1220 plants per m^2 .

Each treatment was replicated four times.

Vertical bars indicate \pm standard of each mean.



experiment at 1220 plants per m^2 an almost horizontal line was obtained.

c) *The rate of advance of disease*

The effect of varying the proportion of the total area occupied by clumps on the rate of advance of damping-off was examined in two experiments, the results of which are shown in Figure 18. In the experiment shown in Figure 18a the clumps were restricted to 0.125 of the total available area, and clearly there is little effect of varying the number of clumps per m^2 on the rate of disease advance (a). As clump numbers per m^2 decline, the results of each treatment tend to become more variable (as shown by the standard errors). This trend is reflected in Figure 18b where the combined results of two similar experiments (with clumps occupying 0.5 of the total area) are shown. Once again, the fluctuations in the values of a which occur between different clumping treatments are not statistically significant.

2. *Varying the proportion of the
total area occupied by clumps while the
number of clumps was held constant*

a) *The occurrence of primary infection foci*

Two experiments were carried out to examine the effect of reducing the proportion of the total area occupied on the total

FIGURE 18.

The effect on the rate of advance of damping-off of varying the number of clumps per m^2 while maintaining constant the area occupied by clumps.

- a) The results of a single experiment where a constant 0.125 of the total area is occupied by clumps. Overall density of 1840 plants per m^2 .

- b) The results of a single experiment where a constant 0.5 of the total area is occupied by clumps. Overall density of 1840 plants per m^2 .

Each data point represents the mean of a minimum of five values.

Vertical bars indicate \pm standard error of each mean.

number of primary infection foci occurring per m^2 , when the number of clumps and total plant density were held constant at 200 clumps and 1840 plants per m^2 respectively. The results were combined together and are presented in Figure 19a as a single set of points. Clearly, considerable variation occurred between replicates of all treatments (only the two most distant points, 0.5 and 0.031, being significantly different from each other), but despite this there is the suggestion of a general trend towards declining numbers of primary infection foci per m^2 with decreasing proportions of the total area occupied.

Of more importance in such clumped arrays is the proportion of clumps actually infected by primary infection foci, as this has considerable influence on the dynamics of disease development. In Figure 19b the above results are expressed in this form, and although only mean values can be shown, (as before the calculation of stand errors is inappropriate), it is readily apparent that there is no consistent relationship between the proportion of clumps infected and the proportion of the total area occupied - approximately the same number of clumps being infected regardless of the area they occupy. The apparent anomaly between the results shown in Figures 19a and b is partly explained by the fact that in large clumps, (that is, where the proportion of the area occupied is large), multiple infections were often recorded - and while these contribute to the total number of primary infection foci per m^2 they have no effect on the proportion of clumps infected.

FIGURE 19.

The effect on the incidence of primary infection foci of varying the area occupied by clumps, while the number of clumps was maintained constant at 200 per m^2 .

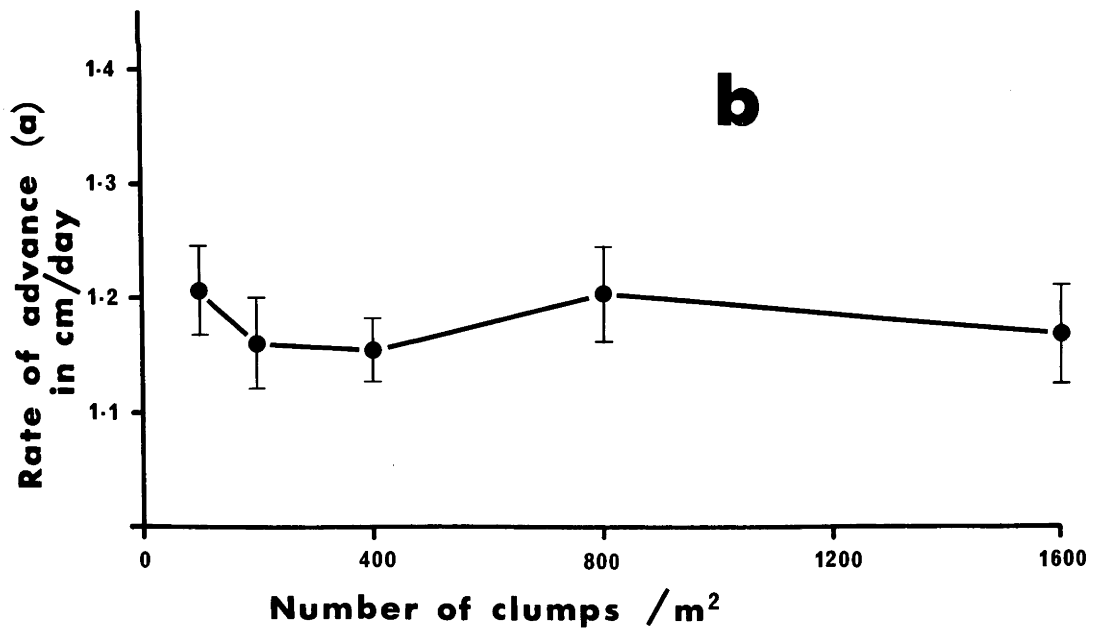
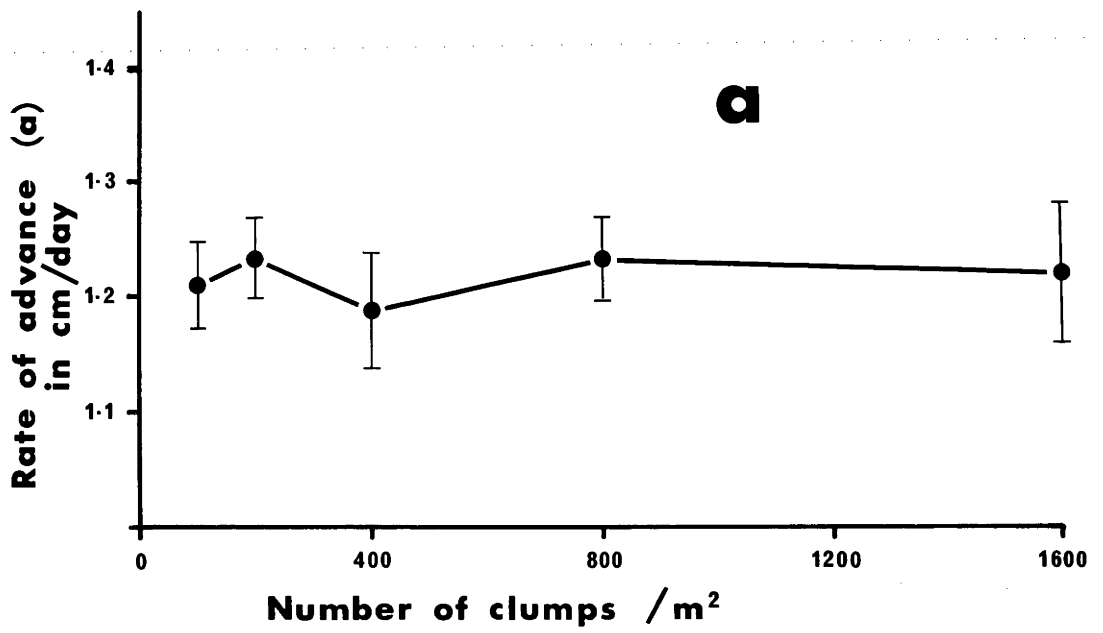
- a) The combined results of two experiments showing the relationship between the proportion of the total area occupied by clumps and the number of primary infection foci per m^2 .

- b) The combined results of the same two experiments as in (a) showing the relationship between the proportion of the area occupied by clumps and the proportion of plant clumps which were infected at one or more foci.

Overall seedling density of 1840 plants per m^2 .

Each data point represents the mean of a minimum of eight values.

Vertical bars indicate \pm standard error of the mean.



b) *The rate of multiplication of disease in randomly inoculated seedling stands*

The effect of varying the proportion of the total area occupied by clumps while maintaining a constant 200 clumps per m^2 was examined in a series of three experiments. In one of these, overall host plant density was held constant at 1220 plants per m^2 , while in the other two a higher density (1840 plants per m^2) was used. The results from the latter two experiments were amalgamated into a single graph line (Figure 20a) in order to increase the number of replicates for the calculation of standard errors of the various means. Standard errors were determined for all mean values. From Figure 20a it is clear that there is a curvi-linear relationship between the apparent infection rate of disease and the proportion of the total area occupied. At low absolute values, increases in the area occupied result in larger increases in r than at higher absolute values. As can be seen from the fitted standard errors, although this curvi-linear relationship is nowhere as marked as that found for host density (see Chapter One of this Section), mean values at the low end of the area occupied range are significantly different (at the 5% level) from those at the higher end. Significant differences of this kind were found for both overall planting densities.

Figure 20b shows the results of two further experiments conducted at a constant 800 clumps per m^2 . Each experiment utilized a different overall planting density (1220 and 1840 plants per m^2). In this case it is apparent that varying the

FIGURE 20.

The effect on the apparent infection rate of damping-off of varying the area occupied by clumps, while maintaining constant the number of clumps per m^2 .

a) The apparent infection rate of damping-off in relation to the proportion of the total area occupied. Stands patterned at a constant 200 clumps per m^2 .

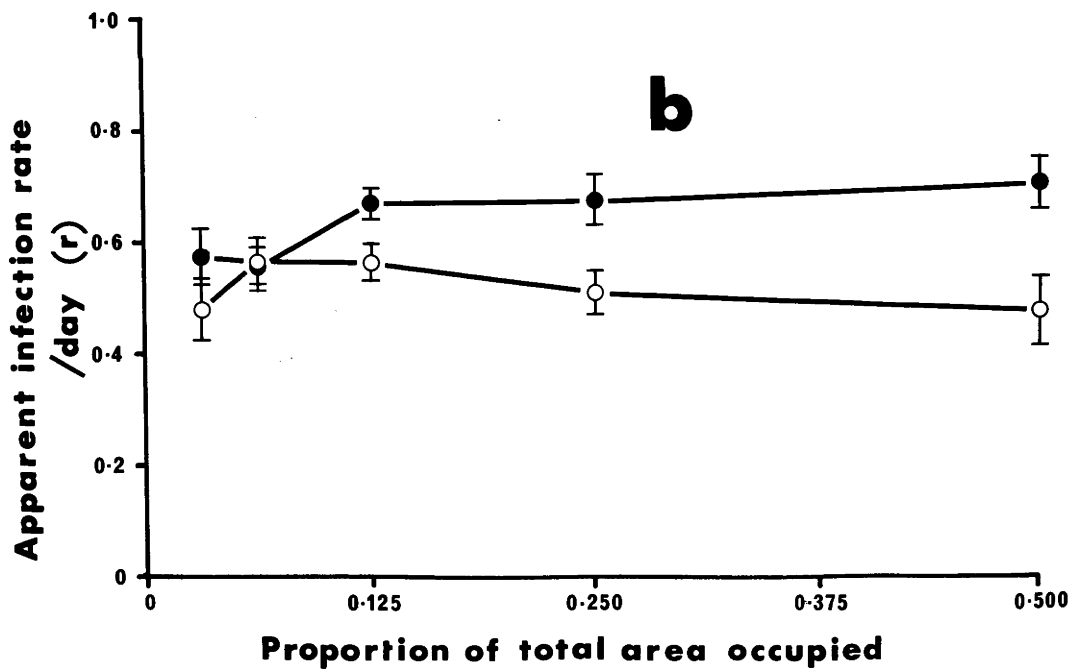
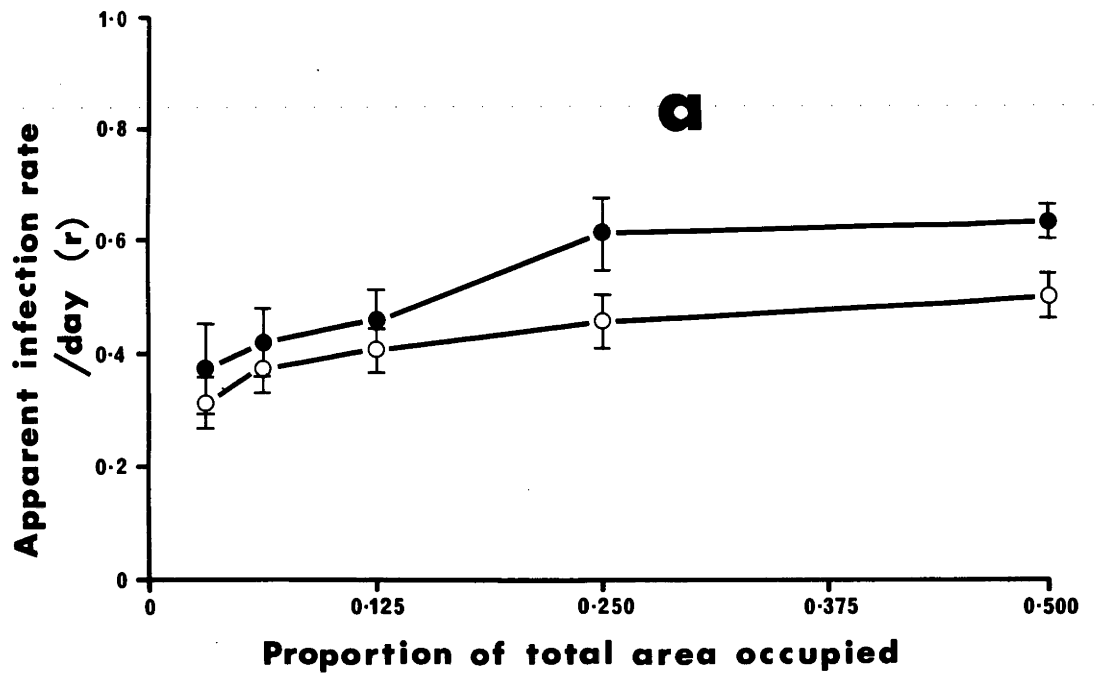
● , the combined results of two experiments at an overall seedling density of 1840 plants per m^2 ; ○ , overall seedling density of 1220 plants per m^2 .

b) The apparent infection rate of damping-off in relation to the proportion of the total area occupied. Stands patterned at a constant 800 clumps per m^2 .

● , overall seedling density of 1840 plants per m^2 ; ○ , overall seedling of 1220 plants per m^2 .

Each treatment was replicated four times.

Vertical bars indicate \pm standard error of each mean.



proportion of the total area occupied has considerably less effect on r values than in experiments conducted at 200 clumps per m^2 . In both experiments, however, apparent infection rates declined at the lowest proportion of the total area occupied. None of these changes were significant.

In all the experiments illustrated in Figure 20, apparent infection rates obtained in stands seeded at 1840 plants per m^2 were consistently greater than those seeded at 1220 plants per m^2 .

c) *The rate of advance of disease*

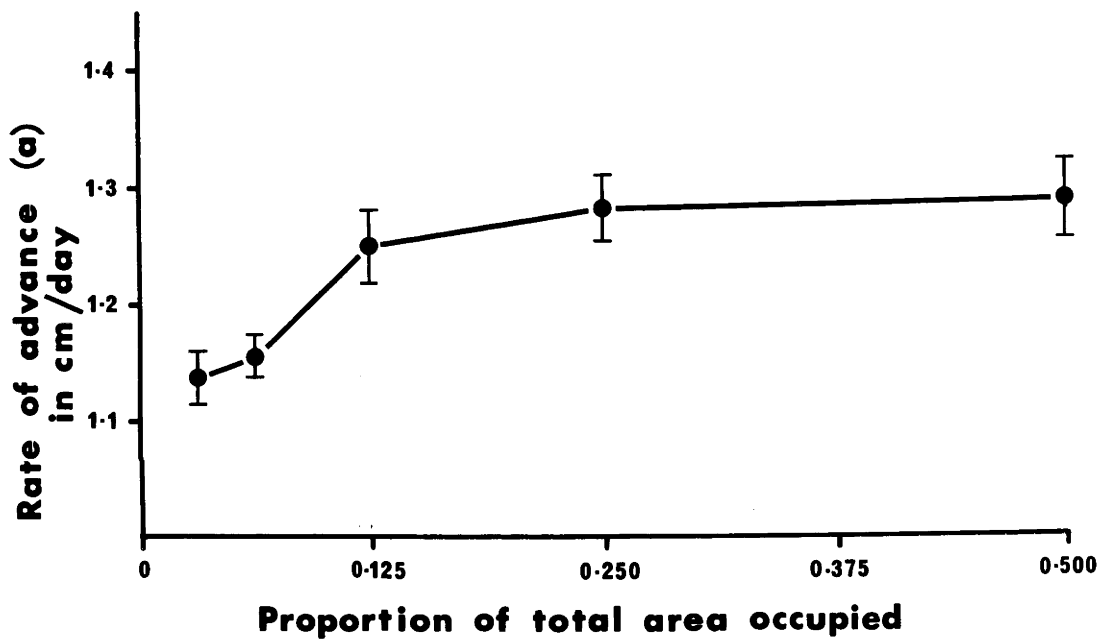
Changes in the rate of advance of disease which occur in response to decreases in the area occupied by clumps are illustrated in Figure 21. Here the results of two separate experiments, at an overall density of 1840 plants per m^2 and a clump density of 200 per m^2 , are shown in a combined form. In both experiments, a weak curvi-linear relationship was apparent, with rates of disease advance increasing as the proportion of the area occupied increased in the range 0 to 0.125. Further increases in the area occupied above this level had little effect on a . Points at the lower end of this curvi-linear interaction were significantly different ($P < 0.05$) from a values obtained for the upper end of the relationship.

FIGURE 21.

The combined results of two experiments showing the effect on the rate of advance of damping-off of varying the area occupied by clumps, while maintaining the number of clumps constant at 200 per m². (Overall seedling density of 1840 plants per m²).

Each data point represents the mean of a minimum of ten values.

Vertical bars indicate \pm standard error of each mean.



CHAPTER FIVE

DISCUSSION OF EXPERIMENTS INVOLVING EPIDEMICS OF DAMPING-OFF

The physical environmental conditions used in all these experiments were intended to prolong the susceptible state in the host plant and thereby minimize the effect of increased resistance with time. The effect of these measures may be determined from the frequency curves shown in Figure 3b. Frequency curves of disease progress relate increments of disease (over the last measurement) more directly to changes in host plant resistance and environmental conditions than do cumulative curves (Kranz, 1974). In so much that the curves in Figure 3b are very uniform and show no dramatic divergence from a normal distribution, they confirm the success of these measures.

1. *Host plant density per se*

a) *The occurrence of primary infection foci*

The arithmetic plots relating the number of primary infection foci to the density of applied inoculum (Figure 2a), are similar in shape to dosage-response curves obtained by other workers who have previously investigated the effect of changing inoculum density on the incidence of soil-borne disease. For instance, Martinson (1963) showed such a relationship for *Rhizoctonia* damping-off, Richardson and Munnecke (1964) for *Pythium* damping-off and Sneh *et al* (1966) for *Rhizoctonia* stem rot of beans. Baker (1971) has analyzed these and other results and has demonstrated by means of simple models how the curvi-linear shape results from a progressive increase in the wastage of inoculum due to multiple infections occurring in the same plants. In the present case there would also be some additional inoculum wastage because secondary infections spreading outward from disease foci pre-empted some potential primary infection sites. Using a geometric model constructed of a lattice of tetrahedra, each point of intersection of lines being the position of a propagule in the soil, Baker and his associates (Baker, 1971; Baker, Maurer and Maurer, 1967) have also shown that four general types of pathogen-host interactions may exist in the soil ecosystem. These interactions may be classified according to the relative degree of mobility of inoculum and infection court (host). Moreover, the slope of log-log plots of inoculum density - disease incidence data provides useful clues to the nature of the particular host - inoculum interaction. Thus,

the slopes of the regression lines for the present data (approximating to unity) are characteristic of non-mobile inoculum interacting with fixed infection courts through intersection with the rhizosphere volume. This is distinct from the need to make actual surface to surface contact, an interaction which, according to Baker *et al*, results in a regression coefficient of lower value. The results obtained here thus support a suggestion of Baker (1971), based on the data of Richardson and Munnecke (1964), that *Pythium irregulare*-induced damping-off might involve a rhizosphere effect, and is consistent also with his analysis of Martinson's (1963) work on *Rhizoctonia solani* infecting radish seedlings.

These experiments on inoculum density are useful then in establishing a general equivalence between the results obtained from this, and other studies employing different methods and materials. More interesting, though, are the results of the parallel experiments conducted in which inoculum density was held constant while host density was varied. It is clear, from the shape of the arithmetic dosage-response curves and from the slopes of the log-log plots (Figure 1) that varying host density has the same effect as varying inoculum density. Starting from a seedling stand of 1800 plants per m² inoculated with 0.5% inoculum, one can halve the number of primary infection events (from approximately 6 down to 3) by reducing the host density to 900 plants per m² just as easily as by reducing inoculum density to 0.25%.

b) *Proportion of damping-off at a particular time*

Previous experimental studies of the effect of host plant

density on damping-off have all been confined to measuring disease incidence at a single arbitrary time after inoculation (Johnson, 1914; Hartley, 1921; Gibson, 1956). The essential inadequacy of such an approach is demonstrated by the results presented in Figure 3a. From this, one can select a linear relationship, or relationships with varying degrees of non-linearity, depending on the time at which the disease incidence is evaluated. The linear relationship which is evident close to the beginning of the epidemic is, therefore, a substantiation of the earlier results published by Hartley (1921) and Gibson (1956), but only in a limited sense. This early relationship possibly reflects an approximately simple proportionality on day *one*, between low host densities and the number of successful encounters with inoculum which result in a primary infection event. During the subsequent three days however, when disease foci are still appearing, rapid secondary spread from early formed foci will pre-empt potential infection sites (thus producing the curvi-linear relationship found in Figure 1a). As the epidemic progresses however, the original occurrence of primary infection foci becomes less and less important as the effects of differential rates of linear advance of the disease come increasingly to dominate the relationship.

c) *Multiplication and advance of damping-off with time*

From experiments with damping-off diseases it has been known for some time that high planting density increases, and low planting density decreases, the overall level of disease impact (Johnson, 1914; Hartley, 1921; Gibson, 1956). In the present work this relationship

was quantified more carefully and it was found that, over a wide range of host densities, the mean rate of advance (α) of the disease front, and the apparent infection rate (r) of damping-off in a randomly inoculated system (which largely depends on the rate of advance), both show curvi-linear responses to increases in host plant density.

In evenly dispersed stands, changes in plant density affect the mean distance between adjacent host plants such that the distance between adjacent hosts (L) is proportional to the reciprocal of the square root of the stand density. That is:

$$L \propto 1/\sqrt{D}$$

When r values (from Figure 4) or α values (from Figure 5) are plotted against L , a simple inverse linear relationship emerges. Thus Figure 22a shows four linear regression lines fitted to plots of the apparent infection rate against the mean distance between adjacent plants. All four lines are statistically significant ($P < 0.05$ and in two cases $P < 0.01$). Similarly Figure 22b shows the simple inverse linear relationship found when rates of advance from the lower density (less than approximately 25,000 plants per m²) end of the range are plotted against the mean distance between adjacent plants. In both cases the relationship may be stated as follows:

$$r \text{ (or } \alpha) = i(1 - L/j)$$

where r is the apparent infection rate (van der Plank, 1963), α is

FIGURE 22.

Epidemic rates of damping-off in monocultures of *Lepidium sativum* plotted against the mean distance between adjacent plants.

a) Apparent infection rate of damping-off.

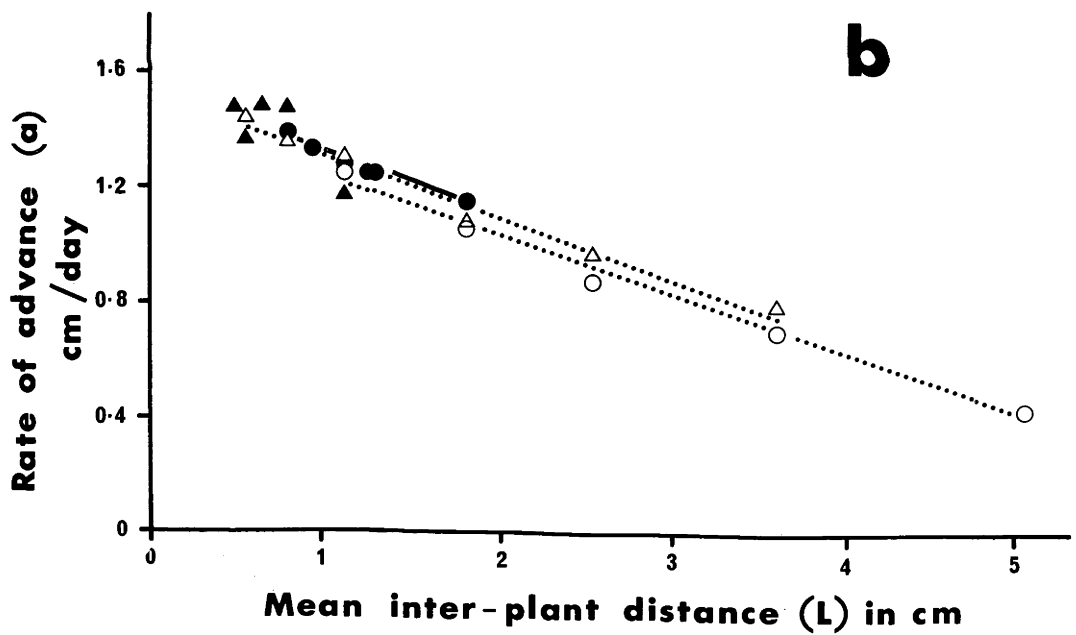
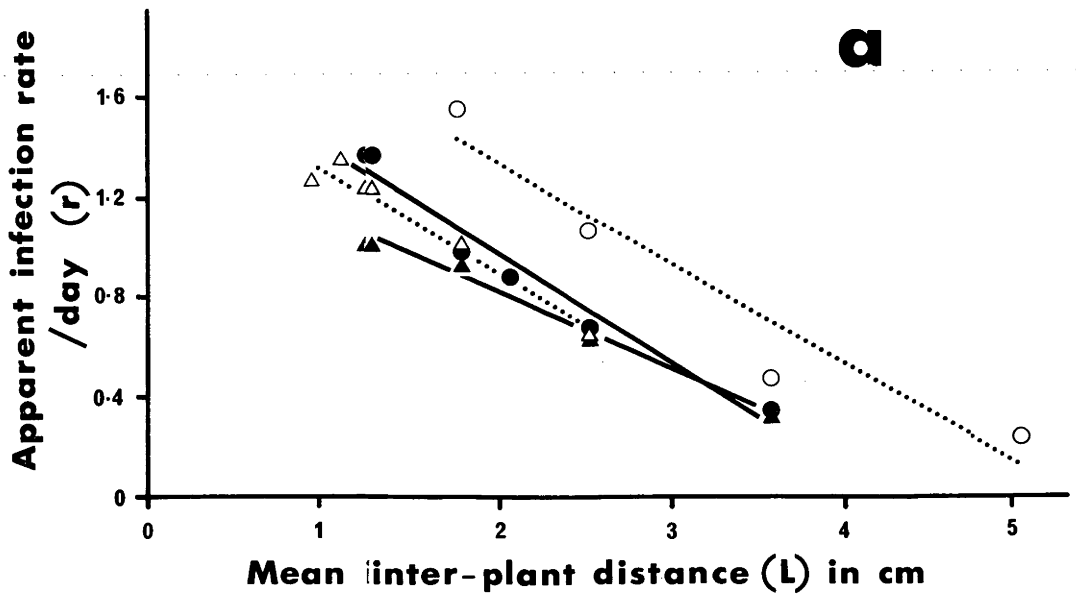
The results of the four experiments shown in Figure 4 are plotted against the mean distance (L) between adjacent individuals and fitted with linear regression lines.

○ , ● , △ , ▲ , results of four separate experiments.

b) Rate of advance of damping-off.

The results of the four experiments shown in Figure 5 are plotted against the mean distance (L) between adjacent individuals and fitted with linear regression lines.

○ , ● , △ , ▲ , results of four separate experiments.



the rate of disease advance, L is the mean distance between adjacent seedlings in the host stand and i and j are constants.

i and j , the intercepts obtained by extrapolating the linear relationship to the r (or α) and L axes respectively, define the rate limits of the disease within the system. Thus, from the i intercepts it appears unlikely that, however much the seedling density is increased, α would exceed 1.6cm per day, or that r , the rate of change of $\log_e(x/1-x)$ per day, would greatly exceed 1.8 for the given conditions. Even though one experiment to measure the rate of advance of the disease was extended to include a host density of 45,000 plants per m^2 , the rate fell short of this theoretical maximum, indicating that the relationship breaks down at very high densities. At such densities, individual host plants are tightly packed together and fungal growth (and thus disease advance), is almost certainly no longer limited by lack of nutrients. Under these circumstances, the rate of advance of the disease is probably determined by the maximum rate at which the physical and chemical processes involved in hyphal growth and extension can take place. In this respect it is interesting to note that Garrett (1970) states that the maximum rate of advance of *Pythium* species through or over the surface of soil is about 1.45cm per day - very close to that determined in this study. Despite these shortcomings, this relationship is nevertheless valid to densities around 20,000 seedlings per m^2 , which greatly exceeds seedling densities normally found in natural situations. In fact, densities above 10,000 seedlings per m^2 are rarely recorded in the literature (results of Yoda *et al* 1963, for *Erigeron canadensis* are exceptional).

The intercept j defines the maximum distance between adjacent host plants above which no increase or advance of the disease can take place. As this value is approached the variability of the results increases to a level where a prohibitive number of replicates would be required in order to obtain meaningful estimates of rate factors. A separate experiment (Figure 7) to determine the maximum distance to which damping-off can grow outwards from a diseased host plant set this at between 3.5 and 4.0 centimetres. While this is considerably less than the distance (at least 20cms) that Blair (1943) found that *Rhizoctonia solani* could travel through soil, it is in very good agreement with the maximum distance (5cms) to which he found mycelial growth could occur through moist sand when supported solely by the food reserves of an agar block. Moreover this value is in reasonably good agreement with the estimates of j obtained from an extrapolation of the apparent infection rate (approximately 5cm) and the rate of advance (approximately 6cm), when due allowance is made for the extra contribution a falling seedling (approximately 2cm) can make to the pathogen's 'reach'.

Throughout these experiments it was apparent that the behaviour of infected seedlings differed between high and low density stands. In high density stands, infected plants tended to be supported by surrounding healthy individuals. In such stands, diseased individuals at the perimeter of an infected area are prevented from falling into the surrounding healthy areas by the sheer density of healthy plants and instead they topple over backwards on to previously infected and collapsed seedlings. In stands of very low density (0 to 2500 plants per m^2) however, infected seedlings

are unaffected by neighbouring individuals and collapse freely in any direction. In this way the contribution that a falling seedling makes to the pathogen's 'reach' is maximal at low host densities and declines as density increases. This contribution is clearly demonstrated in Figure 6 where apparent infection rates in plots in which seedlings were prevented from falling over are compared with rates obtained from control, unsupported plots.

The simple relationship described here between inter-plant distance and the rate of increase or advance of disease does not appear to have been noted before. The only work that specifically mentions inter-plant distance, of which I am aware, is that published by Scott (1956). In studying the epidemiology of white rot of onions caused by *Sclerotium cepivorum*, Scott found that the proportion of plants infected in a series of experimental plots was approximately inversely proportional to their spacing and he related this to the effect of spacing on the transmission of the parasite. Transmission of this soil-borne disease is essentially similar to that of damping-off and it seems probable that other disease systems of this general pattern, for example Panama wilt disease of bananas (Risbeth, 1956) or *Armillaria mellea*, could exhibit the same type of relationship. In the case of *Armillaria*-like organisms, spread from plant to plant occurs by means of rhizomorphs (Tarr, 1972) and it seems reasonable to expect such pathogens to be strongly influenced by the distance between adjacent host plants, especially in an orchard type situation.

2. Mixtures

The most basic result to emerge from this study of the effect of mixtures on the epidemiology of damping-off is that the number of primary infection foci, the apparent infection rate of disease and the rate of advance of damping-off are all reduced in mixtures in comparison with values obtained in monocultures of the susceptible species at the same overall density. At all overall densities examined, the apparent infection rate and the rate of advance of the disease declined progressively as the proportion of resistant plants increased and the proportion of susceptible individuals decreased. In nearly all cases, as the proportion of susceptibles in the mixture continued to fall, the rate of decline of a or r became progressively greater (Figures 10b and 12).

From results of experiments in which only host plant density was varied (Chapter One of this Section) it was anticipated that the density of susceptible or host plants might be important in mixtures and provision was made to test this possibility. In all experiments in which aspects of the epidemiology of damping-off in mixtures of host and non-host plants were investigated, monocultures of the susceptible plant species were grown at a range of densities. This permitted comparisons to be made between the response of the disease to mixtures and the response to monocultures having a total density equal to the *net* density of the susceptible plant component of the mixture. For example, in the case of 50:50 mixtures grown at a density of 900 plants per m² (e.g. Figures 8a, 9a and 11a), as well as the control monocultures of 900 susceptible

plants per m^2 used for comparison in those Figures, further monocultures of 450 susceptible plants per m^2 were also included to enable the above such comparisons to be made. This latter type of control will be referred to as an 'equivalent monoculture'.

In Figure 23, disease parameter values obtained in mixtures are plotted against values of the same parameter obtained in equivalent monocultures of the susceptible species. The diagonal line drawn at 45 degrees from the origin defines perfect proportionality between the two sets of values. Data points falling on or very close to this line are consistent with the hypothesis that the rate value in question was determined solely by the net density of susceptible plants present. Points falling consistently above the line indicate an enhancement of the rate in mixtures over that obtained in equivalent monocultures, while points falling consistently below the line indicate a depression. In Figure 23a the number of primary infection foci occurring in mixtures is plotted against the number of foci occurring in equivalent monocultures of susceptible species obtained from the same experiment. Although only a limited number of data points are available, these all fall on or very close to this line and are thus consistent with the hypothesis that the occurrence of primary infection foci was determined solely by the net density of the susceptible plants present. That is, non-host individuals neither stimulated nor inhibited the formation of disease foci. Similarly from Figure 23c, where rates of advance of damping-off in mixtures are plotted against rates of advance in equivalent monocultures from the same experiments, it is readily apparent that

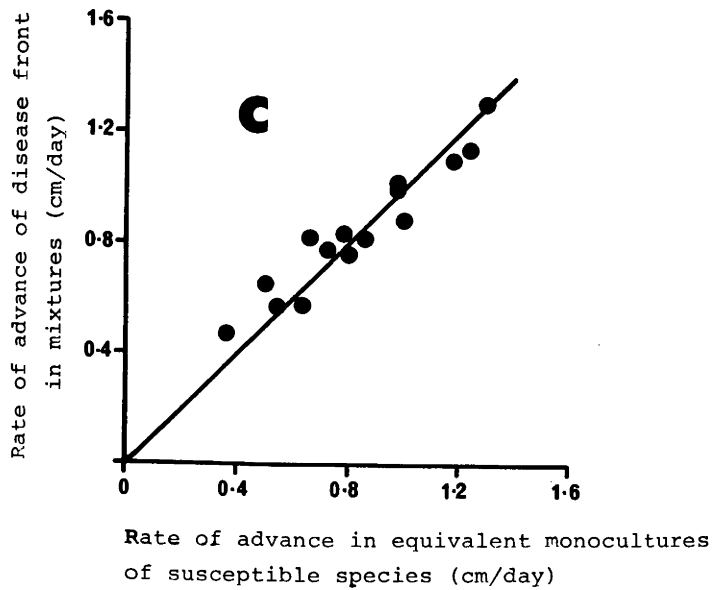
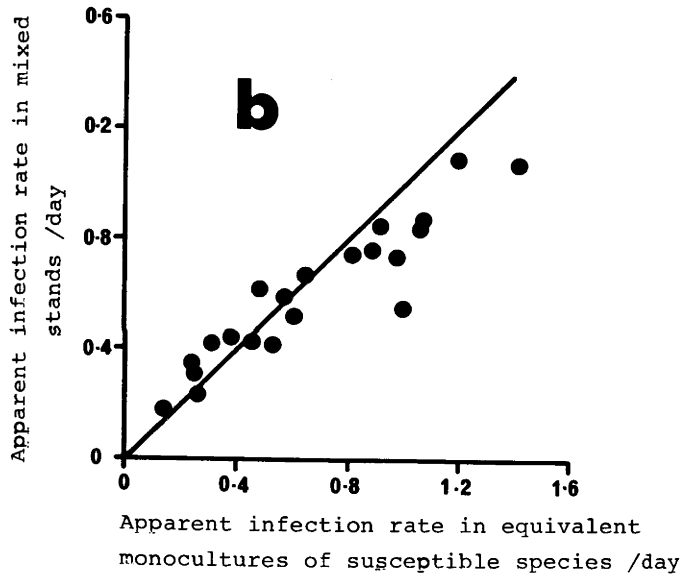
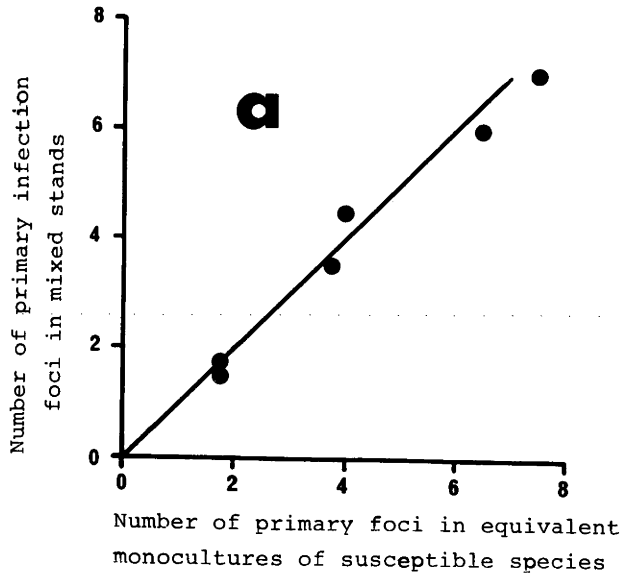
FIGURE 23.

Comparison between epidemic rates in mixtures and equivalent monocultures of the susceptible species.

- a) A comparison between the number of primary infection foci in mixtures and equivalent monocultures.

- b) A comparison of the apparent infection rate of damping-off in mixtures and equivalent monocultures.

- c) A comparison of the rate of advance of damping-off in mixtures and equivalent monocultures.



the rate of advance was determined almost exclusively by the net density of the susceptible component of the mixture. With the possible exception of the lowest density examined (containing a susceptible species component of 450 plants per m^2), this finding was true regardless of the overall density or composition of the mixture in question.

In Figure 23b, apparent infection rates of disease in mixtures are plotted against apparent infection rates in equivalent monocultures obtained from the same experiment. Over the lower half of the range covered the actual plots fall very close to (although consistently above) this line, indicating that the apparent infection rate in low to medium density mixed seedling stands also appears to be determined, in very large part, by the net density of susceptible plants in the mixture. Over the upper part of the range (representing higher stand densities) the plotted points all fall below, and often some considerable distance from the line, thus indicating that some additional factor is at work. Table 2 provides a break-down of these results, from which it can be seen that the greatest departure from proportionality occurs in stands with a relatively high overall density and a high proportion of susceptible seedlings. Indeed, in one experiment, the r value recorded for a mixture containing 75% susceptible plants (overall density of 3600 plants per m^2) was more than 35% below that of an equivalent monoculture.

It seems likely that the observed decline in apparent infection rates in mixtures, at high stand densities, over that occurring

Density (plants/m ²)	Proportion of susceptible plants in mixture		
	0.75	0.50	0.25
900	-	1.3287 1.0712	-
1800	0.8881 0.5454	1.2091 1.1313 1.0804 0.9482 0.8260	1.5639 1.5631
3600	0.7514 0.6307	0.9281 0.7927 0.6506 0.6476 0.5921	0.9573 0.8610
7200	-	0.7693	-

Table 2 The ratio of the apparent infection rate of disease in a mixture to the apparent infection rate in an equivalent monoculture.

in equivalent density monocultures of the susceptible species, was due to some interference with the transmission of inoculum by resistant individuals. It is possible that the resistant plants could have produced some inhibitory chemical which interfered with the linear extension of *P. irregulare* hyphae, but the fact that the rate of advance of disease along a front was so similar in mixtures and the equivalent monocultures makes this explanation very doubtful. It seems more likely that the depression of epidemic rates in just

the randomly inoculated dense mixtures is due to some aspect of the geometry of this particular experimental system which is different in the experiments on disease advance. For instance, it was noted that, in the denser mixed stands, diseased *L. sativum* seedlings were often prevented from falling over by healthy *L. rigidum* plants which propped them up, and that this was most marked in the randomly inoculated stands where disease occurred in discrete pockets rather than as a single large front. As shown in the work on host density *per se* the collapse of seedlings may, in certain circumstances, contribute to pathogen transmission by providing a bridge of nutrient-rich tissue in the direction of uninfected plants. In experiments on the rate of multiplication of disease in randomly inoculated seedling stands the presence of *L. rigidum* may interfere with this.

The observed tendency of apparent infection rates in low density mixtures to be consistently greater than rates in equivalent density monocultures (although not significantly so), may be attributed to an enhancement of fungal growth between susceptible individuals as a result of nutrient leakage from resistant plant roots. Thus Chamblee (1958), in some experiments involving mixtures of orchardgrass and alfalfa, noted incidentally that *Rhizoctonia solani* caused more damage in mixtures of the two species than in pure stands of the susceptible component (orchardgrass). It seems likely here that nutrient leakage of a nitrogenous form from the alfalfa roots was responsible for the enhanced disease impact. *Pythium*-induced damping-off diseases have been shown to react to nutrient leakage in a similar way (Kerr, 1964).

Despite these departures from perfect proportionality, the effect of changes in mixture composition on the apparent infection rate of *Pythium*-induced damping-off is mainly a function of the net density of susceptible plants present. Linear regression lines fitted to plots of apparent infection rates in mixtures (50:50 constant proportion experiments) against the mean distance between adjacent susceptible plants, in all cases showed better fit, than when apparent infection rates in mixtures were plotted against the mean distance between adjacent individuals of both species present.

3. Pattern

In this investigation of the effect of host plant pattern on the epidemiology of *Pythium*-induced damping-off, experiments were at first, designed to determine the effect of such patterns on the apparent infection rate of disease. Only when it became apparent that this system was somewhat more complex than those previously examined (host density *per se* and mixtures), was this investigation expanded to include the effects of host plant pattern on the rate of advance of the disease and the occurrence of primary infection foci. This was done in an attempt to explain some of the unexpected results obtained.

- a) *Varying the number of clumps while the proportion of the total area occupied by clumps was held constant*

In experiments in which the proportion of the total area occupied

was held constant, the density of seedlings within each clump also remained constant, regardless of the number of individuals present in any one clump. It was expected, therefore, that rates of advance and disease increase within such clumps should remain constant throughout the range of clump densities investigated. In fact, from theoretical considerations of the way in which the distance between adjacent clump centres and edges varied with increasing clump number (and a knowledge of the simple inverse relationship found for both rate factors in evenly distributed stands) it seemed that any difference between the treatments would be due to this changing distance separating clumps and that this might result in some decrease in rates as the number of clumps was reduced. Consequently, the results obtained in the experiments investigating the apparent infection rate of disease were entirely unexpected. Rates of advance of damping-off through such arrays appeared to be quite independent of cluster number (Figure 18), while, in experiments investigating the rate of multiplication of disease (Figures 17a and b), all but one host density - total area occupied interaction showed a sharp rise at 100 clumps per m^2 .

As the rate of multiplication of disease was shown in previous work (see 1c above) to be closely correlated with the rate of advance of the disease front, a positive rather than a negative relationship between α and r was expected. This discrepancy probably arises from the differences in the conditions under which the two different parameters were measured. The rate of advance was measured as a disease front moving out from a single infection focus. The apparent infection rate, on the other hand, was measured

in a randomly inoculated stand in which multiple infection foci developed. Examining the number of primary infection foci in the latter system (Figure 15a), it was found that comparable numbers of foci appeared in all different treatments. This is a reasonable outcome, since both the total number of plants available for infection and the proportion of the plot area occupied remained constant. However, as a result of the varying number of clumps into which this constant number of primary infection foci is partitioned, even allowing for the increase in multiple hits at low clump density, the proportion of clumps receiving a primary infection changes drastically (Figure 15b). This, in turn, alters the very important balance between within-clump and between-clump spread and increase within this system. Thus when plants and infection foci are partitioned into a large number of clumps, the majority of clumps are infected as a result of secondary spread between clumps and disease rates are relatively slow. On the other hand, when plants and primary infection foci are partitioned into a small number of clumps, the majority of clumps receive primary infections, most increase of disease results from spread within clumps and disease rates are therefore relatively fast.

- b) *Varying the proportion of the total area occupied by clumps while the number of clumps was held constant*

In contrast to experiments where the number of clumps was varied, primary infection foci did not appear to be important in any of the experiments in which the number of clumps was held constant while the proportion of the total area occupied was varied. In two experiments

conducted at an overall density of 1840 plants per m^2 , although the total number of primary infection foci was significantly reduced between the 0.5 and 0.031 treatments (Figure 19a), this reduction was not directly proportional to the simultaneous reduction in the area occupied by clumps, and hence to the total amount of inoculum to which the plants were exposed. In Chapter Two of this Section it was shown that plant density interacts with the available inoculum to produce a curvi-linear relationship between primary infection foci and host density. Presumably the reduction in clump area was compensated for, to a considerable extent, by the increased effectiveness of inoculum resulting from the concomitant increase in plant density. Moreover, there were also considerably more multiple infections in the larger area clumps, so that the proportion of clumps receiving primary inoculum was ultimately nearly constant throughout the different treatments. As the influence of primary inoculum was constant, apparent infection rates should vary with pattern in the same way as rates of advance of the disease front. This was confirmed by the results of experiments conducted at 200 clumps per m^2 (Figures 20 and 21), with both rates declining increasingly steeply as the clumps became more and more compact. Results obtained at a lower overall density (1220 plants per m^2) to that of the 'core' experiments, or at a higher number of clumps per m^2 (800), showed similar, if somewhat less pronounced, trends to those detailed above. This was to be expected since, at 800 clumps per m^2 , plant distribution is once again becoming more even over the entire plot.

Of the two methods used to vary clumping pattern in this work, altering the area occupied by a fixed number of clumps clearly has a more direct and numerically larger effect on the rate of increase and advance of *Pythium*-induced damping-off, than has alteration of the number of clumps in a fixed proportion of the total area.

SECTION C

CONTROLLED ENVIRONMENT EXPERIMENTS ON EPIDEMICS

CAUSED BY AN AIR-BORNE PLANT PATHOGEN

CHAPTER ONE

GENERAL EXPERIMENTAL METHODS

I. INTRODUCTION

a) *General considerations*

In the previous section a soil-borne pathogen - host plant interaction was studied in the *Pythium irregulare* - *Lepidium sativum* system. The development of disease in that system was basically a function of the occurrence of primary infection foci and the subsequent spread of disease outwards from these points. The simplicity of this effectively two-dimensional system was enhanced by the systemic nature of lesions, each successful infection event causing a single lesion which killed the whole

seedling. In the present section a more complex three-dimensional interaction between an air-borne pathogen and its host plant is studied. In this system, disease infections give rise to small local lesions or pustules which, individually, occupy only a very small fraction of the total foliar area. Transmissions of inoculum may thus occur both between and within plants.

In the study described here, different density populations of barley (*Hordeum vulgare* L.) variety 164 were inoculated with powdery mildew (*Erysiphe graminis* DC. f.sp. *hordei* Marschal) under controlled environmental conditions, in order to evaluate the precise effects of changes, in both host plant density and the composition of mixtures, on rates of epidemic progress within the system.

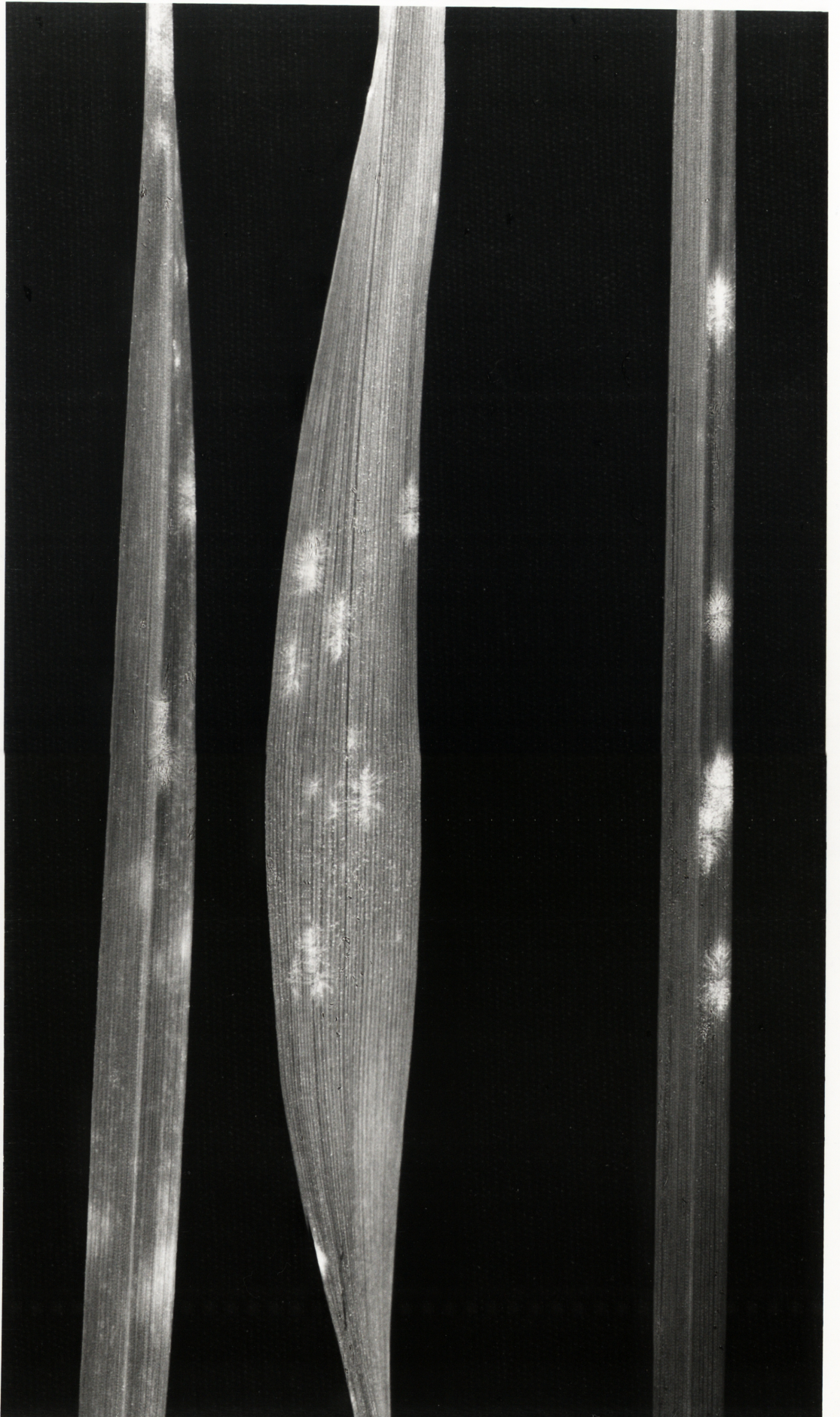
b) *The relevant biology of Erysiphe graminis* DC. f.sp. *hordei* Marschal

Erysiphe graminis is one of the best known examples of a group of diseases known as the powdery mildews, in which the pathogen is a superficial obligate fungus (Plate 2) parasitizing the epidermal cells of hosts by means of intra-cellular haustoria. Under optimal conditions the asexual reproductive cycle of *Erysiphe graminis*, involving dispersal by air-borne conidia, is characterized by extremely short generation times (approximately five days). Although diurnal fluctuations in the number of conidia of *E. graminis* captured in the air have been shown to occur (Gregory, 1952; Hirst, 1953; Hammett and Manners, 1971),

PLATE 2.

The general appearance of powdery mildew pustules
caused by the fungus *Erysiphe graminis* f.sp.
hordei on barley leaves.

(Approximately 1.6 times natural size).



Yarwood (1936) was unable to detect any diurnal cycle of conidial maturation in this species (diurnal cycles of conidial maturation have been observed in other powdery mildews; Yarwood, 1957). Sporulation has been found to be greatest at 20°C, decreasing sharply at higher and lower temperatures. The optimum relative humidity for conidia release is 100% (Ward and Manners, 1974). Germination and infectivity are highest in those conidia produced at approximately 20°C, at high relative humidities (although free water inhibits both germination and mycelial growth) and at moderate light intensities (Yarwood, 1957; Schnathorst, 1965; Ward and Manners, 1974).

Erysiphe graminis f.sp. *hordei* was chosen for use in the experiments described below for the number of advantages which it has over other commonly occurring foliar plant pathogens. Firstly, the short generation time permits the development of 'polycyclic' disease epidemics (Zadoks, 1972) within a reasonably short period of time. Secondly, conidia production by pustules is quite large and continuous over a considerable time span (Hammett and Manners, 1971). Lastly, and perhaps most importantly, conidial germination takes place readily at high relative humidities thus obviating the free water requirement of other air-borne pathogens. Conditions for germination and infection could thus be made available continuously.

II. GENERAL METHODS

1. *Physical environmental conditions*

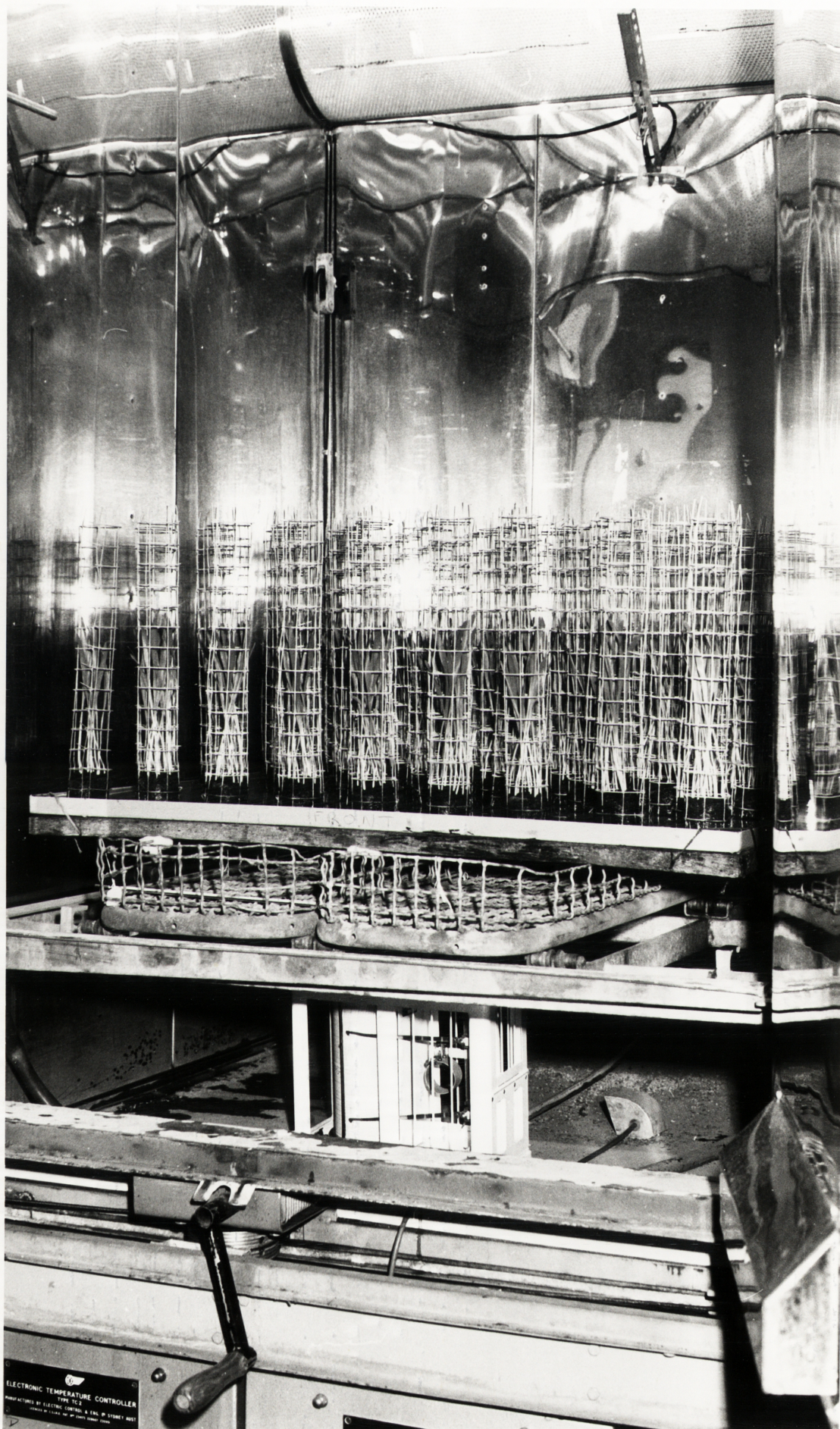
In these experiments, investigating epidemics caused by *E. graminis* f.sp. *hordei*, environmental conditions were controlled by conducting the experiments in LBH type phytotron cabinets (Morse and Evans, 1962). LBH cabinets are identical to the LB cabinets used earlier in the experiments involving *Pythium irregulare*, except that in these the humidity of the air flowing through the plant growing space may also be controlled. The overall dimensions of the cabinets (1.22m wide, 1.58m deep and 3.21m high) permit a plant growing space of 1.64 square metres; the internal chamber being 1.17m wide, 1.40m deep and 1.37m high. Plants are normally supported in removable trays on a platform which can be raised and lowered by a hand winch. Plate 3 provides a general view of an LBH growth cabinet.

a) *Light, humidity and temperature*

Throughout all experiments, plants were grown under an alternating 12hr light - 12hr dark regime, at a light intensity of 90 lumens per m² (at canopy level). The relative humidity of the air within the plant growing space was maintained at 80 ± 2% during the day and 90 ± 2% at night. Temperatures were held constant at 22.5°C during the day and 19.5°C at night.

PLATE 3.

General view of the inside of a LBH phytotron cabinet with a watering tray and plant stand in position.



ELECTRONIC TEMPERATURE CONTROLLER
TYPE TC2
MANUFACTURED BY ELECTRIC CONTROL CO. INC. 10 STREET AVE.
CHICAGO, ILL. U.S.A. PAT. 2,800,000

b) *The pattern of air flow in LBH cabinets*

While environmental variables such as temperature, light and humidity are all recognized to be of importance in the development of epiphytotics (Tarr, 1972), one factor which is commonly overlooked, but also of considerable importance, is wind velocity.

(i) *Pattern of air flow through an empty cabinet* - Air circulation in LBH type phytotron cabinets is generated by a large fan in the base of the cabinet, which forces air up a false back wall, through a baffle to minimize gusting, and then vertically down through the plant growing space (Figure 24a). Mean wind velocity is 0.55 metres per second (Morse and Evans, 1962).

(ii) *Pattern of air flow through a cabinet containing a watering tray* - In all experiments concerning the effect of host plant density on the dispersal and rate of multiplication of disease caused by air-borne pathogens, host plants were grown on a large plastic (P.V.C.) tray, 0.92 metres square, which was placed on top of the removable wire trays within the cabinet. Because of the importance of wind velocity in the liberation and deposition of spores, the effect of the positioning of the P.V.C. tray on air movement within the plant growing space was carefully examined.

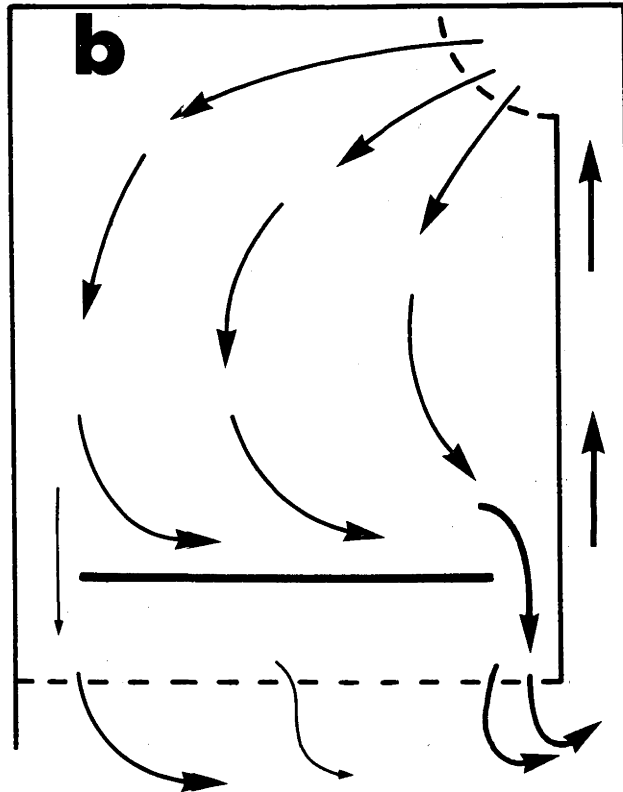
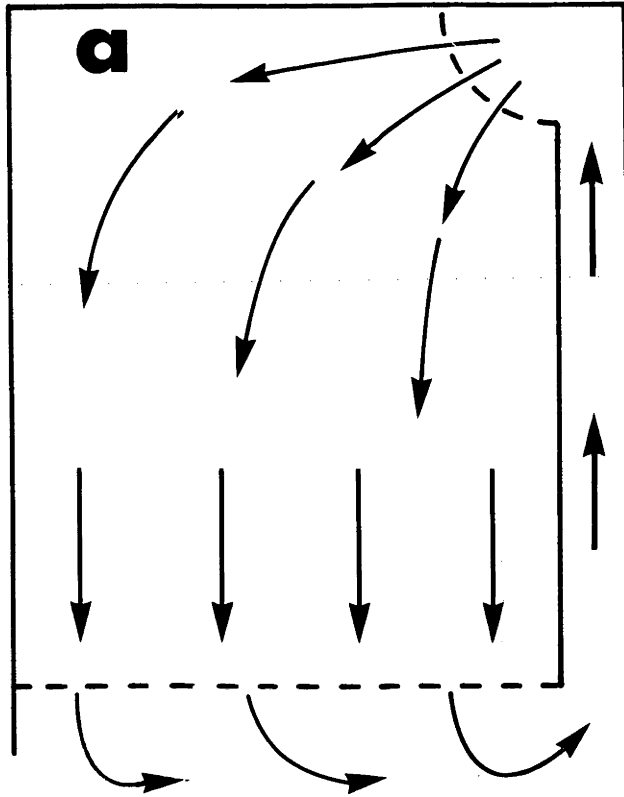
The P.V.C. tray was placed in a series of different positions and the resultant changes in wind velocity and direction were traced. From this, a position for the tray was selected in which the flow of air, instead of being vertically downwards, was

FIGURE 24.

Diagrammatic representation of the pattern
of air flow in LBH phytotron cabinets.

a) Pattern of air flow through an empty
cabinet.

b) Pattern of air flow through a cabinet
containing a watering tray.



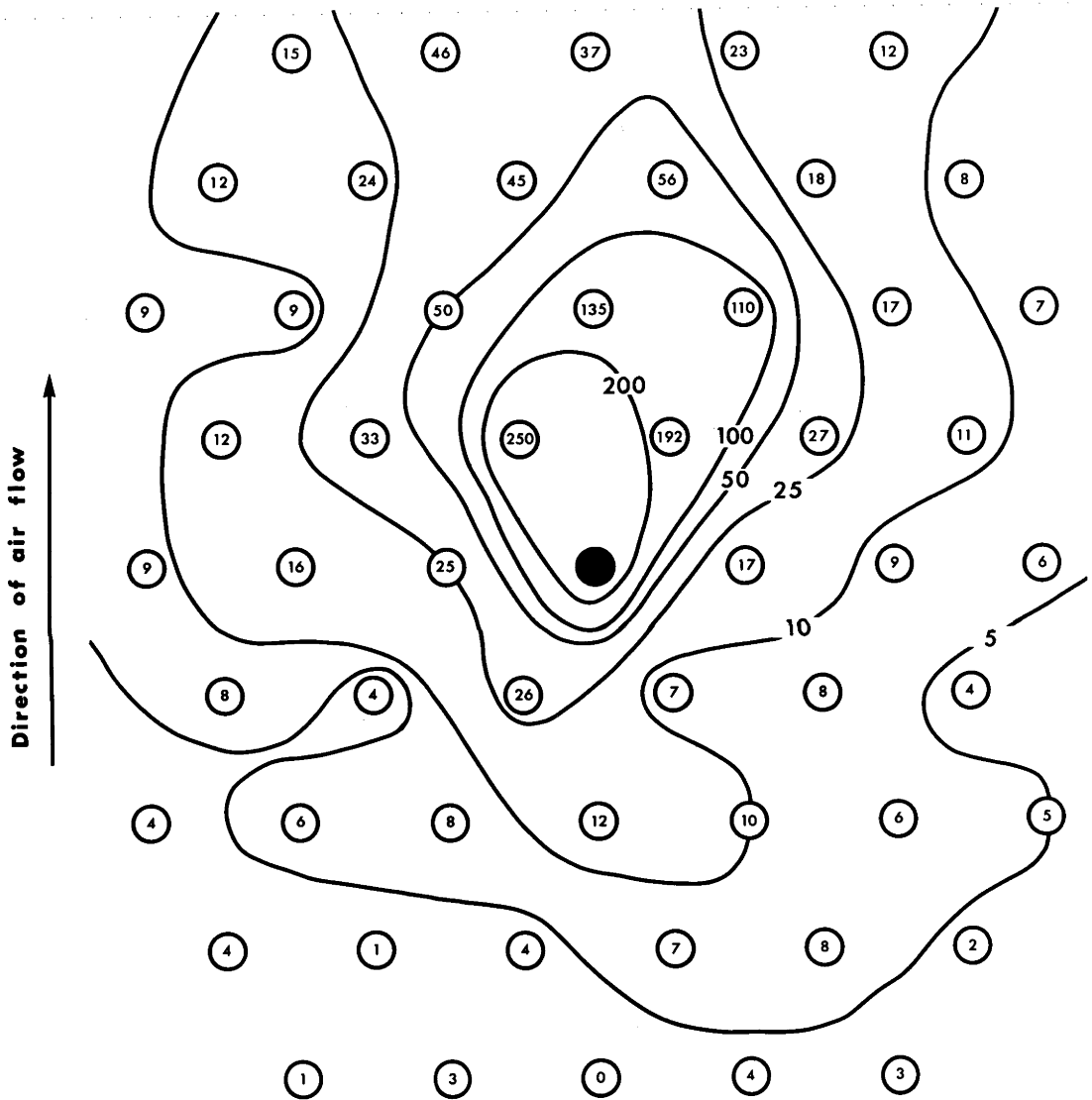
deflected to give a predominantly front-to-back movement in the region directly above the tray (Figure 24b). The optimal position thus determined was one in which the tray was centralized in the horizontal plane and held 0.36m above the cabinet floor. This pattern was confirmed by a simple dispersal experiment in which a barley plant (*Hordeum vulgare*), heavily infected (and infectious) with powdery mildew (*Erysiphe graminis* f.sp. *hordei*), was placed in the centre of a stand of evenly spaced, even-aged, healthy barley plants. Three days later the infectious plant was removed and the rest of the stand was allowed to grow on undisturbed for a further four days. After this time, all conidia successfully transmitted by the original infected plant had formed visible pustules. The plant stand was then taken apart, and the position of each plant, and the number of pustules present was recorded. The pattern of distribution of pustules with respect to host plant position (and thus the pattern of conidia dispersal from the point source) is shown in Figure 25. The 'isosporate' lines, joining plants which received an equal conidia load, graphically confirms the front-to-back wind movement pattern.

(iii) *Pattern of air flow through a cabinet containing experimental plant stands* - As the major variable in these experiments was to be host plant density, wind velocity measurements were made, at three heights (10, 20 and 30 cm) and on two different occasions (six and eighteen days after the start of an experiment), within plant stands of the various densities under investigation. Measurements were made using a Pitot tube supported on a retort stand within the experimental array. The

FIGURE 25.

The pattern of distribution of developing pustules of *Erysiphe graminis* f.sp. *hordei* within a plant stand after three days exposure to an infected, infectious barley plant. The solid lines are 'isosporate' lines joining plants with equal numbers of pustules.

● , barley plant infected and infectious with powdery mildew at the start of the experiment;
○ , barley plants healthy at the start of the experiment. The numbers within the open circles refer to the number of pustules on that particular plant at the end of the experiment.



Pitot tube was connected to an ammeter outside the cabinet and the pattern of wind movement was monitored at each height for three minutes. Maximum, minimum and average readings were obtained in millivolts and converted to wind velocity in metres per second by means of a calibration curve.

The general pattern of wind movement and velocity was the same for all but the most dense stand. Six days after the start of an experiment average wind velocities were approximately the same at the two lower recording positions but somewhat higher at the top of the canopy (Table 3). However, after a further twelve days had elapsed, the average velocity within, and at the bottom, of the

Height of Recording position above tray (cm)	Wind velocity (m/sec)					
	Day 6			Day 18		
	Max.	Aver.	Min.	Max.	Aver.	Min.
10	0.31	0.22	0.03	0.31	0.20	0.03
20	0.49	0.22	0.03	0.49	0.20	0.03
30	0.61	0.25	0.03	0.61	0.25	0.03

Table 3 The maximum, minimum and average velocities of wind moving through a plant stand, measured at three different heights above ground level, six and eighteen days after the start of an experiment. Average values are the mean velocity recorded over a three minute period, at any given height.

plant stand had declined slightly, while that at the top remained constant. Fluctuation and gusting of wind occurred at all three heights, being least noticeable close to 'ground' level. At all heights, wind velocity on occasions dropped almost to zero while gusts considerably greater than the average velocity occurred fairly regularly. Although these velocities are generally lower than those recorded by Hammett and Manners (1973), large numbers of conidia evidently became air-borne, possibly due to the fluctuating nature of the air-stream.

In the most dense stand, wind velocities were noticeably lower than those recorded in less dense stands. In order to overcome this and thus ensure that wind velocity was not a significant variable in these experiments, the velocity of air entering the cabinet was increased (for the duration of the experiment) by taping a thick plastic sheet over most of the lower third of the wind baffle (see Figure 24). This modification resulted in virtually the same pattern of wind movement, fluctuation and gusting in the densest stand as for all other arrays.

2. Establishment of host plant arrays and initiation of disease

a) Stocks of host plant and pathogen

The host plant and the pathogen were both obtained from the

University of Sydney's Research Farm at Castle Hill in New South Wales, courtesy of Dr R. MacIntosh.

The host plant, *Hordeum vulgare* L. variety 164, is a non-commercial barley variety produced during the course of a breeding program. This variety was chosen for its consistent response to attack by mildew, there being no variation in the degree of susceptibility of individual host plants. A small stock of seed was obtained and supplemented by growing further stocks in small field plots.

The pathogen, *Erysiphe graminis* DC. f.sp. *hordei* Marschal, was maintained on barley 164 grown in 10cm pots, being transferred to fresh plants once every three weeks.

b) *Setting up the experimental system*

From a number of preliminary experiments, in which individual barley plants were spaced according to different density arrangements, the impracticality of working with individual plants became apparent. In these experiments, the small amount of host plant tissue available, resulted in low numbers of pustules being recorded with a consequently high variance between samples. This problem was avoided by growing small clusters of ten plants, placed together in the same small pot (the maximum diameter of the cluster being no more than 3cm), such that for practical purposes they could be considered a single 'plant'. The total leaf area of, and placement of individuals within, such a cluster would not be vastly dissimilar to that of a single well-grown plant with tillers. (Of

course, the young leaf tissue would have a different susceptibility to pathogen attack than old leaf tissue).

Throughout this section the unit 'plant' always comprises a small cluster of plants of this type and the distance between plants refers to the placement of these clusters with respect to one another.

(i) *The production of experimental 'plants'* - Individual experimental host 'plants' of the above type, were created by planting ten pre-germinated barley seeds in commercial peat pots (Jiffy-7 peat pots produced by Jiffy Products Ltd, Grorud, Norway). Pre-germination of seed on moist filter paper at 25°C for 48 hours was found to be necessary, as germination of barley seed in wet peat moss was extremely erratic. After planting (Plate 4a), the barley seed was covered with peat moss and the Jiffy pots placed under lights and allowed to grow. Five days later, the young plants reached an age where the primary leaves normally start to bend over (Plate 4b). To maintain the compactness of each plant group, and thus prevent plant stands from degenerating into a mass of interlocking semi-prone leaves, cylindrical supports 5cm in diameter and 38cm tall, constructed out of 2.5cm square mesh wire were placed around the pots (Plate 4c). Eight days after seed germination, plants with fully developed primary leaves were put in the LBH cabinets. In the cabinets, pots were grown in a PVC tray 0.9m by 0.9m and 2.5cm deep. Water levels in this tray were maintained at 2cm by 'topping up' approximately once every two days. No extra nutrients were added as the peat pots were supplied enriched with nutrients.

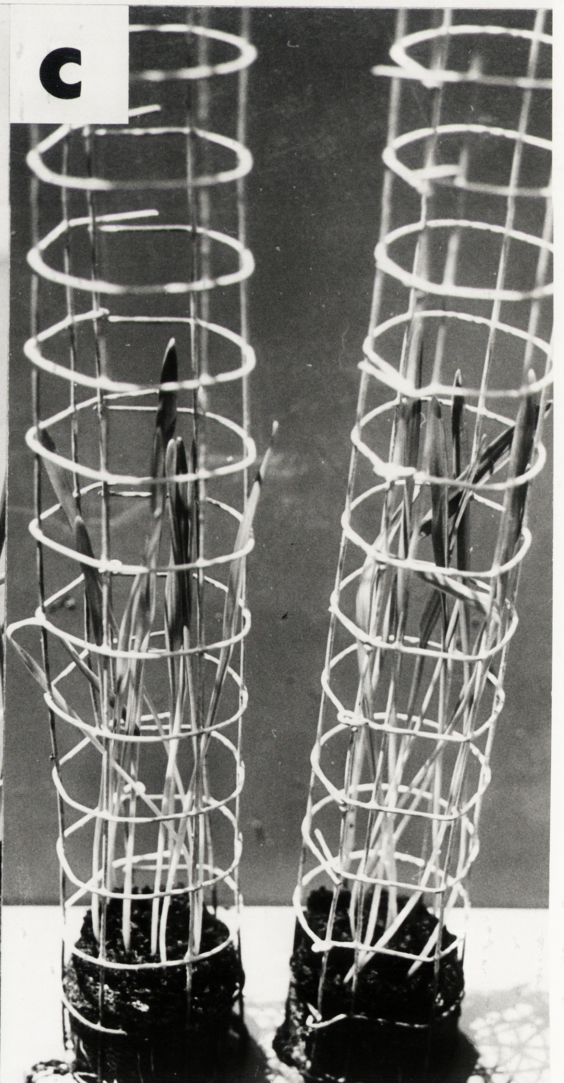
PLATE 4.

Stages in the construction of the experimental 'plants' used in experiments investigating the effect of variations in host plant density on epidemic rates of powdery mildew.

- a) Individual peat pots planted with ten germinating barley seeds.

- b) Five-day-old experimental 'plants' showing the tendency of leaves to fall over.

- c) The same five-day-old experimental 'plants' showing the cylindrical wire supports in place around each 'plant'.



(ii) *Arrangement of host plants in experimental arrays* - Each overall experimental array used had two components which will be referred to as the 'source' array and the 'main' array. Plants in both parts of the total array were arranged in a hexagonal close-packed fashion, the position of individual members being fixed by permanent markers. In all experiments, the distance between adjacent plants in the two front rows (the source array) was maintained at 10.0cm. The foremost of these rows acted as a screen to reduce wind gusting, while plants inoculated with conidia of *E. graminis* f.sp. *hordei* were placed in the second row to provide a primary source of infection for plants in the main array. The distance between adjacent plants in the source array was held constant in order to provide, as far as possible, a uniform primary infection source for all experiments. The density of plants and consequently the distance between adjacent pots in the main array was, on the other hand, varied, with four different experimental densities, (115.5, 51.3, 28.9 and 13.8 plants per m²), being set up. Although wind movement in the cabinets was predominately front-to back, screen plants in the source array were found to acquire some disease and hence contribute to the epidemic. Consequently the final plant densities maintained in the cabinets were 115.5, 62.0, 40.0 and 31.0 plants per m². This produced mean distances between adjacent plants of 10.0, 13.8, 17.0 and 18.8 cms respectively. Figure 26 shows these experimental arrays in a diagrammatic form.

During sampling, watering or any other manipulation of the experiments requiring cabinets to be opened, the power supply was switched off to stop the air fans.

Experiments started with eight-day-old plants and generally ran for a further twenty-four days. At the end of each experiment, the walls and ceiling of the growth chamber were thoroughly washed so that contamination did not carry over from one experiment to the next.

c) *Initiation of disease*

Primary inoculum plants were produced by inoculating eight-day-old plants (primary leaves fully expanded) with conidia of *E. graminis* f.sp. *hordei*. Conidia were obtained directly from a reservoir of infected plants and applied, with a fine paint brush, to 2.5cm segments of two randomly selected leaves per plant (inoculations carried out in this way gave rise to consistently uniform infections). Inoculated plants were then incubated at approximately 22°C for 24 hours in still conditions. Six of these plants were used to initiate disease in the LBH cabinets by placing them in the six central positions of the second row of the source array. Given the pattern of wind flow in the cabinets this resulted in a fairly even number of conidia being released down the entire length of this line source.

3. *Monitoring and assessment of disease*

a) *Overall rate of increase of disease*

The concepts of latent period and incubation period are important

factors in any consideration of the development of epiphytotics. The latent period (p) is the interval of time (in days) from inoculation to the appearance of the first active pustule, while the incubation period (c) is the interval of time (in days) from the arrival of spores on the foliage to the appearance of the first detectable symptoms (van der Plank, 1963). In the progress of the early stages of an epidemic through time, after initial infection from an external source, the amount of all disease, both visible and invisible, remains virtually constant during the latent period. After p days the lesions produce spores and the amount of all disease (visible and invisible) increases. Up to this stage all spores have originated from the initial lesions. The increase in disease at this stage is therefore at a 'simple interest' rate (van der Plank, 1963). After a further p days (i.e. a total of $2p$ after initial infection) these new infections start to erupt and generate spores - and the disease then switches into a 'compound interest' or logarithmic phase.

While visible disease is easily detected, it can be readily appreciated that detection of disease in the early latent phase (before it becomes apparent) is not. For disease that has passed the incubation period and is therefore visible, the logarithmic phase of increase commences $2p + c$ days after initial infection. In the experiments reported here, the latent period of *E. graminis* f.sp. *hordei* was found to be five days, while the incubation period was found to be four days. Artificial epidemics initiated in the LBH type growth cabinets were found to enter a logarithmic phase of disease increase on the fourteenth day after source inoculation.

Disease monitoring commenced early in the logarithmic phase, the first sample being taken on day 16. Three adjacent sampling positions were chosen, approximately 54cms downwind from the primary infection source. The precise distance was not critical as the rate of increase of disease should not be affected by the distance from the primary infection source. The exact location of sampling positions was therefore made to conform with the particular plant array being studied (see Figure 26).

In all plant stands, samples were taken at three different times. These were spaced at four day intervals (day 16, day 20 and day 24) except at the highest host plant density where samples were taken at three day intervals (day 16, day 19 and day 22) because of the rapid progress of the epidemic. On each sampling day, one plant, chosen at random from one of the three sampling positions, was removed and scored immediately to determine the amount of visible disease present. The outline of all disease lesions was traced on thin, clear plastic film, blacked in, and passed through a calibrated Hayashi Denko automatic area meter to determine the total area of lesions. Total leaf area was determined by passing all leaves through the area meter.

b) *Rate of increase of disease due to between-plant transmissions of inoculum*

Disease increase results from the combined development of pustules derived from inoculum transmitted between plants and inoculum transmitted within plants. Of these two components, the

FIGURE 26.

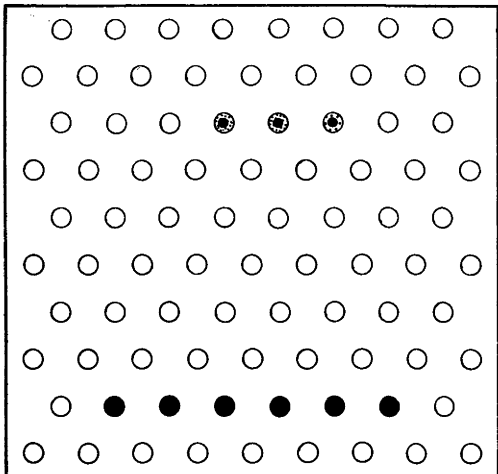
Diagrammatic representation of the pure barley arrays used in experiments investigating the effect of host plant density on epidemic rates of powdery mildew.

- a) Overall density of 115.5 plants per m^2 ;
mean distance between adjacent individuals
of 10.0cm.
- b) Overall density of 62.0 plants per m^2 ;
mean distance between adjacent individuals
of 13.8cm.
- c) Overall density of 40.0 plants per m^2 ;
mean distance between adjacent individuals
of 17.0cm.
- d) Overall density of 31.0 plants per m^2 ;
mean distance between adjacent individuals
of 18.8cm.

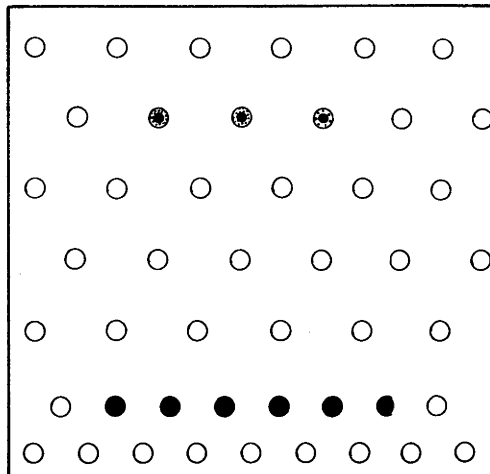
(The bottom two rows of plants in all density treatments comprise the 'source' array).

● , primary inoculum plants; ○ , barley plants
of experimental array; ⊙ + ⊛ , sampling positions
for measurements of overall rate of disease increase;
⊛ , sampling positions for the rate of increase of
disease due to between-plant transmissions.

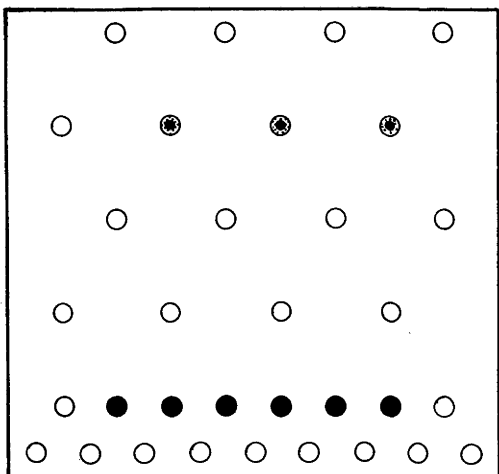
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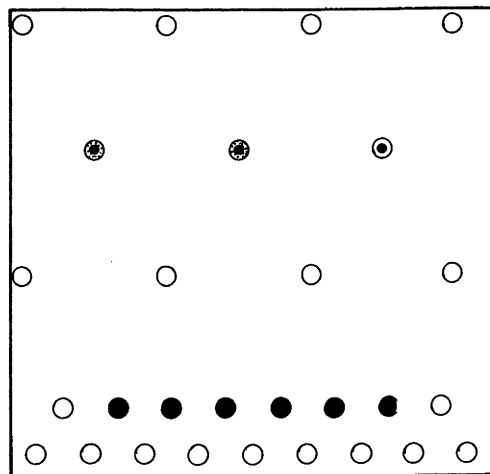
b



c



d



contribution made by between-plant transmissions of inoculum is the only one likely to be directly affected by changes in host plant density. Monitoring for this contribution to disease increase was carried out by using healthy uninfected 14-day-old host plants as replaceable 'traps'. The three adjacent sampling positions, (reduced to two in the lowest density stand) were placed at the same distance from the primary infection source as for the experiments on the overall rate of disease increase. Daily sampling commenced on the fifth day after inoculation of the primary infection source and continued until the twenty fourth day. On each occasion all three trap plants were removed and replaced.

Trap plants were incubated at 22°C for five days under the same environmental conditions as the experiment, during which time, impacted conidia germinated, colonized the leaf surface and formed visible lesions that could be counted. The total leaf area of the trap plants was determined by passing them through the automatic area meter. The number of lesions, standardized as the number per 200 square centimetres of host tissue (the size of an average trap plant), were then added cumulatively day by day (i.e. day 5; day 5 plus day 6; day 5, day 6, plus day 7; etc.).

c) *Rate of increase of disease due to within-plant transmissions of inoculum*

The contribution made to the overall rate of disease increase by inoculum transmissions within plants was examined by monitoring the rate of disease increase in a number of isolated host plants.

Healthy eight-day-old barley plants were infected by brushing fresh conidia, obtained from actively sporulating lesions, on to small segments of the ten primary leaves of each plant. After inoculation, infected plants were held in still conditions at approximately 22°C, for 24 hours before being placed in a growth cabinet. In the cabinet, individual plants were put in a series of 'compartments' formed from sheet plastic screens arranged in the direction of wind flow to prevent cross contamination occurring, while leaving all other variables, especially wind velocity, unaltered. Further external infections were thus prevented. Disease levels, on each of five replicates were determined at four different times after inoculation (day 5, day 12, day 16 and day 22). On each sampling day, six plants chosen at random from each treatment were removed and scored immediately to determine the total area of visible disease present. The outline of all lesions was traced on to plastic film, blacked in, and passed through the automatic area meter. Total leaf area was also determined.

CHAPTER TWO

THE EFFECT OF PLANT DENSITY PER SE ON EPIDEMICS OF POWDERY MILDEW

I. METHODS

Before experimentation was started, it was recognized that a proportion of conidia would impact on the cylindrical wire plant supports. As a result, at barley (host) plant densities less than 115.5 plants per m², empty supports were placed in gaps in the plant stand, so that the number of supports remained a constant independent of plant density. This prevented the possibility of any differential effects occurring between different barley densities due to changes in this factor.

The methods used in these experiments to measure the overall rate of increase of disease, the rate of increase of disease due to between-plant transmissions of inoculum and the overall rate of within-plant disease increase, were all carried out as specified in Chapter One.

II. RESULTS

a) *Overall rate of increase of disease*

The results of three separate experimental runs expressed as the cumulative proportion (x) of diseased tissue plotted against time are shown in Figure 27a. Although only three data points are available per experiment, the distribution of these is entirely consistent with the early stages of typical disease progress curves. These curves were 'straightened' by transforming x to $\log_e (x/1-x)$ in the manner used previously for damping-off epidemics. Figure 27b shows the distribution of these transformed points together with the lines of best fit as determined by linear regression analysis. The slopes of these lines are a measure of the overall infection rate (r_t). The good fit between data points and the regression lines in the three experiments illustrated is typical of all other results obtained in these experiments.

In Figure 28a, overall infection rates (r_t) derived, in the manner illustrated in Figure 27, from the slopes of the linear

FIGURE 27.

Detailed results of three experiments investigating the overall increase of powdery mildew of barley with time.

a) The proportion x of all tissue diseased plotted against time.

b) $\text{Log}_e (x/1-x)$ plotted against time and fitted with linear regression lines.

● , ▲ , ■ , three different barley stand densities.

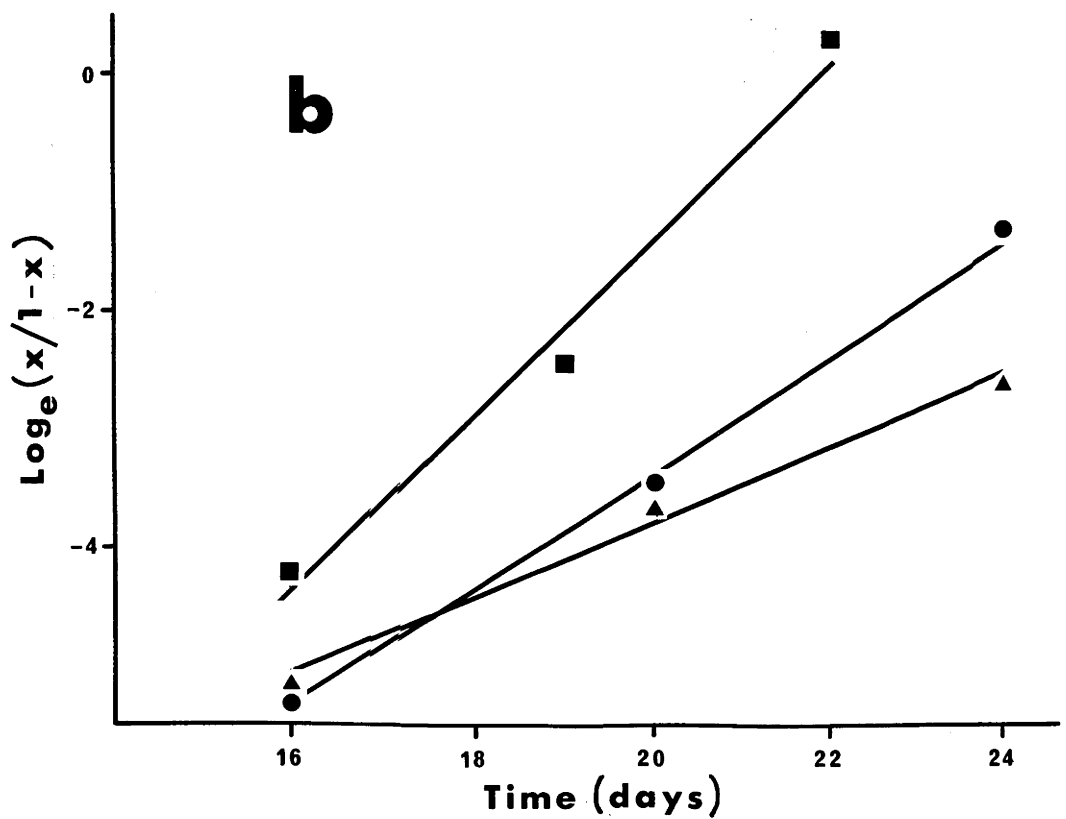
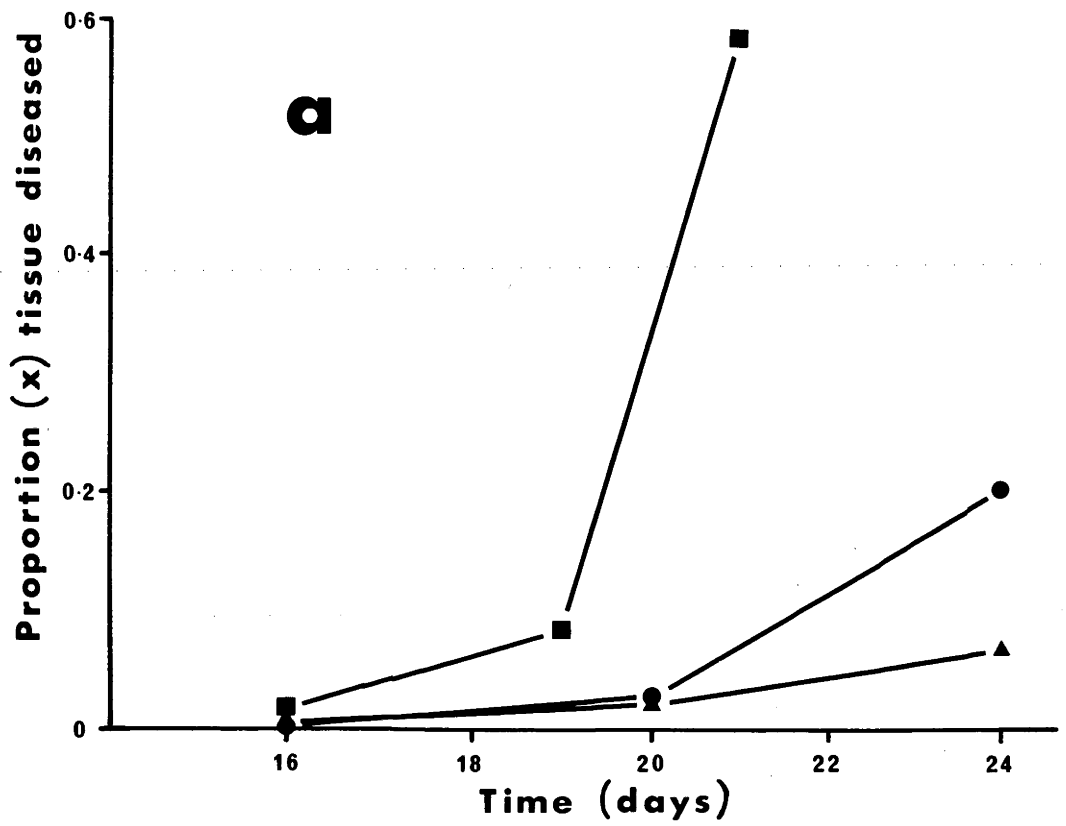


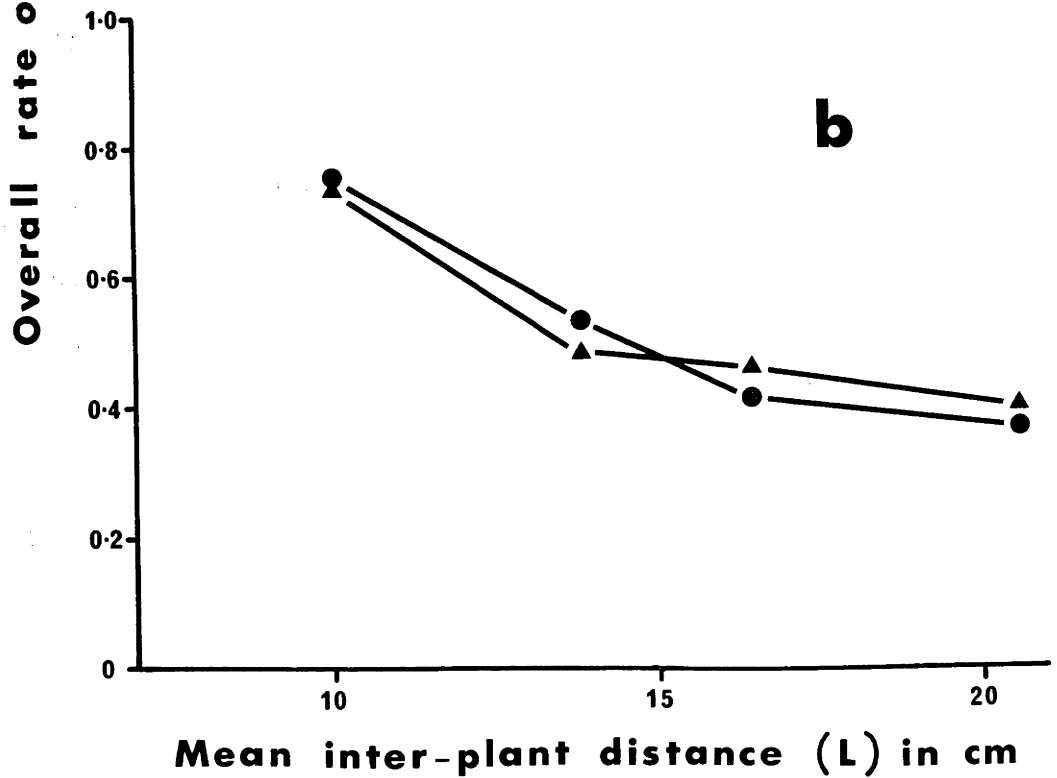
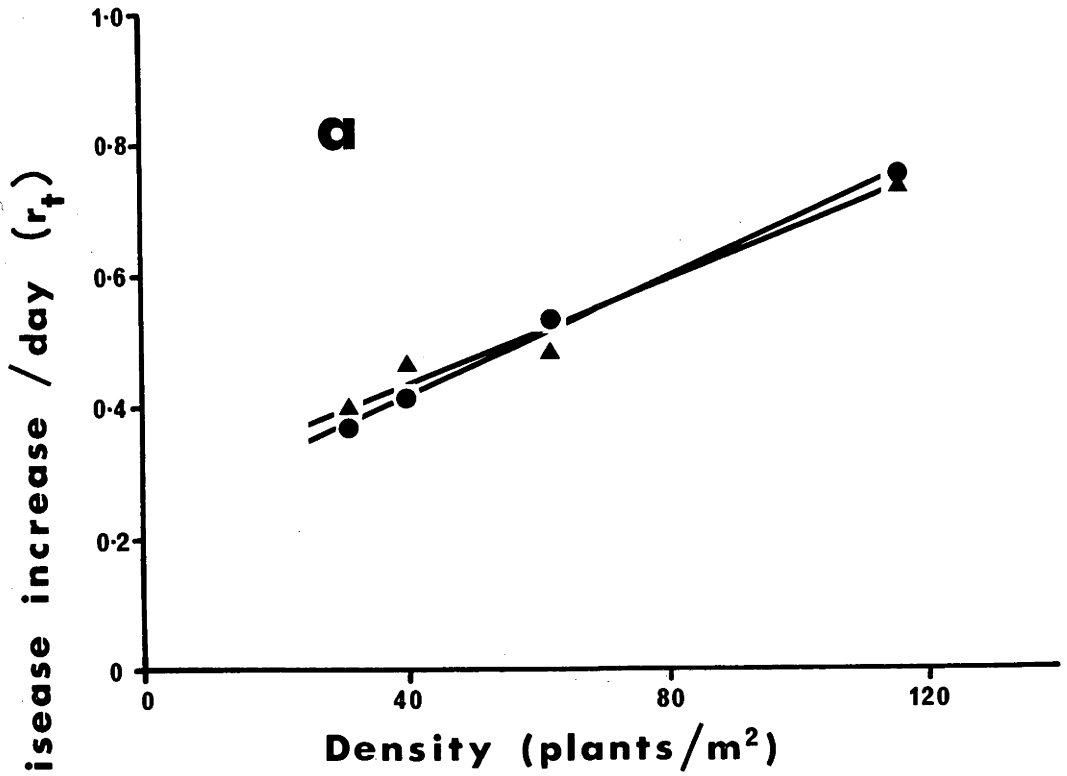
FIGURE 28.

The combined results of a number of separate experiments investigating the overall rate of increase of powdery mildew (r_t) in a range of different density barley stands.

- a) The effect of varying host plant density on the overall rate of disease increase. Lines fitted by linear regression analysis. (Each data point represents the results of a single experiment).

- b) The overall rate of disease increase plotted against the mean distance (L) between adjacent plants.

● , ▲ , results from two different phytotron cabinets.



regression lines of a total of eight separate experiments (four different barley densities; two different growth cabinets) are plotted against plant density. From the distribution of the individual rate values it is clear that there is good agreement between the results obtained from the two different growth cabinets. In both cases, lines fitted by linear regression analysis were statistically significant (in one case $P < 0.05$; in the other $P < 0.01$). There was no evidence of any consistent deviation of the data points away from the linear relationship. Constant increments in plant density produced constant increments in the overall rate of increase of disease within the stand over the range of densities studied.

Rate values were also plotted against the mean distance between adjacent plants (L), which resulted in a shallow curvi-linear relationship (Figure 28b). Increases in the mean distance between adjacent barley plants within the range 10 - 15cms (115.5 - 50 plants per m^2), produced large reductions in the rate of increase of disease, but increases in L above this range had progressively less effect on the rate of disease increase.

b) *Rate of increase of disease due to between-plant transmissions of inoculum*

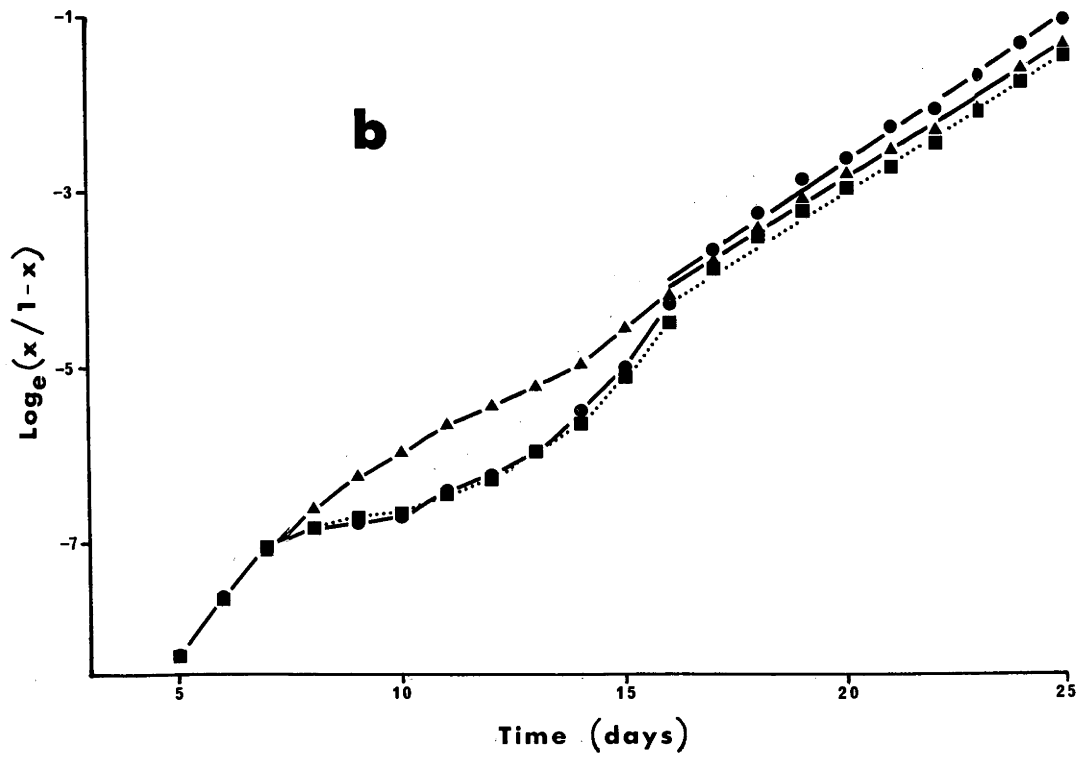
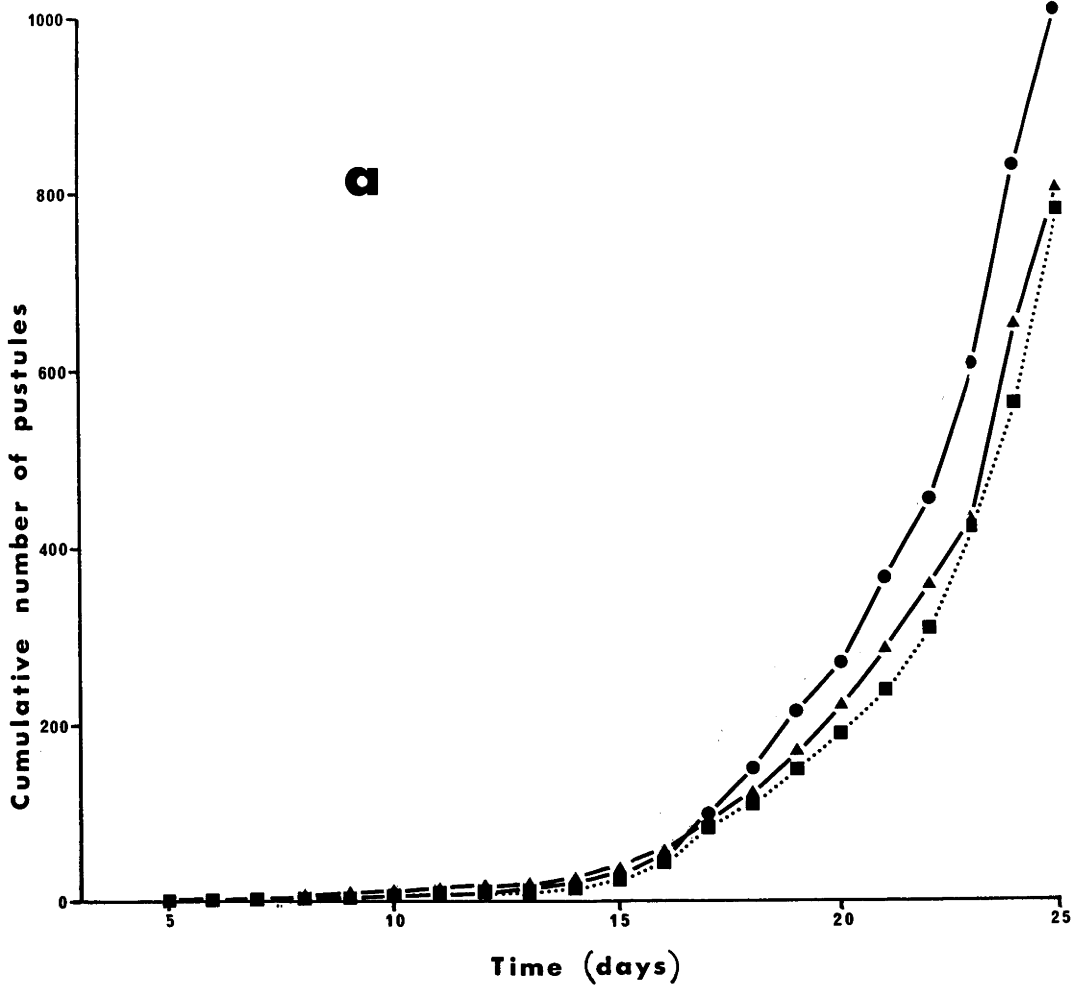
In Figure 29a the cumulative number of successful transmissions, expressed as the number of pustules per 200 square centimetres of leaf tissue (the size of an average trap plant), is plotted against time. The three curves are for the three sampling positions within one particular experiment and are in good agreement with one another.

FIGURE 29.

Detailed results of a single experiment investigating the rate of increase of disease due to between-plant transmissions of inoculum (r_b) with time.

- a) The cumulative number of pustules recorded (successful transmissions), at three different sampling positions within the one experiment, with time.
- b) $\log_e (x/1-x)$ plotted against time and fitted with statistically significant linear regression lines over the period day 16 to day 25. (x is the cumulative number of pustules recorded expressed as a proportion of the total possible number; x is thus equivalent to the proportion of host tissue diseased).

● , ▲ , ■ , three different sampling within the same experiment.



During the first four days, while disease in the source plants was in the latent phase, no conidia were produced and thus there was no increase in disease due to between-plant transmissions. From the fifth until the thirteenth day after inoculation, the number of conidia released into the air and thus the number of pustules produced (assuming a direct correlation exists between the number of conidia released and the number of successful transmissions) increased very slowly. After day 14, however, the number of pustules occurring on trap plants increased very rapidly.

Previously (see above), disease progress curves were straightened by transforming x , the proportion of tissue diseased, to $\log_e(x/1-x)$. This simple transformation is based, however, on the knowledge that when all plants are totally infected x must equal unity. The value x in the logistic function need not necessarily be expressed in terms of the area of plant tissue diseased. Thus, disease curves of several epiphytotics of wheat rust have been straightened with this function, in which x is the cumulative number of spores trapped at any given time, expressed as a proportion of the maximum cumulative spore level (Romig and Dirks, 1966; Dirks and Romig, 1970; Eversmeyer and Burleigh, 1970). In the present series of experiments the number of successful between-plant transmissions was recorded in terms of the number of pustules produced, so that the contribution of between-plant transmissions to the overall rate of disease increase could be estimated. From a careful investigation of the area of young *E. graminis* f.sp. *hordei* pustules and the maximum number of individual pustules which can occur per square centimetre of leaf tissue, it was estimated

that 4000 5-day-old pustules would have effectively covered 200 square centimetres of leaf tissue (the size of an average trap plant). Using this value, which is in reasonably good agreement with that which may be obtained from the 'standard area' diagrams of Grainger (1947), it was possible to convert the cumulative number of pustules to x , a proportion of the maximum cumulative spore level (in these experiments this is equivalent to the proportion of the host tissue diseased).

In Figure 29b the cumulative curves of pustule numbers shown in Figure 29a were straightened in the manner described above. It is clear, from the linear relationship that occurs between $\log_e(x/1-x)$ and time, that from day 16 onwards the epidemic has entered a logarithmic phase. During this time interval, lines fitted to points by linear regression analysis were all statistically significant ($P < 0.01$) having regression coefficients of 0.336, 0.301 and 0.308. These coefficients or line gradients, are a measure of the rate of increase of disease due to between-plant transmissions (r_b).

At an earlier stage in the development of the epidemic (day 5 to day 12), the inoculum source is constant and the epidemic is in a simple interest phase. Plotting $\log_e(x/1-x)$ against time for such simple interest phases results in a curvi-linear relationship between $\log_e(x/1-x)$ and time, with constant increases in time bringing smaller and smaller increments in $\log_e(x/1-x)$. This is particularly well shown by the early part of the top curve in Figure 29b.

In Figure 30a, values derived in the above manner from the results of eight separate experiments, (four different host densities in two different growth cabinets), are plotted against the density of plants in the stand. Despite differences in absolute rates (due possibly to minor differences in wind velocity) the results obtained confirmed the great similarity between the two cabinets. Lines fitted by linear regression analysis to the appropriate sets of points are significant at the 1% level. At the same time, however, the mean square for deviations from regression is considerably greater than the mean square for deviations within groups, resulting in a significant variation from the computed regression line. Such deviations may mean either that r_b is a curvi-linear function of density or that there is a large amount of random statistical variation around the regression line (Sokal and Rohlf, 1969). In fact, visually the points do appear to lie on a shallow curve although regarding the relationship between r_b and plant density as a linear one would still provide reasonable predictive value over the range of densities investigated.

To enable subsequent comparison between these results and those involving disease gradients, rates of increase of disease due to between-plant transmissions were also plotted against the mean distance L between adjacent plants (Figure 30b). In this case, a clear inverse curvi-linear relationship was apparent. At small inter-plant distances, increases in the distance between adjacent individuals produced a rapid decline in r_b . As the mean distance between adjacent plants continued to increase however, there were progressively smaller reductions in the rate factor.

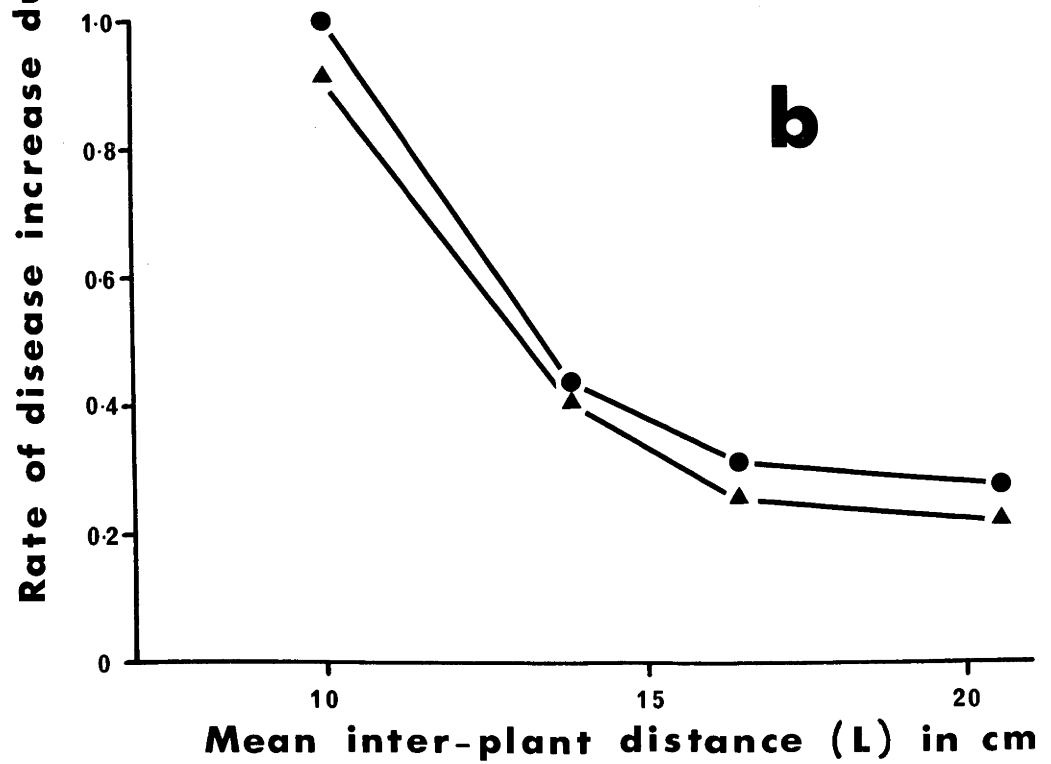
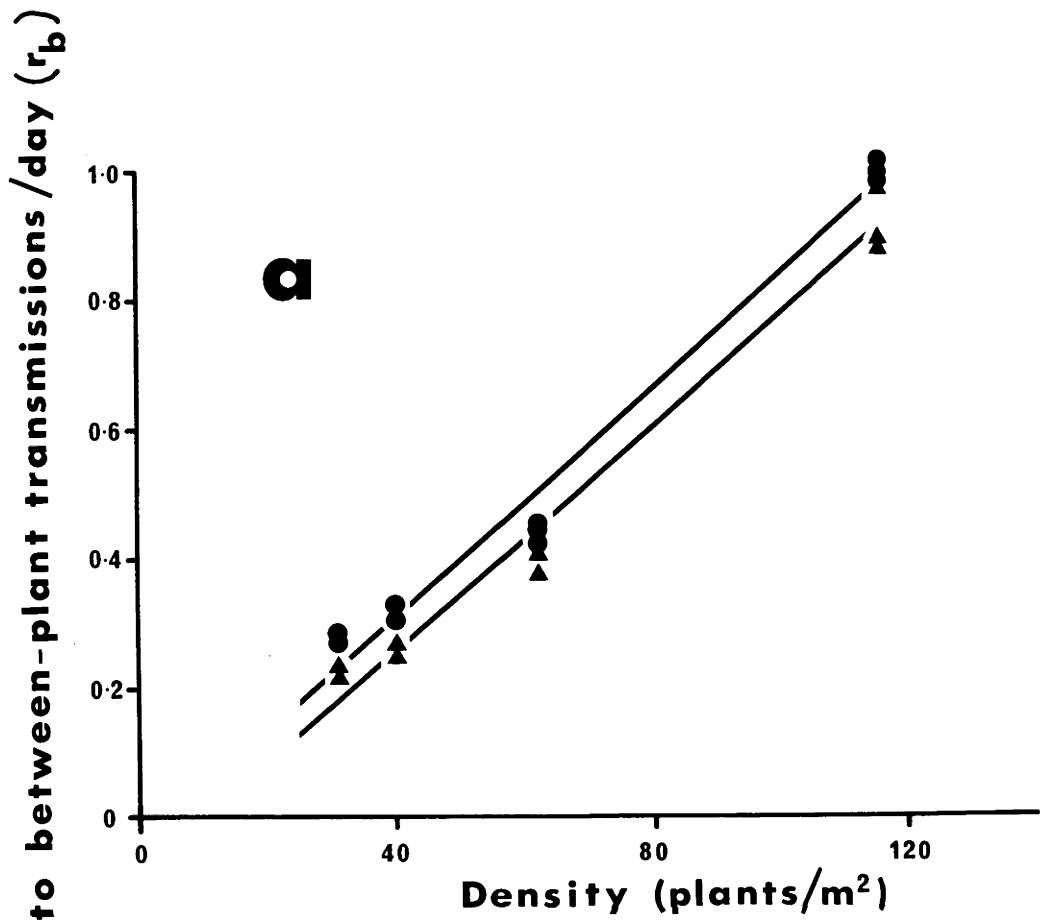
FIGURE 30.

The combined results of a number of separate experiments investigating the rate of increase of disease due to between-plant transmissions of inoculum (r_b) in a range of different density barley stands.

- a) The effect of varying host (barley) plant density on the rate of increase of powdery mildew due to between-plant transmissions of inoculum. Lines fitted by linear regression analysis. (The results for different host stand densities are from different experiments).

- b) The mean rate of increase of powdery mildew due to between-plant transmissions of inoculum plotted against the mean distance (L) between adjacent plants.

● , ▲ , results from two different phytotron cabinets.



c) *Rate of increase of disease due to within-plant transmissions of inoculum*

The results of an experiment designed to determine the level of disease increase in plants protected from external infection are illustrated in Figure 31a, where the proportion x of tissue diseased is plotted against time in days. The disease level on day five was directly attributable to the initial amount of inoculum, while levels on the three subsequent sampling days arose through growth of individual pustules and the initiation of new pustules within each plant. When this curve was straightened by transforming x to $\log_e(x/1-x)$ the line shown in Figure 31b was obtained. A line of best fit was determined for the distribution of all but the first sampling point (this was omitted as disease increase between day 5 and day 10 was exclusively of a simple interest type). From the gradient of this line it was estimated that the mean rate of disease increase within isolated experimental plants is 0.12 per day.

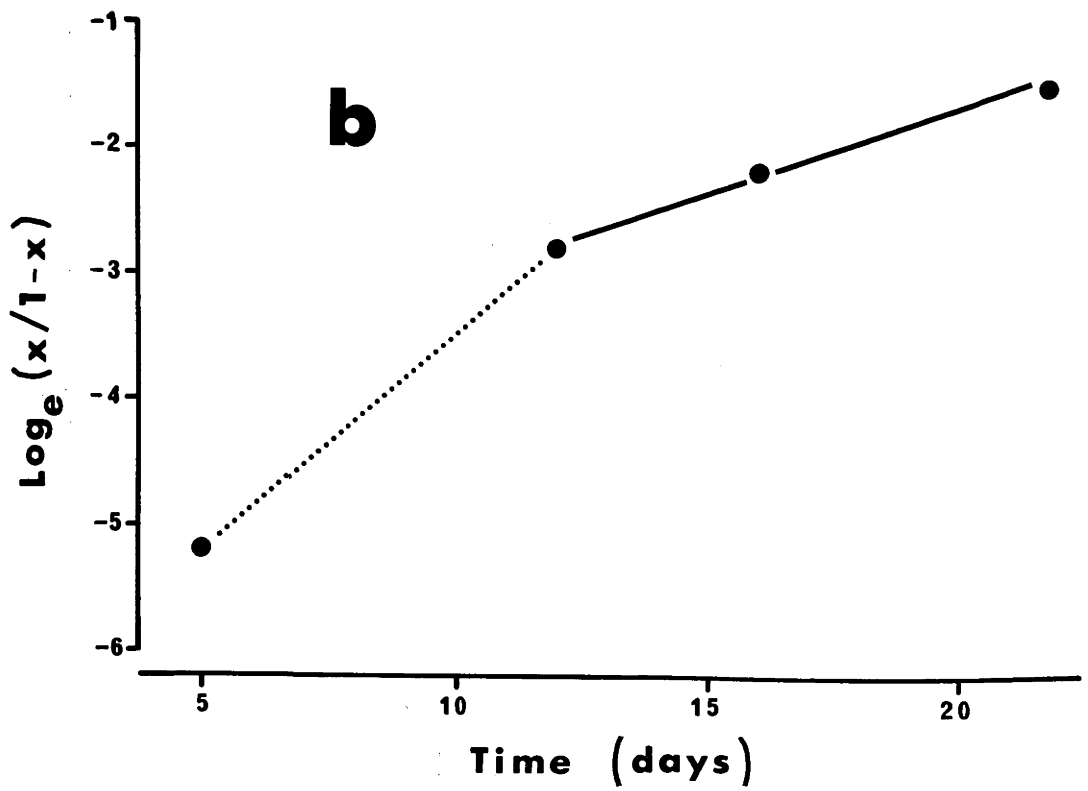
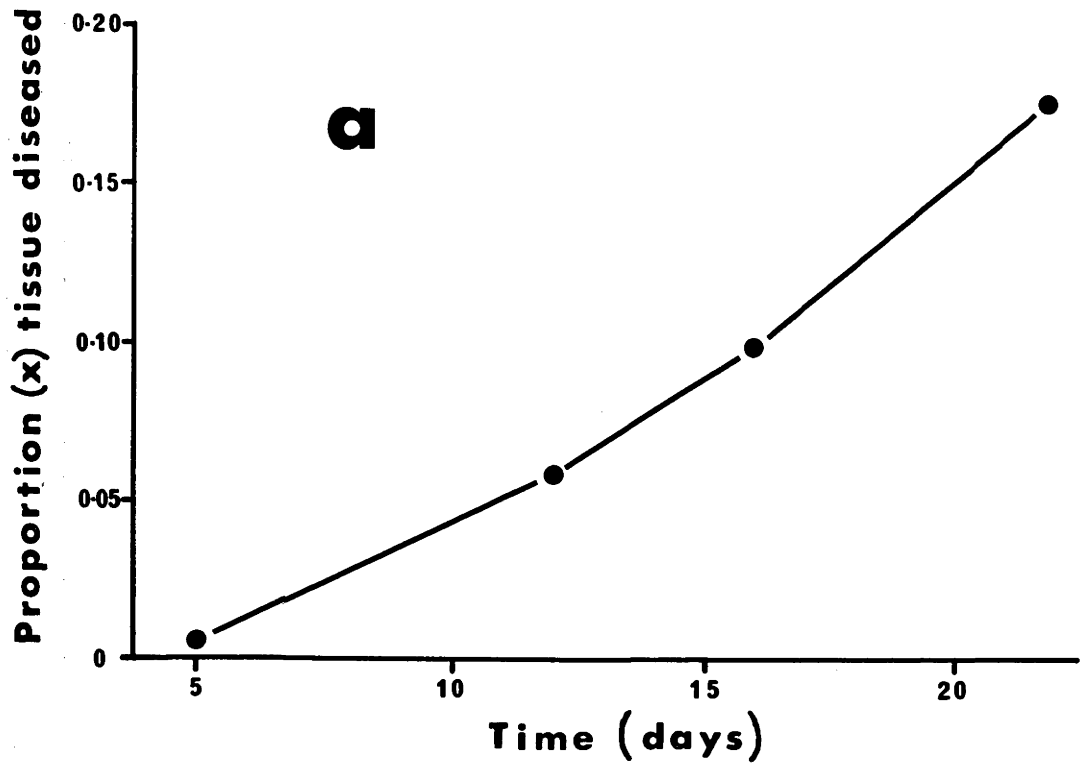
FIGURE 31.

The rate of increase of disease in plants
protected from external infections.

a) Proportion (x) of all tissue diseased
plotted against time.

b) $\text{Log}_e (x/1-x)$ plotted against time and fitted
with a linear regression line over the period
day 12 to day 22.

All data points are the mean of six values.



CHAPTER THREE

*EPIDEMICS OF POWDERY MILDEW IN MIXTURES OF SUSCEPTIBLE
AND IMMUNE PLANTS*

I. METHODS

1. *Plant varieties used*

The present chapter describes an investigation of the effect of mixed species stands on the epidemiology of disease caused by powdery mildew of barley, using the same basic *Hordeum vulgare* - *Erysiphe graminis* f.sp. *hordei* system employed in the previous chapter but elaborated by the addition of a second plant species - bread wheat (*Triticum aestivum* L.). Early trials showed this species to be entirely immune to *Erysiphe graminis* f.sp. *hordei*, the pathogen used in these experiments. The wheat variety used,

Olympic, is a midseason maturing variety which showed a comparable growth rate, (under the conditions of the experiment), to *Hordeum vulgare* variety 164 (the host) and was readily available from commercial sources. Wheat 'plants' were prepared in the manner previously described for barley 'plants' (see Chapter One).

2. *Planting patterns used*

The basic planting pattern used in these experiments (a hexagonal close-packed array) was the same as that for pure barley stands so that the latter could act as controls. Overall stand density was held constant for all experiments at 115.5 plants per m^2 , while the relative proportions of barley (host) and wheat (non-host) plants were varied. In the 'main array', two different methods of distribution of barley and wheat plants were used - random and patterned in rows. In both cases the arrangement of the 'source array' was standardized. The first row consisted entirely of wheat plants, while the second row was entirely barley plants. The six central plants in this row were inoculated at the start of the experiment (see Chapter One) and acted as the primary disease source.

a) *Random placement*

Plants were arranged in a hexagonal close-packed array so that the mean distance between adjacent plants was 10.0cms. The position of the three experimental sampling plants was fixed centrally in the

stand, adjacent to one another and approximately 54cm downwind from the primary infection source. Barley and wheat plants were assigned to the remaining positions in the given array in the following random manner. Differently marked pieces of paper representing barley and wheat plants were placed in a box in the desired proportions, mixed, and then drawn out randomly one at a time. In this way, the identity of the plants at each point in the array was determined working on a back-to-front, left-to-right basis (Figure 32). Replicates of any particular treatment used exactly the same array. Three different barley - wheat mixtures were used (Table 4).

Proportion of barley to wheat plants	Barley plant density /m ²	Wheat plant density /m ²	Mean distance between adjacent barley plants (cms)
0.492/ 0.508	56.8	58.7	15.30
0.363/ 0.647	41.8	73.7	18.33
0.305/ 0.695	35.2	80.3	21.09

Table 4 The relationship between the proportion of barley to wheat plants, barley density, wheat density and the mean distance between adjacent barley plants in the random mixtures used in these experiments (overall plant density: 115.5 plants per m²).

FIGURE 32.

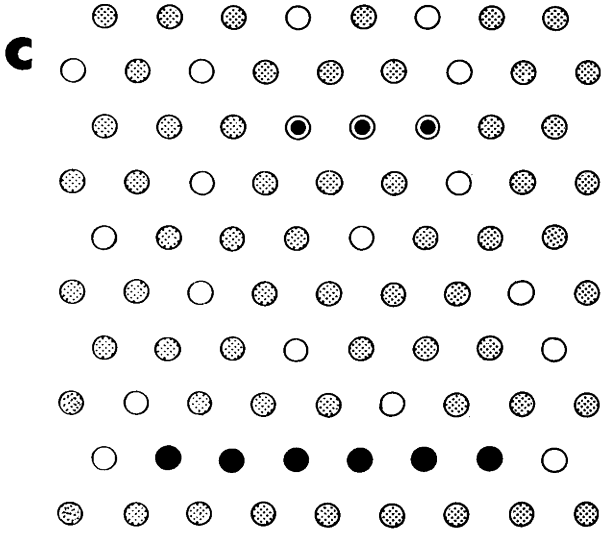
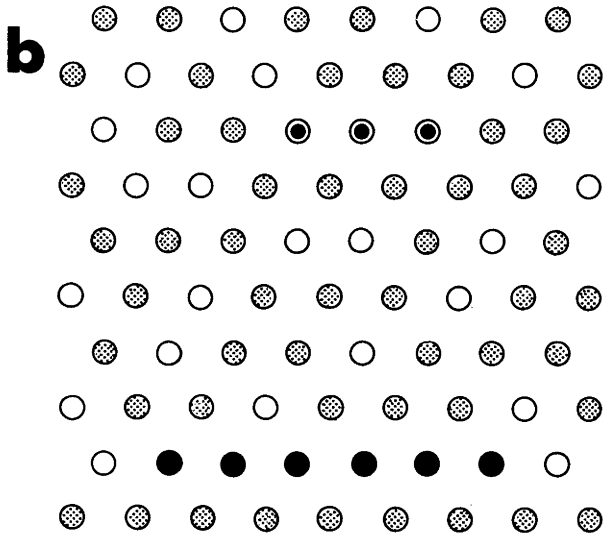
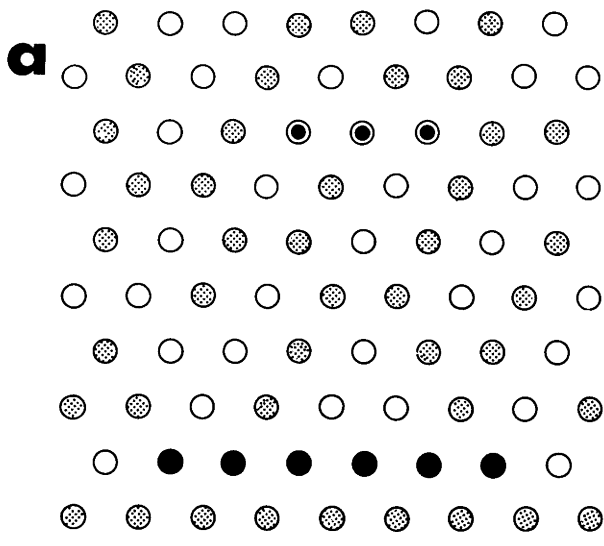
Diagrammatic representation of the random placement of barley and wheat plants used in experiments on the effect of mixtures on epidemic rates of powdery mildew.

a) 0.492:0.508 mixtures of barley and wheat.

b) 0.363:0.647 mixtures of barley and wheat.

c) 0.305:0.695 mixtures of barley and wheat.

● , primary inoculum plants; ⊙ , barley sampling plants; ○ , barley plants of the experimental array; ⊗ , wheat plants of the experimental array.



Direction of air flow

b) *Placement in rows*

In experiments where barley and wheat plants were placed in rows, these were arranged parallel to the primary infection source and at right angles to the direction of wind flow. Three different arrangements were used - stands consisting of alternate rows of barley and wheat, stands composed of one row of barley alternating with two rows of wheat, and stands composed of one row of barley alternating with three of wheat (Figure 33). The proportions of the susceptible (barley) plants in these three different patterns are shown in Table 5.

Number of rows of barley to the number of wheat	Proportion of barley to wheat plants	Barley plant density /m ²	Wheat plant density /m ²	Mean distance between adjacent barley plants (cms)
1:1	0.469/ 0.531	54.1	60.9	16.00
1:2	0.293/ 0.707	33.8	81.7	21.33
1:3	0.281/ 0.719	32.5	83.0	27.67

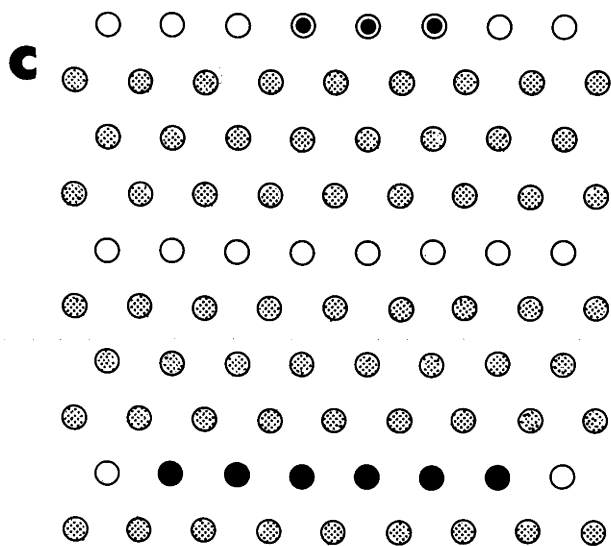
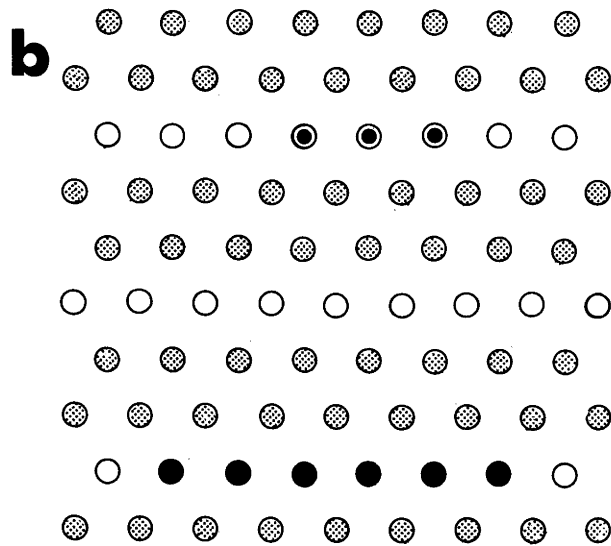
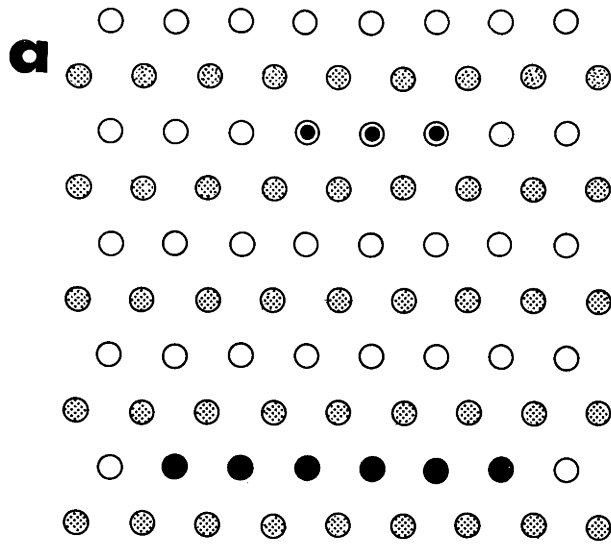
Table 5 The relationship between the proportion of barley to wheat plants, barley density, wheat density and the mean distance between adjacent barley plants in mixtures patterned in rows (overall plant density: 115.5 plants per m²).

FIGURE 33.

Diagrammatic representation of the rows of barley and wheat plants used in experiments on the effect of mixtures on epidemic rates of powdery mildew.

- a) One row of barley alternating with one row of wheat.
- b) One row of barley alternating with two rows of wheat.
- c) One row of barley alternating with three rows of wheat.

● , primary inoculum plants; ● , barley sampling plants; ○ , barley plants of experimental array; ⊙ , wheat plants of experimental array.



In all stands at least one row of barley occurred between the source and sampling rows. This meant that the actual distance from the source to the sampling row varied between 54 and 71cms. This was unimportant, as the factor under investigation was the relative rate of disease increase which should be independent of the precise location in the array.

II. RESULTS

a) *Overall rate of increase of disease*

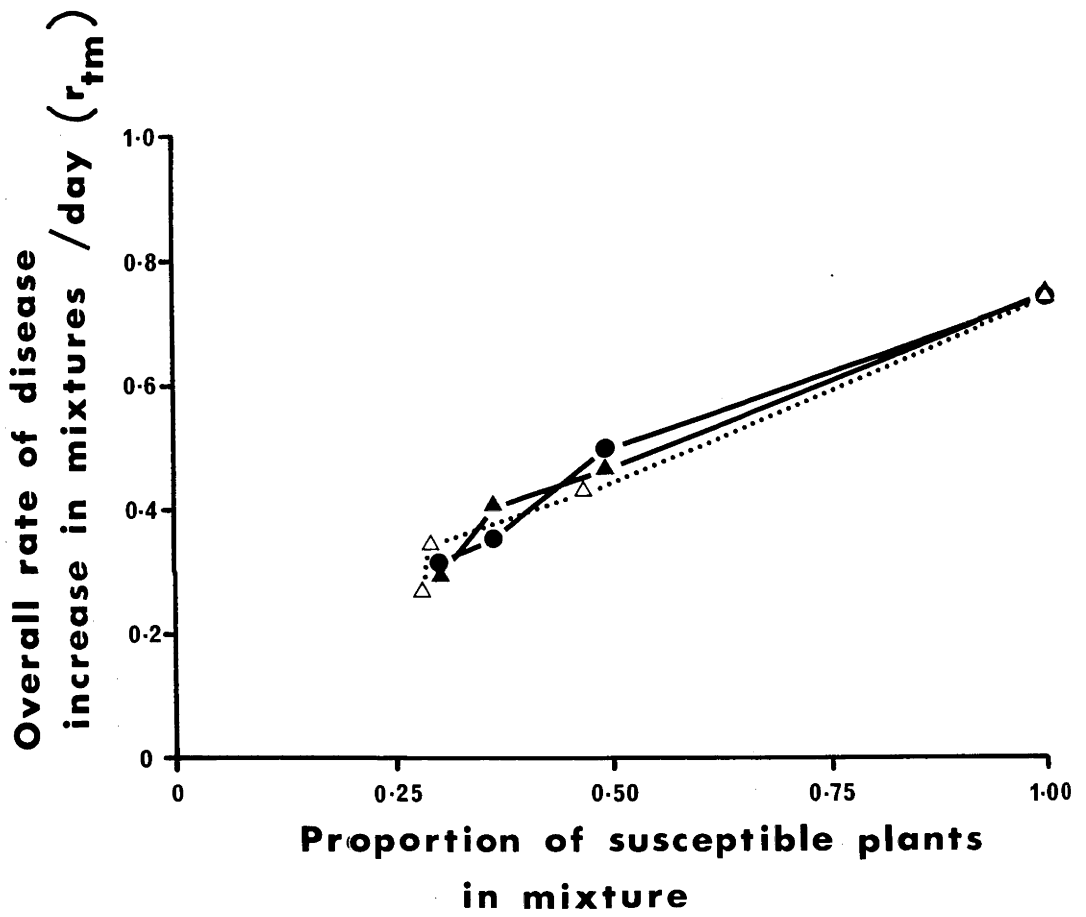
The overall rate of disease increase was monitored in the same manner as that previously described for pure stands. The proportion (x) of tissue diseased, when plotted against time, produced curves similar to those shown in Figure 27a. These were straightened using the modified logistic function $\log_e (x/X-x)$, where x is the proportion of tissue diseased and X is the proportion of susceptible individuals in the population (as used in Chapter Two, Section B). Figure 34 shows the results of a number of separate experiments carried out to investigate the effect of the proportion of barley plants on the rate of disease increase in a mixture of susceptible and resistant plants. Results from the two different cabinets (the two solid lines) and the two different planting patterns (solid and dotted lines) are in reasonably good agreement with one another. In all cases, the overall rate of increase of

FIGURE 34.

The combined results of a number of separate experiments showing the effect of the proportion of susceptible (barley) plants in a mixture of barley and wheat plants on the overall rate of disease increase in mixtures (r_{tm}).

● , ▲ , results from random mixtures of barley and wheat in two different phytotron cabinets; Δ , results from mixtures patterned in rows.

Each data point represents the results of a single experiment.



disease for the *entire* stand declined as the proportion of immune wheat plants in the stand increased. The lines are reasonably straight over the range of proportions examined but show a marked departure from a simple one-for-one relationship between the rate factor and the proportion of barley plants.

b) *Rate of increase of disease due to between-plant transmissions of inoculum*

In experiments in which the number of pustules resulting from between-plant transmissions was monitored, cumulative curves were obtained similar to those illustrated in Figure 29a. For each replicate of each density treatment, cumulative pustule numbers were transformed (using $\log_e(x/X-x)$), plotted against time and fitted by linear regression analysis. From the slopes of these lines of best fit, measures of the rate of increase of disease due to between-plant transmissions of inoculum were obtained.

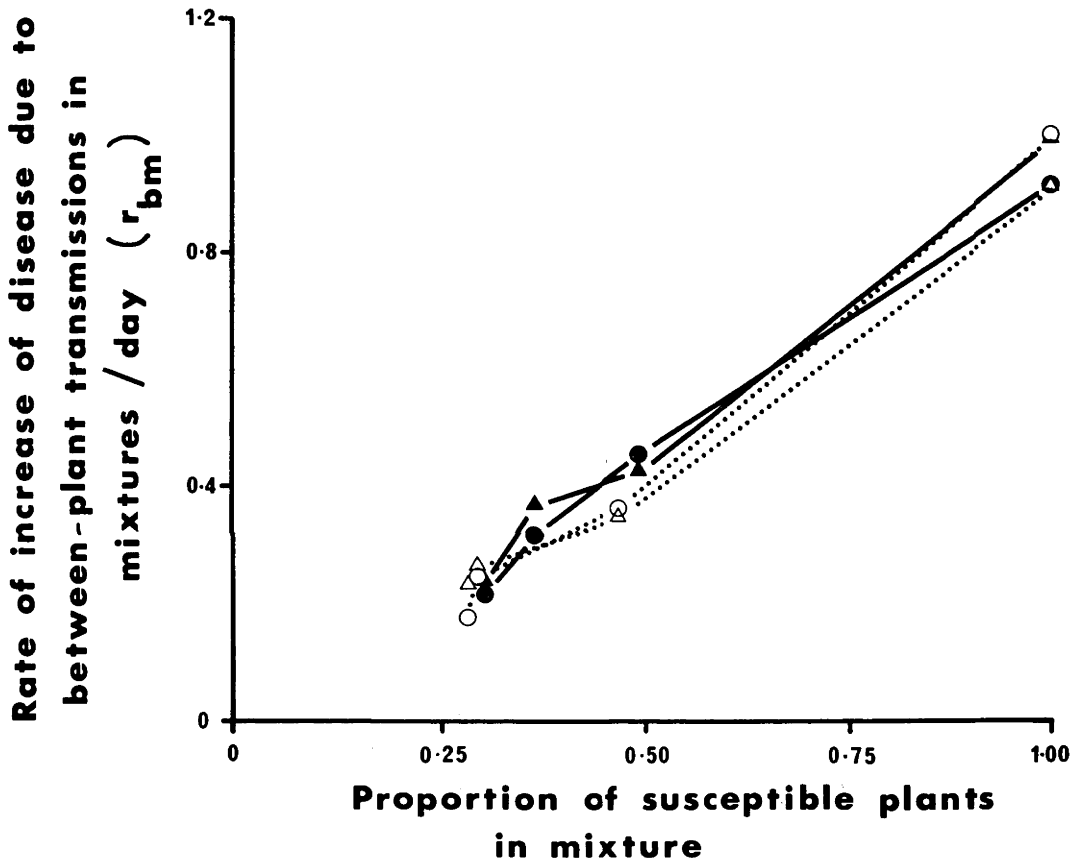
In Figure 35, mean rate values, derived in this way from the results of a number of separate experiments, are plotted against the proportion of susceptible (barley) plants in the mixture. Solid lines represent results obtained for random barley/wheat distributions while dotted lines represent those obtained in experiments where barley and wheat plants were arranged in rows. In both cases there is good agreement between duplicate experiments and between the two different planting patterns. For both patterns, the rate of increase of disease due to between-plant transmissions declined as the proportion of wheat plants increased and the proportion of barley plants decreased.

FIGURE 35.

The combined results of a number of separate experiments showing the effect of the proportion of susceptible (barley) plants in a mixture of barley and wheat plants on the rate of increase of disease due to between-plant transmissions of inoculum in mixtures (r_{bm}).

● , ▲ , results from random mixtures of barley and wheat in two different phytotron cabinets; ○ , △ , results from mixtures of barley and wheat patterned in rows in two different phytotron cabinets.

Each data point represents the mean results of a single experiment.



c) *The interception of inoculum by wheat and barley plant barrier rows*

The essential difference between mixtures and monocultures of barley plants of the same density as the net density of barley plants in the mixture is the presence of wheat plants. These plants may act as filters removing conidia from the air and thus rendering them inoperative as inoculum. To investigate the effect of wheat plant barriers on the downwind dispersal of conidia from a line source, a large experiment with six sub-parts was devised. Diseased infectious barley plants, forming a line source at right angles to the direction of wind flow, were placed at the front of the cabinet so that adjacent plants were touching each other. Sampling plants (three at each distance) were placed 17.5, 44.2 and 71.0 cms downwind from this source so that no plant was shielded by any other. Barrier rows of wheat were placed 17.5, 26.5 and 35.5 cms from the source in the same hexagonal array used during the earlier experiments. The experimental set-up is illustrated in diagrammatic form in Figure 36. In addition to wheat, the trapping efficiency of a row of barley was compared with that of a row of wheat. Each sub-experiment was allowed to 'operate' for six hours after which time the barley plants were removed and incubated at 22°C for 5 days to allow pustules to develop. Environmental conditions were maintained constant within and between sub-experiments.

Figure 37 shows the results of these experiments. In Figure 37a the mean values at each distance are plotted. Considerable variation occurred between experiments in the number of conidia

FIGURE 36.

Diagrammatic representation of the experimental set-up used to investigate disease dispersal gradients.

- a) Downwind disease dispersal from a line source; no barrier rows present.
- b) Downwind disease dispersal from a line source; one barrier row of wheat close to the line source.
- c) Downwind disease dispersal from a line source; one barrier row of wheat some distance from the line source.
- d) Downwind disease dispersal from a line source; one barrier row of barley close to the line source.
- e) Downwind disease dispersal from a line source; two barrier rows of wheat present.
- f) Downwind disease dispersal from a line source; three barrier rows of wheat present.

● , primary inoculum plants of the line source;
○ , barley sampling plant; ⊗ , wheat plants of barrier rows; ⊙ , barley plants of barrier row.

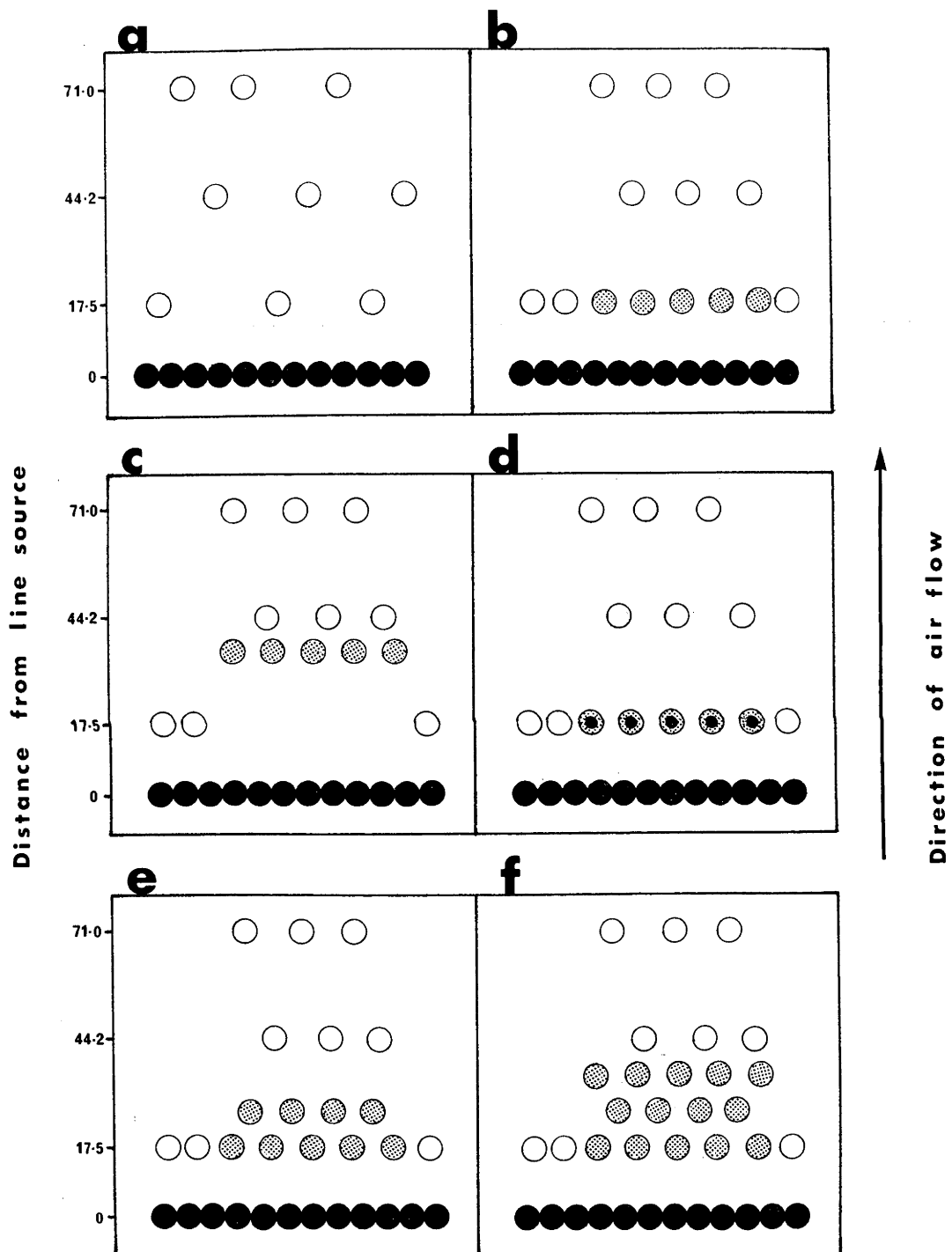


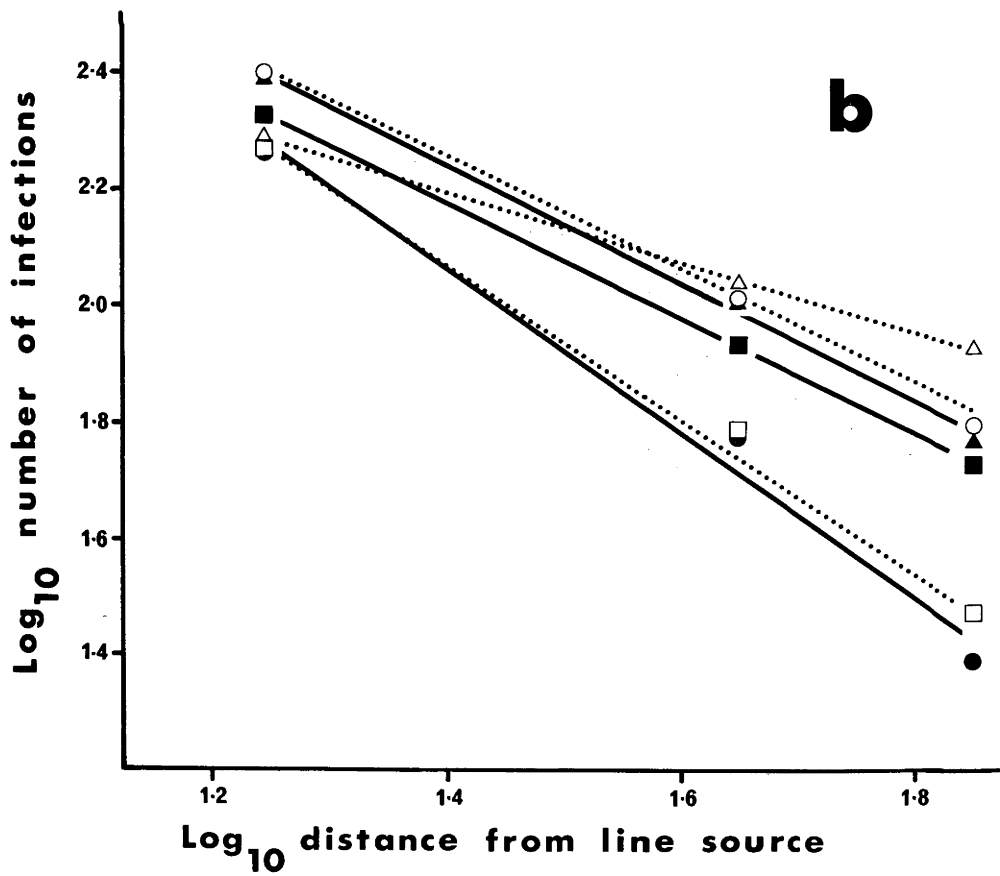
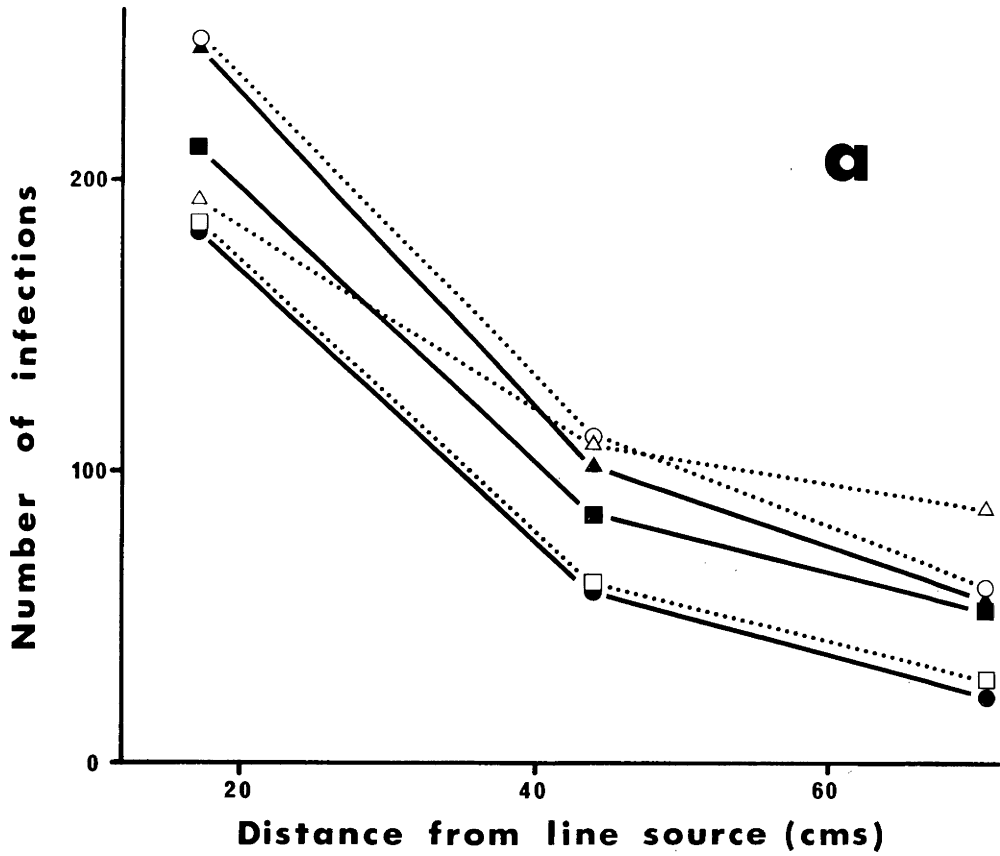
FIGURE 37.

The effect of the presence of barrier rows on downwind dispersal of *Erysiphe graminis* f.sp. *hordei*.

a) The number of infections plotted against the distance from the line source.

b) \log_{10} of the number of infections plotted against \log_{10} of the distance from the line source.

Δ , no barrier rows; \blacktriangle , one row of wheat close to the line source; \circ , one row of wheat some distance from the line source; \blacksquare , one row of barley close to the source; \square , two rows of wheat; \bullet , three rows of wheat.



trapped at all distances, including the 17.5cm sampling station which was unshielded from the line source. In Figure 37b, \log_{10} of the number of successful transmissions is plotted against \log_{10} of the distance from the disease source. This is equivalent to plotting logarithms of: $y=a/x^b$, and has the effect of substantially straightening the line. As can be seen from the Figure, the disease gradient is fairly shallow in the control situation where no barrier plants were present, somewhat steeper where one barrier row was present and even steeper when two or three rows were present.

CHAPTER FOUR

*DISCUSSION OF EXPERIMENTS INVOLVING EPIDEMICS OF
POWDERY MILDEW*1. *Epidemic rates*

Van der Plank in his three books concerning epidemics of plant disease (1963; 1968; 1975) defines three main types of infection rate: the logarithmic infection rate, the apparent infection rate and the basic infection rate.* Logarithmic infection rates are

* Other infection rates defined by van der Plank, for example the corrected infection rate, are only refinements of the basic three in which allowance is made for growth of the crop, removals etc.

applicable only when the proportion of tissue diseased is small ($x < 0.05$). When the proportion is not small, an apparent or basic rate may be determined. The apparent infection rate, r , is based on x_t the proportion of infected tissue regardless of whether it is in the latent, infectious or dead phase. R , the basic infection rate is based, on the other hand, on x_i the proportion of tissue that is exclusively in the infectious phase. As already pointed out, while visible disease is easily detected, neither the detection of disease in the latent period nor the determination of the proportion which is no longer infectious is easy. In the experiments described here, the rates determined are not precisely equivalent to either van der Plank's r or R . In the work concerning *Pythium irregulare*-induced damping-off of *Lepidium sativum*, the rates determined were virtually apparent infection rates as latent periods were extremely short - a matter of a few hours. In epidemics in which the latent period is noticeably longer than this, as in the present case, the determination of apparent infection rates is virtually impossible. Equally, accurate estimation of R is very difficult, as pre-infectious and post-infectious disease, both of which should be excluded from computations of R , are impossible to separate easily from actively sporulating tissue. The infection rates determined in these experiments fall somewhere between van der Plank's apparent and basic infection rates and are based on the proportion of tissue which is visibly infected.

2. Host plant density per se

The most important result to emerge from this study of host plant

density is that, over the range of densities examined, there appears to be a linear relationship between the overall rate of increase of powdery mildew and the density of barley plants* in the stand. This relationship can be stated as follows:

$$r_t = a (1 + D/c)$$

where r_t is the overall rate of increase of disease, D is the density of plants in the host stand, and a and c are constants.

Attempts to extend this relationship outside the range of densities investigated, to determine a and c , the intercepts obtained by extrapolating to the r_t and D axes respectively, result in seemingly anomalous findings. When $D=0$, $r_t = 0.25$; that is the rate of increase of disease is 0.25 when host plant density is zero - an impossible result for an obligate parasite. Consideration of other results presented in this area allows these apparent inconsistencies to be reconciled to a large extent.

Transmission of disease caused by *E. graminis* f.sp. *hordei* occurs when conidia, 'released' from active pustules, successfully infect and form new pustules on previously healthy tissue. These transmissions may be conveniently classified into two types on the basis of where they originate and where they cause disease. Conidia produced by pustules outside a given host plant, but which impinge

* In the discussion of these results 'plants' are, in fact, groups of ten individual seedlings (see Chapter One).

on and infect that particular plant, result in between-plant transmissions; while conidia produced by pustules which re-infect other parts of the same plant, result in within-plant transmissions.

One of the most noticeable differences in the relationship between the overall rate of disease increase and barley density (Figure 28a) and the rate of disease increase due to between-plant transmissions of inoculum and barley density (Figure 30a), is the tendency for r_t values to form a straight line whereas r_b values tend to form a shallow curve (although a statistically significant linear regression line may be fitted to the points; $P < 0.01$). Moreover, while at low plant densities the rate of disease increase due to between-plant transmissions of inoculum is considerably lower than the overall rate of disease increase, at high plant densities this situation is reversed. Most of these anomalies probably derive from differences in the experimental sampling system used to obtain estimates of r_t and r_b .

A measure of under-estimation occurs in r_b values due to the fact that disease was evaluated after five days, whereas in experiments designed to measure r_t values some disease lesions may have been expanding in size for up to twenty days. (This should result in a constant under-estimation of r_b values regardless of stand density). A more significant discrepancy results from the problem of inoculum wastage. Inoculum wastage occurs as a result of multiple 'hits' or infections. At high plant densities where the number of conidia in the air is large and increasing rapidly, considerable numbers of multiple hits would normally occur if sample traps were not replaced daily. Equally at low barley densities where

the absolute number of conidia in the air is lower, the number of multiple hits would also be less. r_b values were obtained by fitting van der Plank's $\log_e(x/1-x)$ transformation to cumulative additions of the number of successful infections. While this transformation allows for the declining amount of healthy tissue available for infection (van der Plank, 1963), it does not make allowance for the number of multiple hits that would have occurred if sample plants had not been changed daily. If allowance is made for this lack of multiple hits, (by applying Gregory's (1948) multiple infection transformation to raw data), r_b values at high plant densities were found to be disproportionately over-estimated in comparison with those at low plant densities. Furthermore, overlapping infections occurring as a result of adjacent pustules merging with one another after day 5 will also tend to reduce r_b values more at high plant densities than at low plant densities. The combination of these factors could thus be expected to flatten the slight curve apparent in Figure 30a and thus produce a rectilinear distribution of data points with a shallower slope similar to that found for the relationship between r_t and plant density.

Although between-plant transmission of inoculum obviously makes a significant contribution to the overall rate of disease increase in a plant stand, it is only one of the two factors to be considered. The second component of the overall rate of disease multiplication is the contribution made by disease increase due to within-plant transmissions of inoculum (r_w). Obviously, to simplify measurement of disease increase due to within-plant transmissions it is necessary to isolate plants so that between-plant contact is prevented. Such a

situation, although idealized, may be regarded as an extremely low density stand in which individual plants are spaced so far apart that between-plant transmissions are virtually eliminated. In the actual experiment performed here a mean value of $r_w = 0.12$ was obtained. Such a rate would occur at very low barley densities (i.e. when D approximates to zero), and thus partly explains the seemingly impossible suggestion (from an extrapolation of Figure 28a) that r_t would equal 0.25 when D was virtually zero. The contribution that the rate of disease increase due to within-plant transmissions of inoculum makes to the overall disease rate is obviously relatively important at low barley densities (where the contribution made by r_b is low), and is relatively insignificant at high barley densities where the contribution made by between-plant transmissions is much greater.

3. Mixtures

a) *Mixtures in general*

The results discussed here attempt to analyse in some detail the way in which disease rates in mixtures decline as the proportion of susceptible plants decrease, and to investigate the added advantages, if any, of mixtures over equivalent density monocultural controls of the susceptible (barley) species.

Taken over the range of susceptible plant proportions for which actual values are available (0.25 to 1.00), the results

obtained lie reasonably close to a straight line (Figure 34). Considered over the entire range of possible proportions (0 to 1), however, they show considerable deviation (up to 58%) from a simple linear relationship between the overall rate of disease increase for the mixture as a whole and the proportion of susceptible plants in the mixture. While it is readily apparent that in a 'mixture' consisting entirely of immune plants the rate of disease increase must be zero, it is not immediately clear what value r will have when all but one plant in the stand is immune. The rate of increase of disease on that solitary plant could be quite high if the plant becomes infected (as great as 0.12 here) but the rate in the stand as a *whole* will be very low - not noticeably greater than zero. (Disease rates recorded here are for the entire mixture).

In Table 6, the values shown in Figure 34 were used to calculate coefficients of determination (equal to the square of the correlation coefficient) for the overall rate of increase of disease in the mixture regressed against the proportion of susceptible plants (m) on both arithmetic and logarithmic scales. Coefficients of determination always lie between 0 and 1 and indicate how closely an equation fits the actual data; the closer these values are to 1 the better the fit (Sokal and Rohlf, 1969). Such values were thus determined for the following equations:

$r_{tm} = r_t + bm$ and $r_{tm} = r_t + b \log_e m$, where r_{tm} and r_t are the rates of increase of disease in mixed and pure stands respectively, m is the proportion of susceptible barley plants in the mixture and b is the regression coefficient (slope of the line). From a visual

Pattern	Coefficient of determination of linear regression lines based on:	
	Arithmetic absc.	Natural log absc.
a) <i>Random</i>		
Cabinet I	0.97	0.99
Cabinet II	0.96	0.98
b) <i>Patterned in rows</i>	0.96	0.94

Table 6 Coefficients of determination for the fit of data to the two equations: $r_{tm} = r_t + bm$ & $r_{tm} = r_t + b \log_e m$.

inspection of Table 6 it is clear that there is little to choose between the predictive value of the two formulae, although in two out of three cases it appears that the regression of the overall disease rate against a log-scaled abscissa gave a marginally better fit.

Extrapolation of the linear relationship between the rate of increase of disease in mixtures of susceptible and immune plants and $\log_e m$ suggests that when the proportion of barley plants in the mixture reaches 0.128 (mean value), the rate of increase of disease in the crop as a whole will be virtually zero. Extrapolation of the arithmetic relationship between the rate of increase of disease in the mixture and m produces, on the other hand, the unreasonable suggestion that disease continues to increase at a rate of 0.147

(mean value) even when all plants in the 'mixture' are immune.

Overall then, the results presented here suggest that there is a relationship between the rate of increase of disease in mixtures and the proportion of barley plants present, best described by the equation $r_{tm} = r_t + b \log_e m$. Had mixtures with lower proportions of susceptible plants than those tested here been used (that is, less than 0.25), it is probable that the log relationship would have shown a much better fit than the arithmetic relationship as a log scale has a much greater 'straightening' effect between the values 0 and 0.25 than between 0.25 and 1.

Because of the central role played by the overall proportion of barley plants in determining disease rates in mixtures, in Figure 38, rates of overall disease increase for mixtures (obtained from Figure 34) are plotted against the net density of barley plants present (solid graph lines). As can be seen, there appears to be a more or less linear relationship between the disease rate and the susceptible plant density. The nature of the planting pattern, whether random or patterned in rows, had no apparent effect on the relationship.

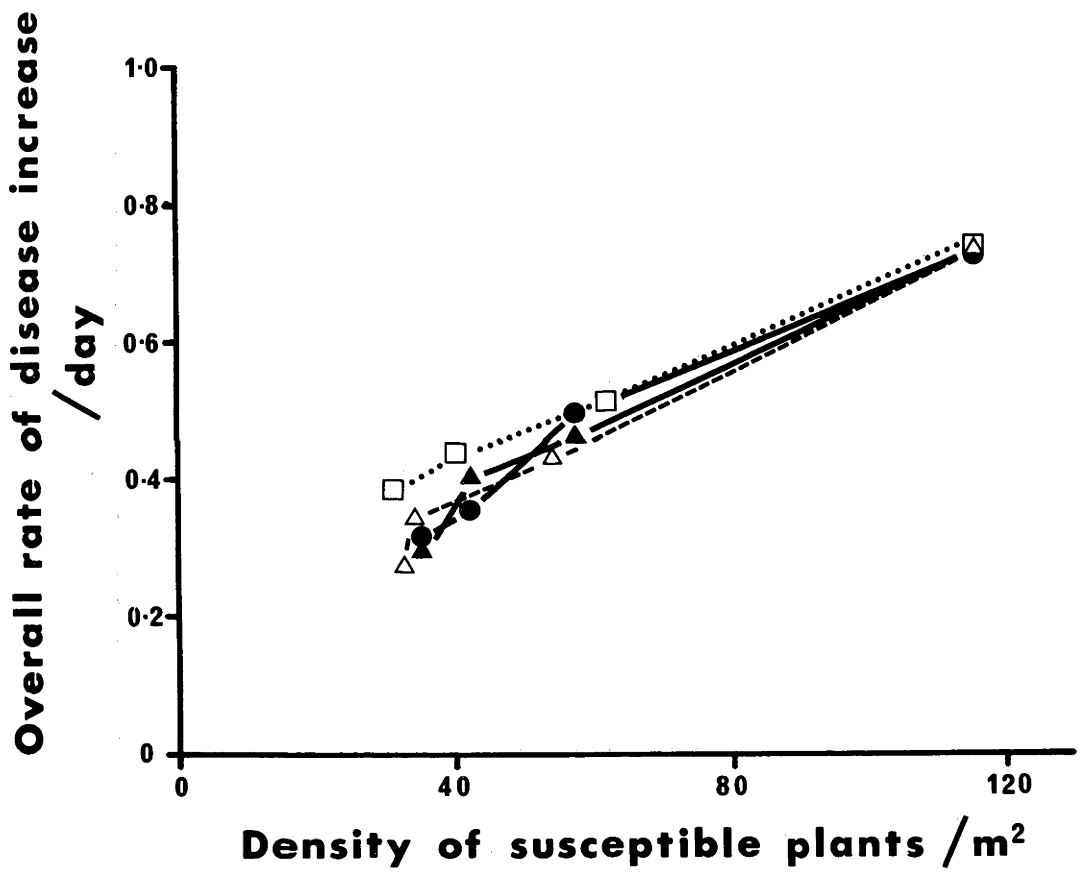
In order to compare disease rates in mixtures with disease rates in barley stands of lower overall density, the mean overall disease rate values obtained earlier for pure stands of barley of different density (Figure 28a) were also included in Figure 38 (dotted graph line). These results provide 'controls' against which the effectiveness of a mixture in reducing disease may be judged. In mixtures where there is a high proportion (0.492) of barley plants,

FIGURE 38.

The results shown in Figure 34 redrawn to show the relationship between the overall rate of increase of disease in mixtures and the density of susceptible (barley) plants in the mixture.

Solid lines (● , ▲) - overall rates of disease increase in random mixtures (r_{tm}) from two different phytotron cabinets; broken line (Δ) - overall rate of disease increase in mixtures (r_{tm}) patterned in rows.

The dotted line (□) is a mean for the overall rate of disease increase in pure stands of barley, r_t , (data from Figure 28a) and is included here for comparison purposes.



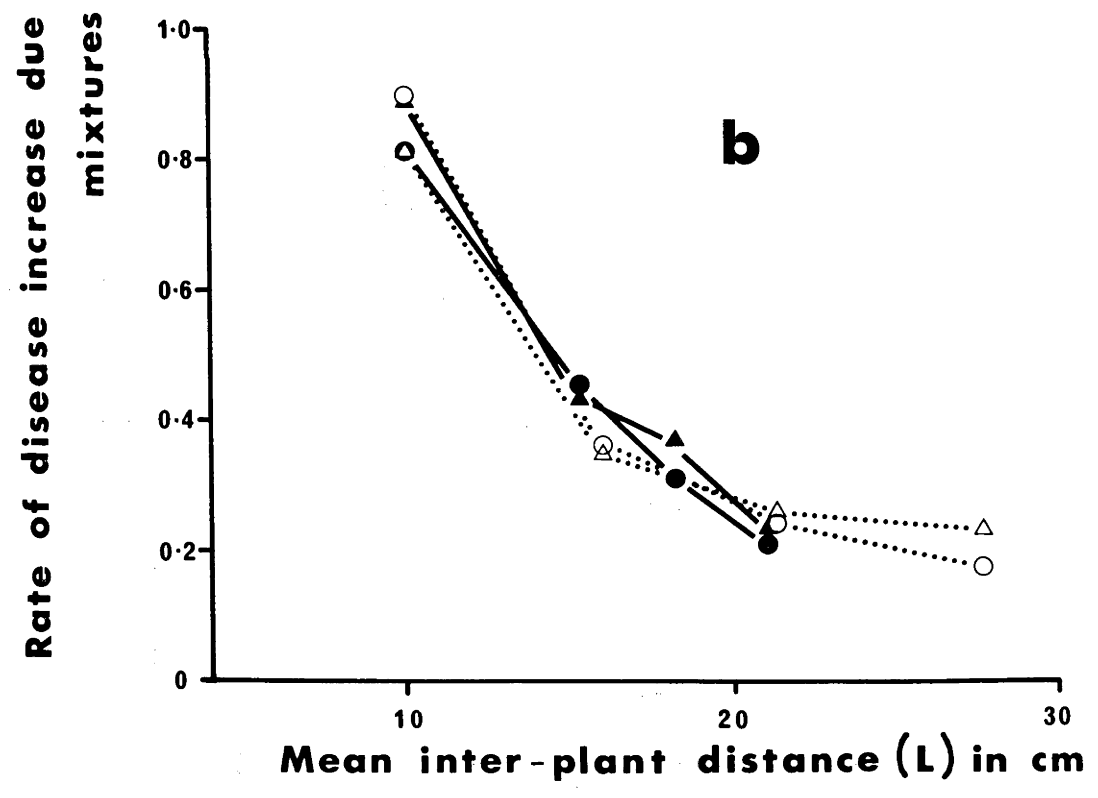
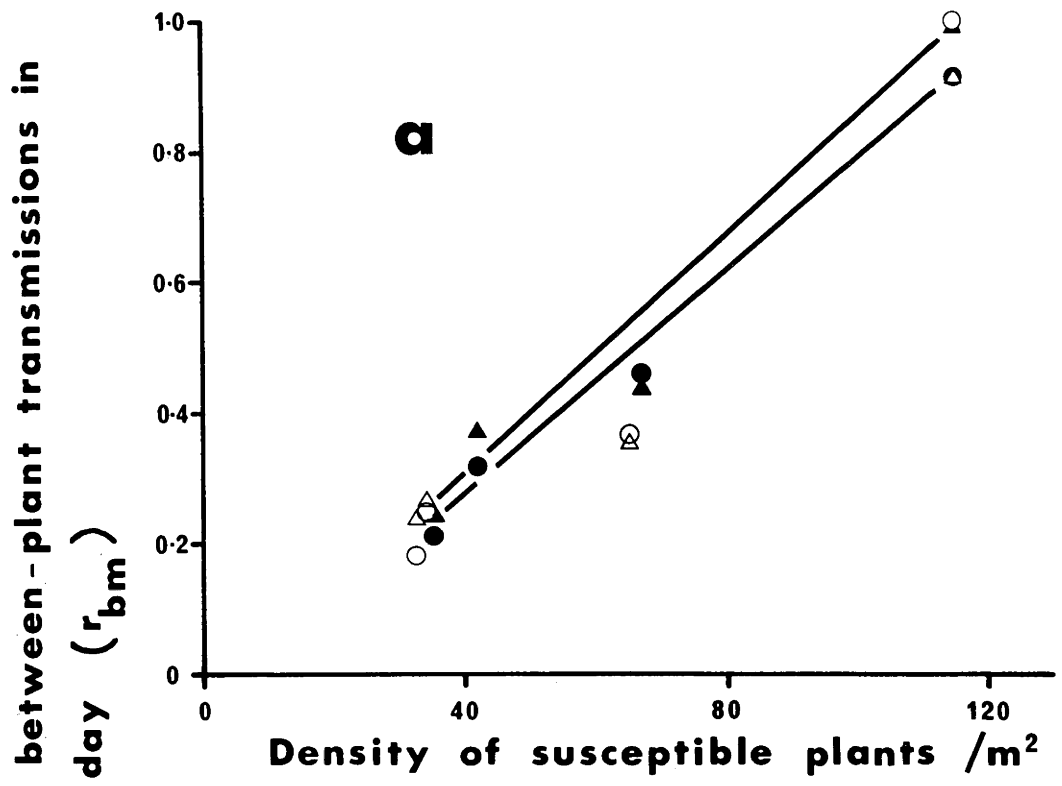
the rate of increase of disease is not noticeably affected by the presence of immune wheat plants. As the proportion of susceptible plants in the mixture continues to decline (0.362 to 0.305 to 0.281) the rate of increase declines more rapidly than in monocultures with similar densities of susceptible plants. Additionally the slopes of regression lines fitted to the results from mixtures (0.0051, 0.0052 and 0.0053) are consistently greater than that for pure barley stands (0.0043), indicating that the difference between the rate of increase in pure stands and in mixtures will continue to increase as the proportion of susceptible individuals decreases. Obviously barley plants gain some advantage from mixtures in addition to that which derives from the reduction in host density *per se*. The most likely source of this advantage lies in the role that the non-host wheat plants play in intercepting air-borne inoculum. This is discussed later.

By comparing the relative rates of decline of r in pure stands with those in mixtures, an estimate of the relative importance of the reduction in susceptible plant density and the insertion of immune plants in reducing disease rate values can be obtained. Reducing barley density from 115 to 40 plants per m^2 in pure stands resulted in a decline in r_t from 0.75 to 0.44 - a reduction of 41.3%. A similar drop in the density of the barley fraction of a mixture resulted in r_{tm} falling from 0.75 to 0.36 - a decline of 52.3%. Obviously most of the decline in the rate of disease increase in the mixture is due to the reduction in density of the susceptible individuals that occurs, while only a relative minor advantage (approximately 10% reduction) derives from the presence of immune plants.

These results conflict somewhat with those obtained when rates of disease increase in mixtures due to between-plant transmissions of inoculum (obtained from Figure 35) are plotted against the net density of susceptible plants present in the mixture (Figure 39), and then compared with r_b values obtained in pure host plant stands (see Figure 30a). It is clear from a comparison of these two Figures (Figures 30a and 39), that r_b values are very similar in mixtures and pure stands with a similar net density of susceptible plants although, on the average, rates due to between-plant transmissions are marginally less in mixtures. This is particularly apparent in mixtures with the lowest proportion of barley plants (and thus the lowest net density of susceptible plants). That a larger difference in r_b values between mixtures and their equivalent monocultures was to be expected, is suggested by not only the difference in overall disease rates between mixtures and their equivalent monocultures but also from experiments involving the effect of rows of wheat plants on the dispersal gradient of *E. graminis* f.sp. *hordei*. The reason for this lack of difference in r_b values between mixtures and pure stands of similar susceptible plant density is not fully understood, although it is possible that in mixtures with a high proportion of resistant plants, these plants reduce the number of conidia in the air and thus reduce the number of multiple hits that occur. This would maximize r_b values in mixtures. In pure stands of low overall density, on the other hand, no interference occurs, and thus the number of multiple hits occurring is likely to be greater. This would reduce r_b values in pure stands. The interaction of these two factors

FIGURE 39.

- a) The results shown in Figure 35 redrawn to show the relationship between the rate of increase of disease due to between-plant transmissions of inoculum in mixtures (r_{bm}) and the density of susceptible plants in the mixture. Linear regression lines fitted to the results for random mixtures (● and ▲) are statistically significant.
- b) The same results redrawn to show the relationship between the rate of increase of disease due to between-plant transmissions of inoculum in mixtures (r_{bm}) and the mean distance (L) between adjacent susceptible plants in the mixture.
- , ▲ , results from random mixtures of barley and wheat, two different phytotron cabinets; ○ ,
△ , results from mixtures patterned in rows, two different phytotron cabinets.



could minimize the difference between r_b values in mixtures and pure stands. Additionally, the monocultural 'control' experiments were not carried out at the same time as the mixture experiments and thus slight variations in environmental conditions in the growth chambers may have produced some of the observed discrepancies.

In Figure 39b, r_b values obtained in mixtures are plotted against the mean distance between adjacent susceptible individuals so that comparisons may be made between these results and those obtained for disease gradients (see below).

b) *The effect of patterning in mixtures*

In the experiments conducted here there was no consistent difference in disease rates between mixtures in which the barley fraction was planted in rows and mixtures in which they were randomly placed. In the mixtures patterned in rows, lines of barley plants were placed at right angles to the direction of wind flow thus resulting in a minimum rate of disease increase for such a pattern. In natural conditions, where winds tend to vary considerably in direction, it is possible that rates in such mixtures might be greater than in random situations due to the greater opportunity for successful transmission of the pathogen when the wind is blowing along rather than across rows.

c) *Disease dispersal gradients*

Downwind dispersal gradients in the LBH cabinets (measured in terms of the number of pustules successfully formed) showed the

logarithmic decline with distance from inoculum source characteristic of most air-borne pathogens. The value of b (-0.602), the coefficient of the linear regression of log number of effective conidia against log distance from the source, was somewhat less than is commonly recorded for point sources (Gregory, 1968), but according to Meredith (1973) this is to be expected, as with line, strip and area sources, infection falls off less rapidly with increasing distance. Moreover, in these experiments, the unidirectional nature of the wind flow would tend to strengthen this tendency towards a shallower disease gradient.

The two species used in these experiments differed to some extent in their early growth stages, with wheat leaves being considerably thinner than those of barley. Despite this, examination of the trapping capacity of single rows of wheat and barley showed no significant difference in the steepness of the disease gradient ($b = -1.017$ and -0.987 respectively). An explanation for this apparent inconsistency is to be found in the relative surface areas of barley (136.2cm^2) and wheat (97.3cm^2) plants and the efficiency of conidial impaction. In general, impaction efficiencies are influenced by three main factors - wind velocity, spore diameter and target size - with efficiencies increasing as wind velocities and spore diameters increase and target sizes decrease (Gregory, 1961; Chamberlain, 1967). In the present situation wind speed and spore diameter are held constant while target size, particularly width, varied between barley and wheat. As the relative number of conidia trapped was the same for barley and wheat it is readily apparent that the trapping efficiency

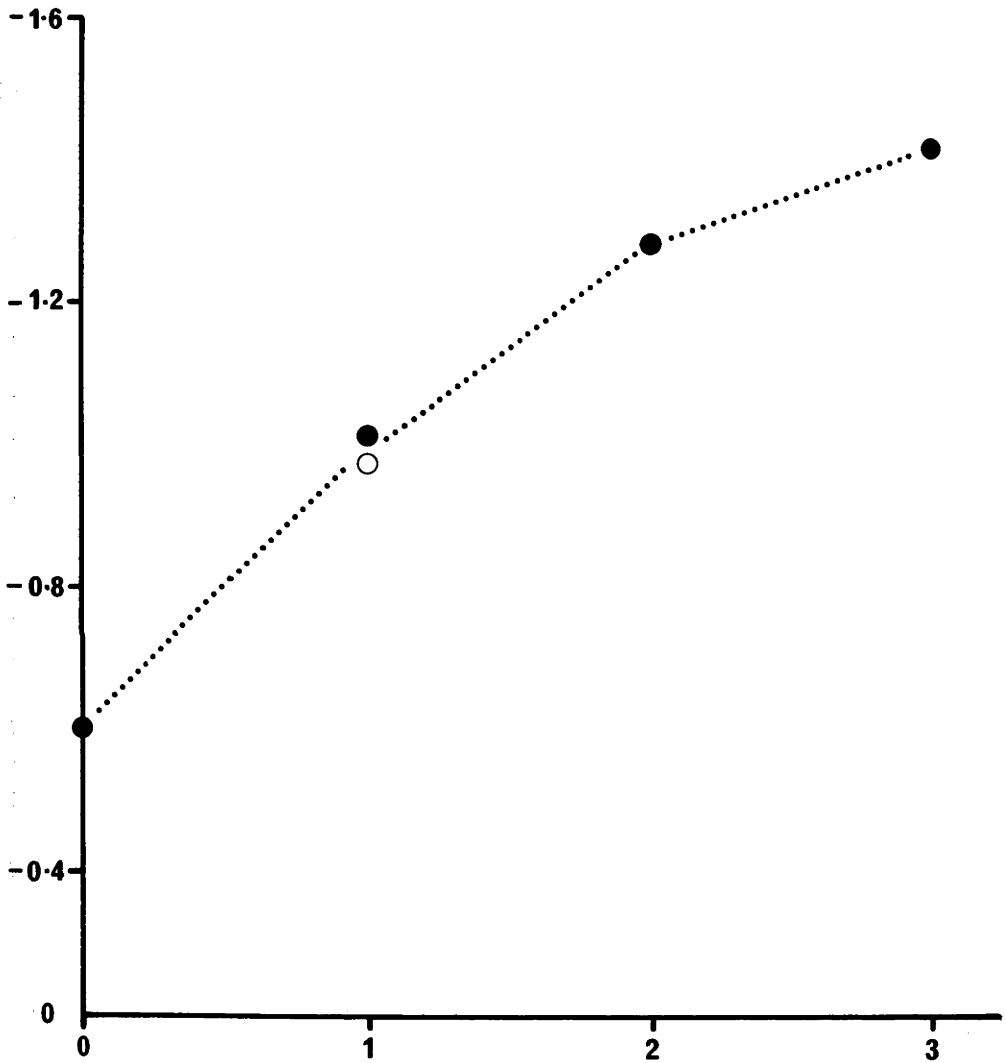
per unit area was higher for wheat than barley, but this was offset by the larger total area of the barley plants. Thus although the host and non-host plants in these experimental mixtures were morphologically dissimilar they behaved essentially the same.

The position of protective screens, whether close to the infectious source or close to the infectable 'target', is apparently unimportant, screens in both these positions having the same effect on the steepness of the disease gradients ($b = -1.017$ and -0.975 respectively). The effect of increasing the number of barrier rows in a screen did, however, have a considerable effect on the number of conidia capable of penetrating the screen (and thus on the disease gradient). In Figure 40, the regression coefficient of the disease gradients shown in Figure 37b are plotted against the number of intervening barrier rows. A curvi-linear relationship between these two factors was found. In experiments in which no barrier rows were present the ratio of the number of successful infections at the sampling point closest to the source to that furthest from the source was 2.3. When one barrier row was interposed between the closest and furthest sampling points (see Figure 36b) the ratio increased to 4.3. The deployment of additional barrier rows in the same region (that is, between the closest and furthest sampling points; Figure 36e and 36f) produced progressively less effect on the resulting number of infections at the furthest sampling point. Part of this decline in the effectiveness of barrier rows as screens is due to the sequential absorption of conidia as they pass through successive bands of

FIGURE 40.

The effect of increasing the number of barrier rows of wheat on the steepness (regression coefficient) of the disease dispersal gradient.

Regression coefficient of disease gradient



Number of barrier rows

vegetation (Dimond and Horsfall, 1960). Thus some of the vegetation in the second and subsequent rows will 'screen' air which has already been 'screened' by the first row. A second barrier row will therefore, trap a smaller proportion of the spores passing it than the first, while a third row will trap a smaller proportion than the second.

In Figures 30b and 39b, where the rate of increase of disease due to between-plant transmissions of inoculum is plotted against the mean distance between susceptible plants, curves similar to those of Figure 37a are apparent. This is not unexpected as, at any given point, the rate of increase of disease due to between-plant transmissions is determined by the sum of all incoming inoculum; conidia arriving from nearby active pustules making a greater contribution than those from more distant ones. More pustules will be close by in high density stands than in low density ones, resulting in the observed decline.

Results obtained for overall rates of increase of disease and rates of increase due to between-plant transmissions of inoculum tend to suggest that, over the range of densities examined, interference with inoculum transmission by immune wheat plants was not very significant. The results discussed above for experiments specifically designed to examine disease gradients suggest, on the other hand, that the presence of immune plants may significantly reduce the amount of inoculum passing between host plants. This contradiction may be partly explained by differences in the experimental design of the two types of experiment. In experiments

measuring disease gradients directly, the number of plants present was considerably less than the number present in experiments designed to measure disease rates (approximately 25 versus 85) and this would inevitably lead to a greater wind velocity (and possibly greater turbulence) through such stands and thus a flattening of the dispersal and hence the disease gradient. Moreover, this flattening would be most pronounced in the experiment where no barrier rows were present. As more and more were added, dispersal gradients would probably steepen as a result of reduced wind velocity alone. Two other differences between these experiments which may be of considerable significance are, firstly, the possibility for lateral transmission of conidia between adjacent plants in disease rate experiments (this would also flatten disease gradients, particularly in arrays patterned in rows), and secondly, the difference in time during which 'trap' plants were exposed to inoculum. In disease gradient experiments, this was only six hours and nearly all viable conidia impinging on host plants would form visible pustules. In experiments measuring disease rates however, trap plants were changed or monitored far more infrequently and thus there was a greater chance of inoculum wastage due to multiple infection events. This would also flatten disease gradients.

SECTION D

**THE EFFECT OF DISEASE ON COMPETING MIXTURES OF
HOST AND NON-HOST SPECIES**

THE EFFECT OF DISEASE ON COMPETING MIXTURES OF HOST
AND NON-HOST SPECIES

I. INTRODUCTION

In the two preceding experimental sections (Sections B and C) the effect of host plant density, mixtures and planting pattern on epidemics of a soil and an air-borne plant pathogen have been examined. In both the *Pythium irregulare* - *Lepidium sativum* and the *Erysiphe graminis* f.sp. *hordei* - *Hordeum vulgare* systems, although plants were grown together in the same experimental array, there was no effective competition between individuals of the same or different species. In the *Pythium* system this was because experiments were concluded before the plants had made much growth while in the *Erysiphe* system 'plants' were grown in separate well-spaced pots with an excess of water and nutrients. This simplified

the processes of elucidating the general principles involved in changes in host plant density without the added complication of competitive interactions between neighbouring individuals (particularly in mixtures). However, it is realized that such competitive interactions are an important facet of any natural ecosystem.

The present chapter describes an investigation of the effect of disease on the competitive interaction between a pair of species in a mixture, using the same pathogen, *Erysiphe graminis* f.sp. *hordei*, and the same pair of plant species, *Hordeum vulgare* (host) and *Triticum aestivum* (non-host), as in the previous section (Section C). The results of preliminary trials suggested that this combination of species would be interesting, as the host species was found to be the more aggressive species in disease free competition.

II. METHODS

1. *Experimental procedures*

A series of experiments were carried out to investigate the effects of inoculating *E. graminis* f.sp. *hordei* on to *Hordeum vulgare* and *Triticum aestivum* grown separately and together to determine the effect of the pathogen on the competitive interaction of the plants. The two plant species were planted according to a

replacement series (de Wit, 1960) in the following proportions of barley and wheat respectively: 0.75:0.25, 0.50:0.50, 0.25:0.75. In addition monocultures of both species were also grown.

a) *Setting up and planting of pots*

Pure and mixed populations of barley and wheat plants were grown in 15cm diameter plastic pots filled with soil. The soil used was prepared by heat sterilizing a heavy loam at 180°C for 12 hours (to release nutrients and kill any extraneous seeds and fungal pathogens) and then mixing this with medium coarse river sand and peat moss in a ratio of 4:2:1 respectively. This resulted in a fairly light well draining experimental soil. In all but one experiment no additional nutrients were added. In the single case in which plants were allowed to flower and set seed, a small amount of the fertilizer 'Multigrow' (a nitrogen-phosphorus-potassium based mixture) was added at the time of soil preparation.

In all experiments, seed of the two species was sown in the required proportions (allowance being made for the poorer germination rate of the wheat) to a uniform depth of 1.5cms. In general, only one overall density was used within each experiment although this was varied between experiments. Between four and nine replicates were used in different experiments, depending on the number of harvests. All experiments were carried out in glasshouses, with the pots placed in copper trays lined with plastic sheeting to permit bottom watering. Since all seed was sown randomly, it was impractical to cope with edge effects on a per pot basis by leaving

outer plants unharvested. To minimize edge effects on individual pots these were, however, all packed closely together, while edge effects on the array as a whole were minimized by re-randomizing the positions of individual pots approximately once every ten days. About five days after planting, seedlings had grown sufficiently for a check of the germination rate to be made. In all cases this was satisfactory. At this stage all pots were fitted with wire supports constructed of 5cm square wire mesh to prevent plants from falling over.

All pots were grown together in the same glasshouse until after the first harvest, when half the remaining pots of each treatment were transferred to a second glasshouse for inoculation and subsequent disease development. In this way, disease was prevented from spreading to the control (non-disease) portion of the experiment. During the course of a single experiment, conditions in the two glasshouses (with and without disease present) were maintained as far as possible the same, by placing pots in similar positions within similarly oriented glasshouses. The minimum temperature in the two glasshouses was also standardized. Conditions inevitably changed between experiments which were carried out at different times and there was, in fact, a slight suggestion that wheat was better able to compete successfully with barley during experiments carried out in summer than those carried out in winter. Part of a typical experimental array is shown in Plate 5.

b) *Inoculation of pots*

In experiments where more than one harvest was to be taken,

PLATE 5.

General view of a typical competition experiment
between barley and wheat plants showing the close-
packed placement of individual pots.



inoculation with conidia of *E. graminis* f.sp. *hordei* was carried out immediately after the first harvest. Pots were infected by shaking small clumps of heavily diseased barley plants about 0.5m above the experimental array and allowing the conidia to settle on to the foliage. It was found that this provided a fairly uniform infection of all host plants regardless of their frequency in any pot. In most experiments, disease, once established, was allowed to progress naturally thereafter. In one experiment, however, plants were deliberately inoculated with a heavy dusting of conidia. The large amount of disease which developed from this inoculation was then controlled by twice weekly sprayings of Karathane.

c) *Harvesting*

At each sampling time, plants were harvested by being cut off at ground level, dried in a forced-draught drier at 70°C for ten days and then weighed. In mixed pots the two species were separated out prior to drying.

2. *Theoretical considerations*

To measure competition between two plant species, the performance or yield of the two species grown together in mixtures is compared with the performance of the same species grown in monoculture. For an accurate analysis of competitive interactions, however, yield differences are insufficient, as different plant species may differ in the efficiency with which they utilize the limited growing factors.

The yield of each species in a mixture is therefore weighted by dividing by the efficiency factors (yield per unit area) found for each species in monoculture (Ennik, 1970; Sandfaer, 1971) so that comparisons of the performance of species are based on relative yields (de Wit, 1960).

a) *Relative yield*

The relative yields of species *a* grown in mixture with species *b* (r_a) and of species *b* grown in mixture with species *a* (r_b), are defined by:

$$r_a = \frac{O_{ab}}{M_a} \quad \text{and} \quad r_b = \frac{O_{ba}}{M_b}$$

where M_a and M_b are the yields per unit area of species *a* and species *b* grown in monoculture; O_{ab} and O_{ba} are the yields of species *a* and *b* respectively, harvested per unit area of the mixture of the two species.

b) *Relative yield total*

The 'relative yield total' of a mixture of species *a* and *b*, RYT_{ab} , is the sum of the relative yields of the component species ($\text{RYT}_{ab} = r_a + r_b$). The relative yield total approach developed by de Wit and his associates (de Wit, 1960; de Wit and Ennik, 1958; de Wit and van den Bergh, 1965; de Wit, Tow and Ennik, 1966) provides three different models of competition. The simplest of these is characterized by RYT values of unity. When RYT equals unity, the

two species present in the mixture either occupy the same ecological niche (under the conditions of the experiment) and thus compete for precisely the same growing factors or requisites simultaneously, or the two species are competing for a limiting resource or resources. In circumstances when RYT is greater than unity, one or both species are less affected by interspecific competition than would be expected from the effect of crowding for the same resources alone. RYT values significantly greater than unity are not common in the literature, although high values may be recorded in legume-grass interactions (for example, Ennik (1970) recorded a value of 6.72 for a white clover/perennial ryegrass interaction). Finally when RYT is less than unity, one or both species are more affected by interspecific competition than would be expected from the effect solely of competing for the same growth resources. RYT values less than unity thus imply a direct antagonism between the two species in the mixture.

c) *Relative replacement rate*

A measure of the relative competitiveness of the two species of a mixture is provided by the 'relative replacement rate' which may be calculated by determining the double quotient of the relative yields of species *a* and species *b* at some harvest and a reference harvest:

$${}^{nm}P_{ab} = \frac{{}^n r_a / {}^m r_a}{{}^n r_b / {}^m r_b} = {}^n r_a / {}^n r_b \times {}^m r_b / {}^m r_a$$

${}^{nm}P_{ab}$ is then called the relative replacement rate of species *b*

by species a at the n^{th} harvest with respect to the m^{th} harvest (de Wit and van den Bergh, 1965; van den Bergh and Ennik, 1973).

In a competitive situation, the relative success of one species with respect to the other may thus be monitored by plotting the relative replacement rate against time to obtain 'course lines' (van den Bergh and Ennik, 1973). The angle the course line makes with the horizontal is a measure of the rate at which species a replaces species b . An upward sloping line would indicate that species a is replacing species b ; a horizontal line indicates that the two species are competing equally; and a downward sloping line indicates that species a is being replaced by species b . The relative replacement rate is only one measure of the competitiveness of species in a mixture. Examples of other measures are 'relative crowding coefficients' (de Wit, 1960; van den Bergh, 1968; van den Bergh and Ennik, 1973) or 'aggressivity indices' (McGilchrist and Trenbath, 1971) which also provide very similar measures (Trenbath; pers. comm.) of the degree of success of competitive interactions between two plant species. The main difference between these values lies in whether the competitive ability of one species with respect to another is expressed in terms of the amount of 'space' (*sensu* de Wit, 1960) occupied (relative crowding coefficient) or the rate at which one species replaces the other (relative replacement rate). In the experiments detailed here the competitiveness of species in mixtures is expressed in terms of their relative replacement rate.

III. RESULTS

a) *The effect of host plant density on the incidence and impact of disease*

The results of a single experiment investigating the effect of the presence or absence of powdery mildew on the interaction between barley and wheat in mixtures, examined over a range of three different planting densities (5,600, 11,200 and 16,800 plants per m²) are shown as replacement diagrams in Figure 41. The replacement diagrams plot the relative proportion of the two species in the mixture at seeding against the yield, in grams of dry matter per pot, obtained 35 days after planting.

A comparison between yields of healthy and diseased barley shows that the presence of disease caused a significant reduction in yield in nearly all cases. The effect of powdery mildew on barley yields becomes increasingly apparent as plant density increases, which implies that increases in host plant density have resulted in a higher level of disease and thus disease impact on the plants. This is clearly illustrated in Figure 42, where the total dry matter per pot produced in monocultures is plotted against the three overall densities used. These results suggest that, for the particular experimental conditions used, a simple linear relationship exists between the total dry weight per pot and the density of wheat monocultures, regardless of whether this species had grown in the presence or absence of disease. (The

FIGURE 41.

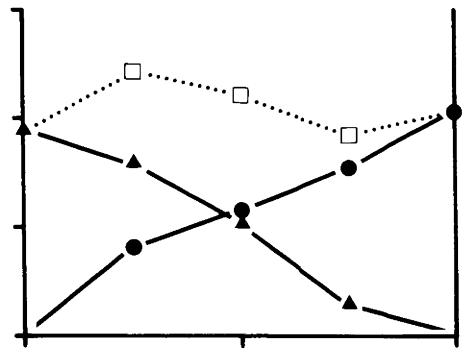
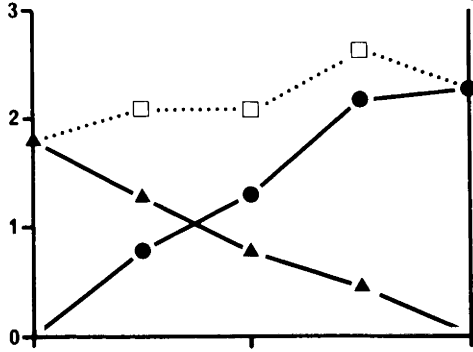
Replacement series diagrams for a competition experiment between barley and wheat showing the effect of the presence or absence of powdery mildew of barley on the outcome of the competitive interactions between the two plant species. Experiments were conducted at three different overall planting densities: 5,600, 11,200, and 16,800 plants per m².

- ▲ , yield of wheat (grams per pot);
- , yield of barley (grams per pot);
- , total yield (grams per pot).

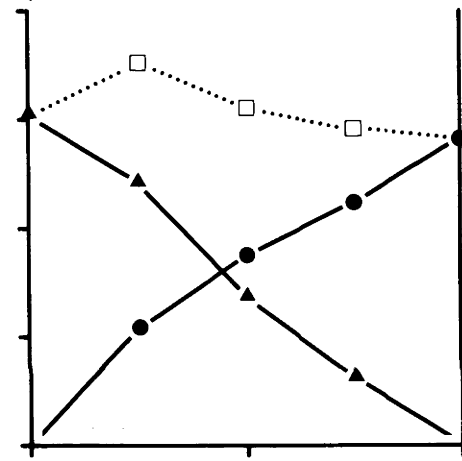
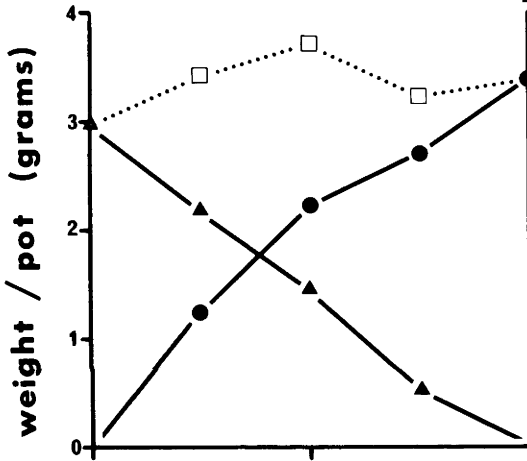
HEALTHY

DISEASED

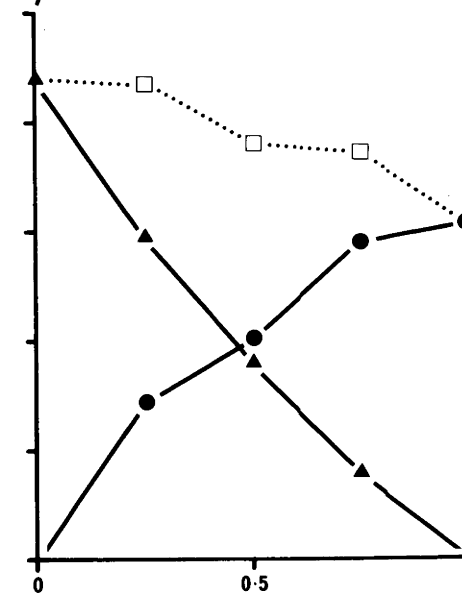
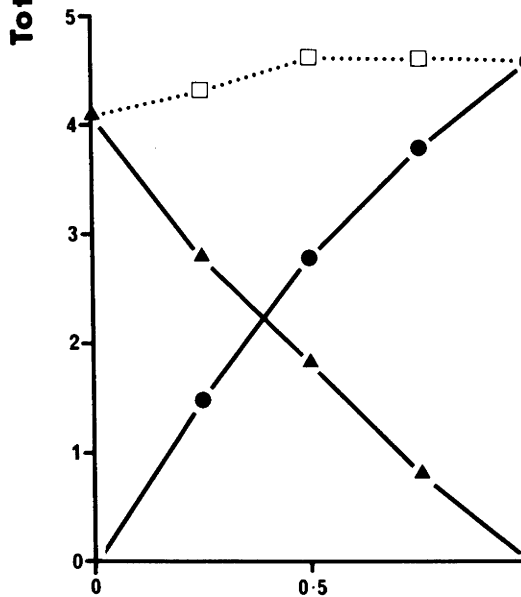
5600 plants / m²



11200 plants / m²



16800 plants / m²

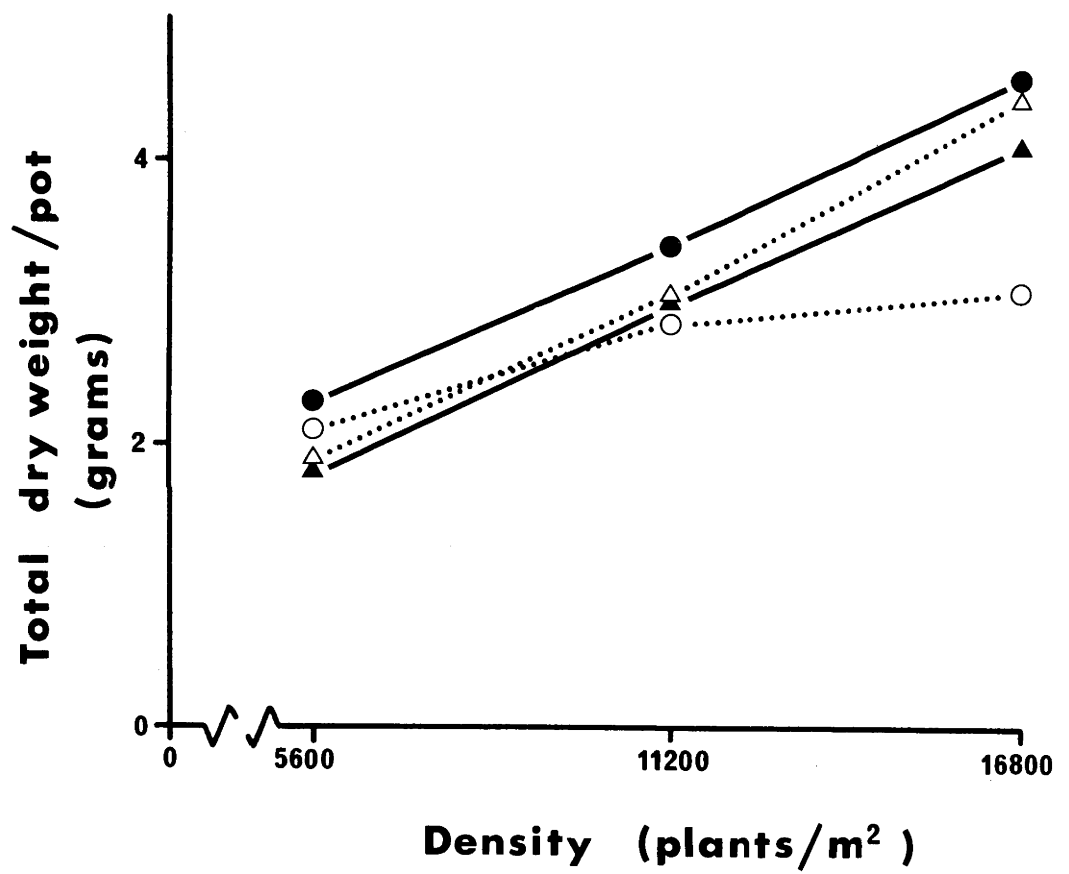


Proportion of barley in the mixture

FIGURE 42.

The effect of varying plant density on the total dry matter production of monocultures of barley and wheat in the presence and absence of powdery mildew of barley.

○ , barley inoculated with *Erysiphe graminis* f.sp. *hordei*; △ , wheat inoculated with *Erysiphe graminis* f.sp. *hordei*; ● , uninoculated barley; ▲ , uninoculated wheat.



minor difference in relative position of the two slopes is probably due to small differences in the environmental conditions within the two glasshouses). By contrast, while pure stands of barley grown in the absence of disease showed a similar relationship between the density of the stand and total dry weight per pot, pure stands grown in environments in which disease was present showed increasing divergence from this linear relationship as stand density increased.

b) *The effect of disease on the relative competitive abilities of host and non-host plants during the early vegetative growth phase*

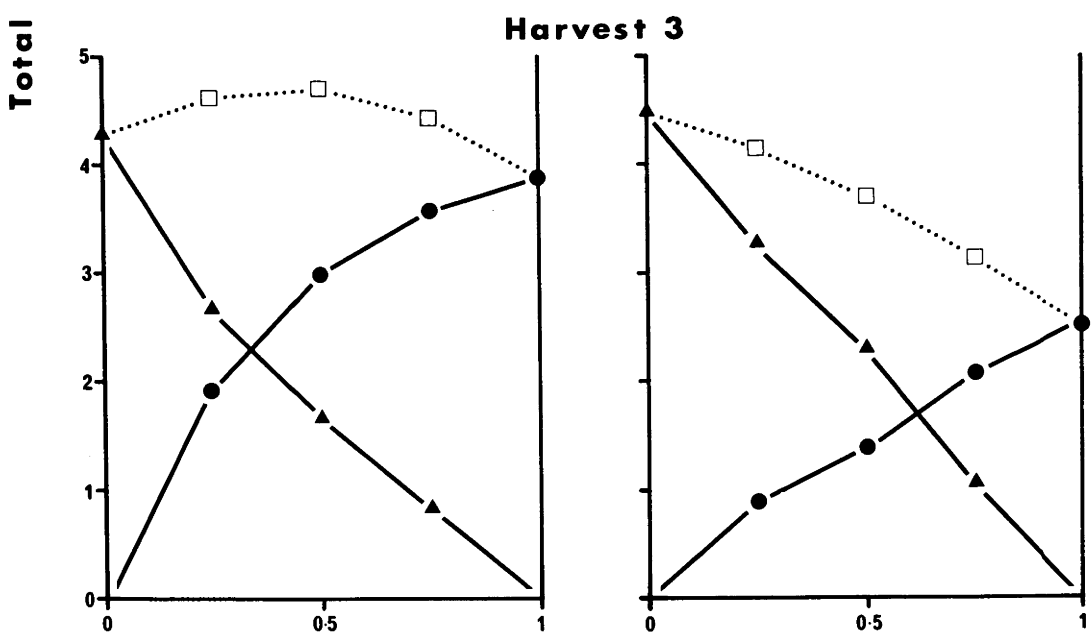
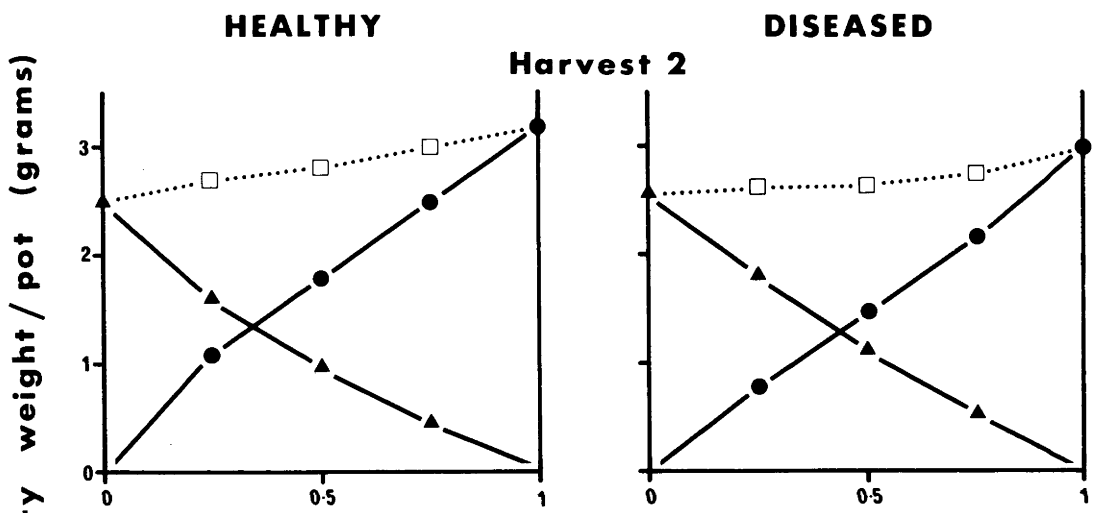
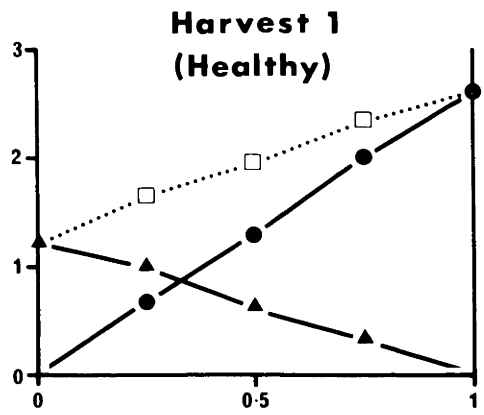
(i) *Light application of inoculum* - Figure 43 shows the results of a barley/wheat competition experiment (overall density: 16,800 plants per m²) in which half the experimental array was inoculated with a light dusting of conidia of *E. graminis* f.sp. *hordei* (immediately after the first harvest) and the resultant disease was subsequently allowed to develop unhindered. At every harvest, four replicates of each treatment were sampled and their gross yields averaged to give the points shown in Figure 43a. The first harvest was taken eight days after seed was sown while the second and third harvests were taken 38 and 59 days later. From the straightness of the lines in the first replacement diagram (Figure 43a) it can be inferred that no competition had occurred between the two species at that stage. A comparison of the yield of the barley in uninoculated and inoculated stands shows that while the disease had only a minor impact on the yield of

FIGURE 43a.

Replacement series diagrams for a competition experiment between barley and wheat showing the effect of powdery mildew of barley on the relative competitive abilities of barley and wheat plants during the early vegetative growth phase. (Half the experiment was inoculated immediately after the first harvest and the resultant disease was allowed to develop unhindered).

Overall plant density of 16,800 plants per m^2 .

- ▲ , yield of wheat (grams per pot);
- , yield of barley (grams per pot);
- , total yield (grams per pot).



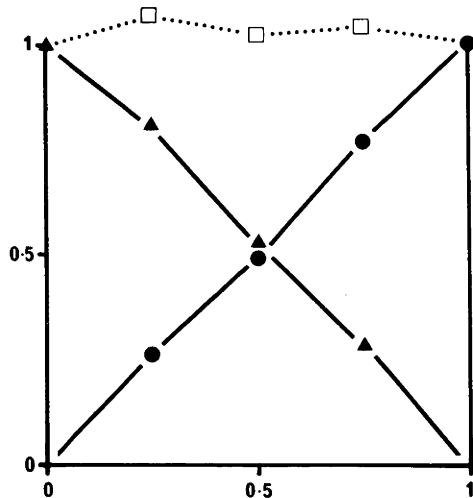
Proportion of barley in the mixture

FIGURE 43b.

Replacement series diagrams for the competition trial shown in Figure 43a with the gross yield values transformed to relative yields, to permit fairer comparisons between the rates of change of yields in the presence and absence of powdery mildew of barley. (Half the experiment was inoculated immediately after the first harvest and the resultant disease was allowed to develop unhindered).

- ▲ , relative yield of wheat;
- , relative yield of barley;
- , relative yield total.

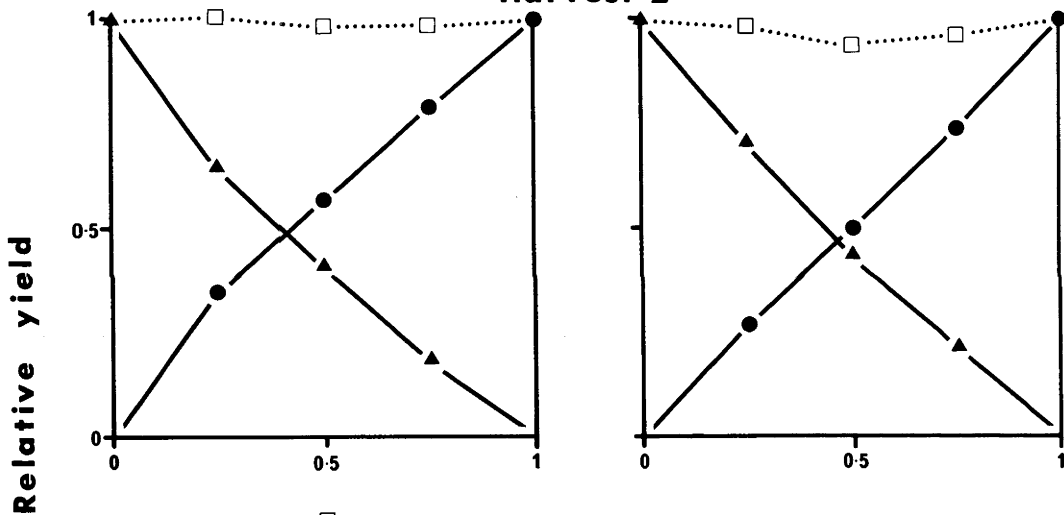
**Harvest 1
(Healthy)**



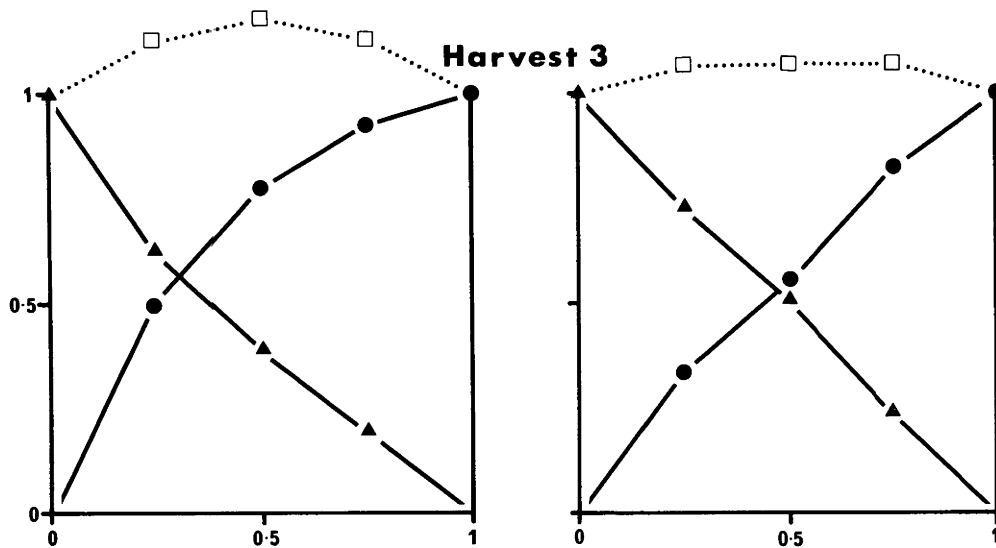
HEALTHY

DISEASED

Harvest 2



Harvest 3



Proportion of barley in the mixture

barley at the second harvest, by the third harvest the disease impact on yield was quite substantial.

In Figure 43b these gross yield values are transformed to relative yields to permit fairer comparisons between the rates of change of yields in disease and non-disease situations. Relative yield total (RYT) values obtained from summing the relative yields of barley and wheat in the various mixtures are all close to unity and thus indicative of two species competing for exactly the same resources (RYT values calculated in this way are very seldom exactly equal to unity, due to experimental errors: van den Bergh and Ennik, 1973). Although the monocultural yields of healthy barley and wheat are reasonably well matched (Figure 43a), the curvature of the lines in the healthy replacement series diagrams of Figure 43b (particularly the third harvest) indicate a better performance by barley than wheat in mixtures of the two species. In the presence of disease, on the other hand, the convexity of the barley replacement line has been markedly reduced while the concavity of the wheat replacement line has been all but lost. This is apparent at both the second and third harvests.

By calculating values of P , the relative replacement rate, it is possible to place a numerical value on these competitive differences between the two interacting species. In Figure 44, the relative replacement rate (${}^{n1}P_{bw}$) is plotted against time, to obtain a 'course line' for the barley (b), with respect to the wheat (w), using the relative proportions of the two species at the first harvest as the comparative base. From these course lines,

FIGURE 44.

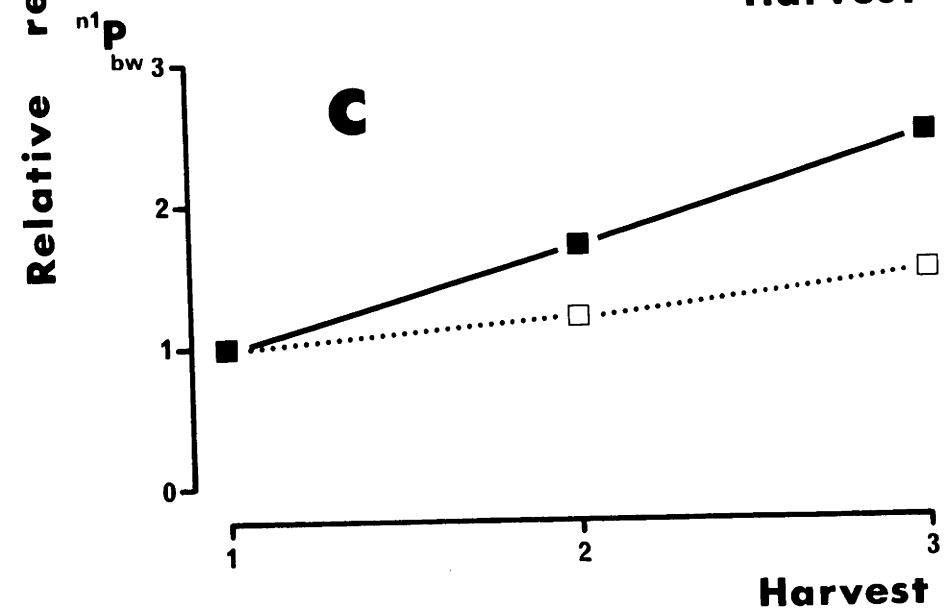
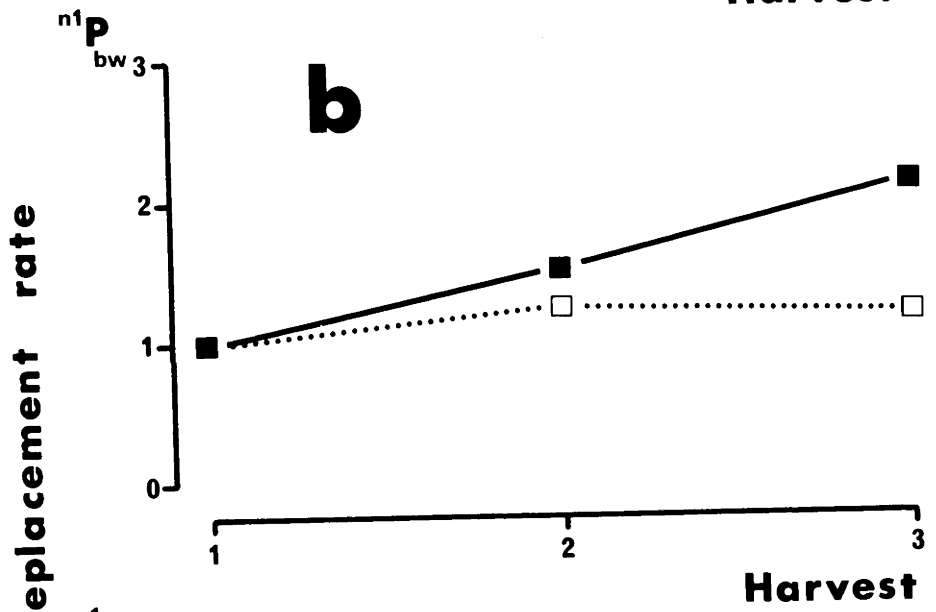
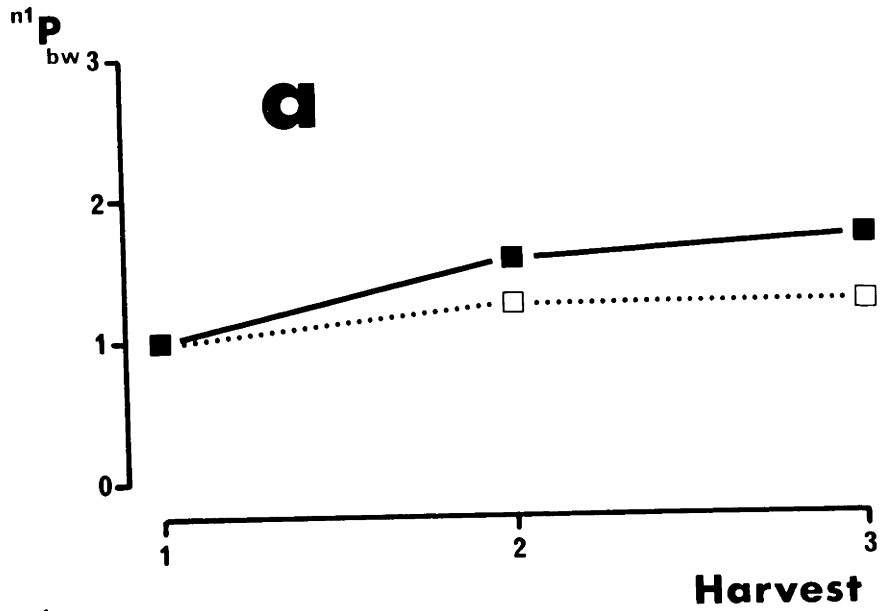
'Course' lines for the relative rate of replacement of wheat by barley in the presence and absence of powdery mildew of barley, using the relative proportions of the two species at the first harvest as a comparative base. (Three harvests; overall plant density of 16,800 plants per m²).

a) 0.75:0.25 mixtures of barley and wheat.

b) 0.50:0.50 mixtures of barley and wheat.

c) 0.25:0.75 mixtures of barley and wheat.

■ , healthy mixtures; □ , diseased mixtures.



which provide a measure of the rate at which barley replaces wheat, it is apparent that in the absence of disease the barley component of all mixtures gains at all harvests. However, in the presence of *Erysiphe graminis* f.sp. *hordei* the competitive ability of barley is reduced to a level more closely comparable to that of wheat.

The results of a second competition experiment between barley and wheat, conducted at the same overall density of 16,800 plants per m², is shown in Figure 45. In this experiment five successive harvests were taken and the results expressed in terms of the relative replacement rate of barley with respect to wheat. The results obtained, although somewhat more variable than those illustrated in Figure 44, again show substantial reductions in the rate of replacement of wheat by barley when barley plants are infected with disease.

(ii) *Heavy application of inoculum followed by disease irradiation* - Figure 46 shows the results of an experiment conducted at an overall density of 11,200 plants per m² (each data point is a mean of four replicates). The first harvest was taken 8 days after sowing, and immediately after this half the remaining stand was heavily inoculated with conidia of *E. graminis* f.sp. *hordei*. A week later the inoculated barley plants had developed a heavy mildew infection with numerous pustules present on all leaves. On the tenth day after inoculation, this disease was irradiated by several applications of the fungicide Karathane. The second and third harvests were taken after a further 10 and 31 days respectively.

A comparison of the uninoculated and inoculated stand replacement

FIGURE 45.

'Course' lines for the relative rate of replacement of wheat by barley in the presence and absence of powdery mildew of barley, using the relative proportions of the two species at the first harvest as a comparative base. (Five harvests; overall plant density of 16,800 plants per m²).

- a) 0.75:0.25 mixtures of barley and wheat.
- b) 0.50:0.50 mixtures of barley and wheat.
- c) 0.25:0.75 mixtures of barley and wheat.

■ , healthy mixtures; □ , diseased mixtures.

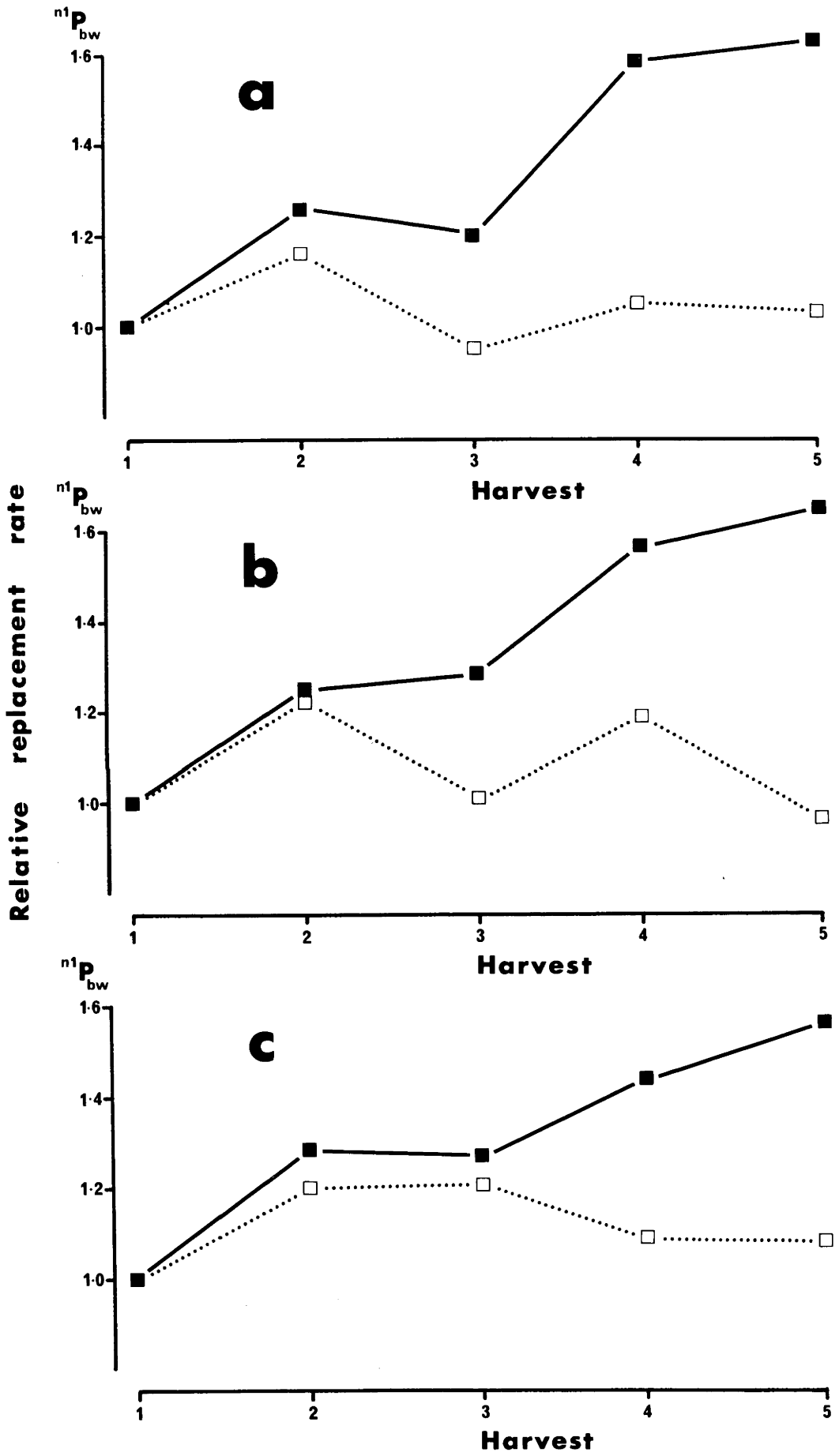
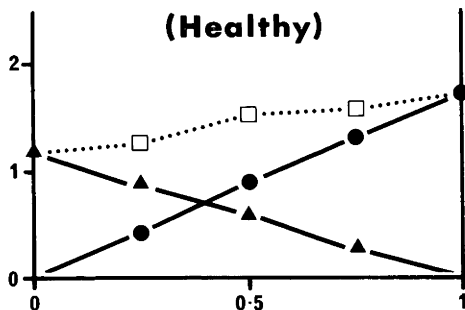


FIGURE 46.

Replacement series diagrams for a competition experiment between barley and wheat showing the effect of a heavy application of inoculum of *Erysiphe graminis* f.sp. *hordei* followed by early disease irradiation. Half the experiment was inoculated immediately after the first harvest; disease was irradiated ten days later. (Overall plant density of 11,200 plants per m²).

- ▲ , yield of wheat (grams per pot);
- , yield of barley (grams per pot);
- , total yield (grams per pot).

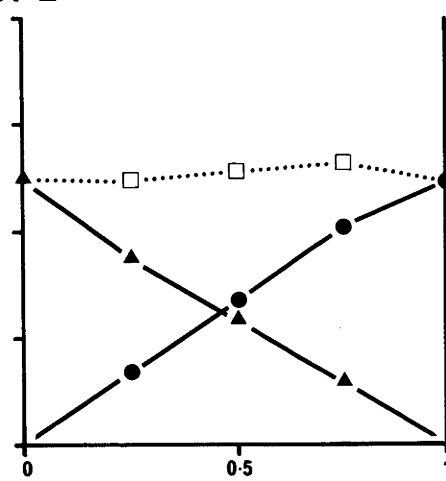
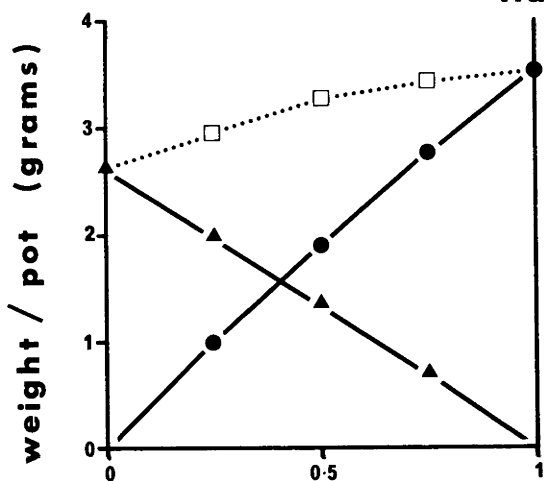
**Harvest 1
(Healthy)**



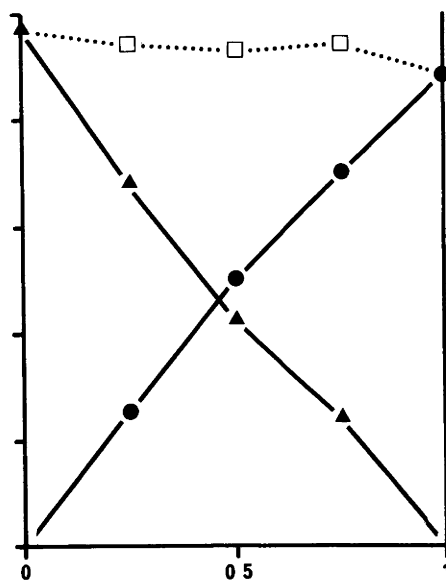
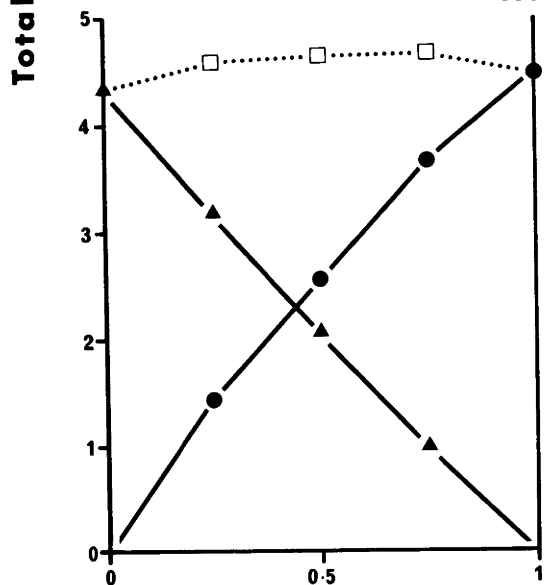
HEALTHY

DISEASED

Harvest 2



Harvest 3



Proportion of barley in the mixture

diagrams at the second harvest clearly illustrates the substantial drop in dry matter production per pot that occurred as a result of disease infection. Dry weight values for the barley monocultures or fractions of mixtures subjected to disease were, on average, 26.7% less than those in healthy stands. Once the barley in the disease situation was protected from further damage however, it was able to recover rapidly and by the time of the third harvest there was no significant difference between the healthy and disease treatments.

c) *The effect of disease on the relative competitive abilities of host and non-host up to the time of grain filling*

The results of a single competition experiment (overall density: 2,200 plants per m²) in which both species were allowed to flower and set seed before harvesting, is shown in Figure 47. Each combination of species, in each treatment, was replicated nine times. Half the experiment was given a light inoculation with conidia of *E. graminis* f.sp. *hordei* eight days after seed germination and disease was then allowed to develop unhindered. It was noted however, that while the proportion of host tissue diseased rose rapidly at first, as air temperatures increased (this experiment was carried out in early summer) and plants matured, the disease slowly declined. By the time of flowering and grain filling no active disease was apparent.

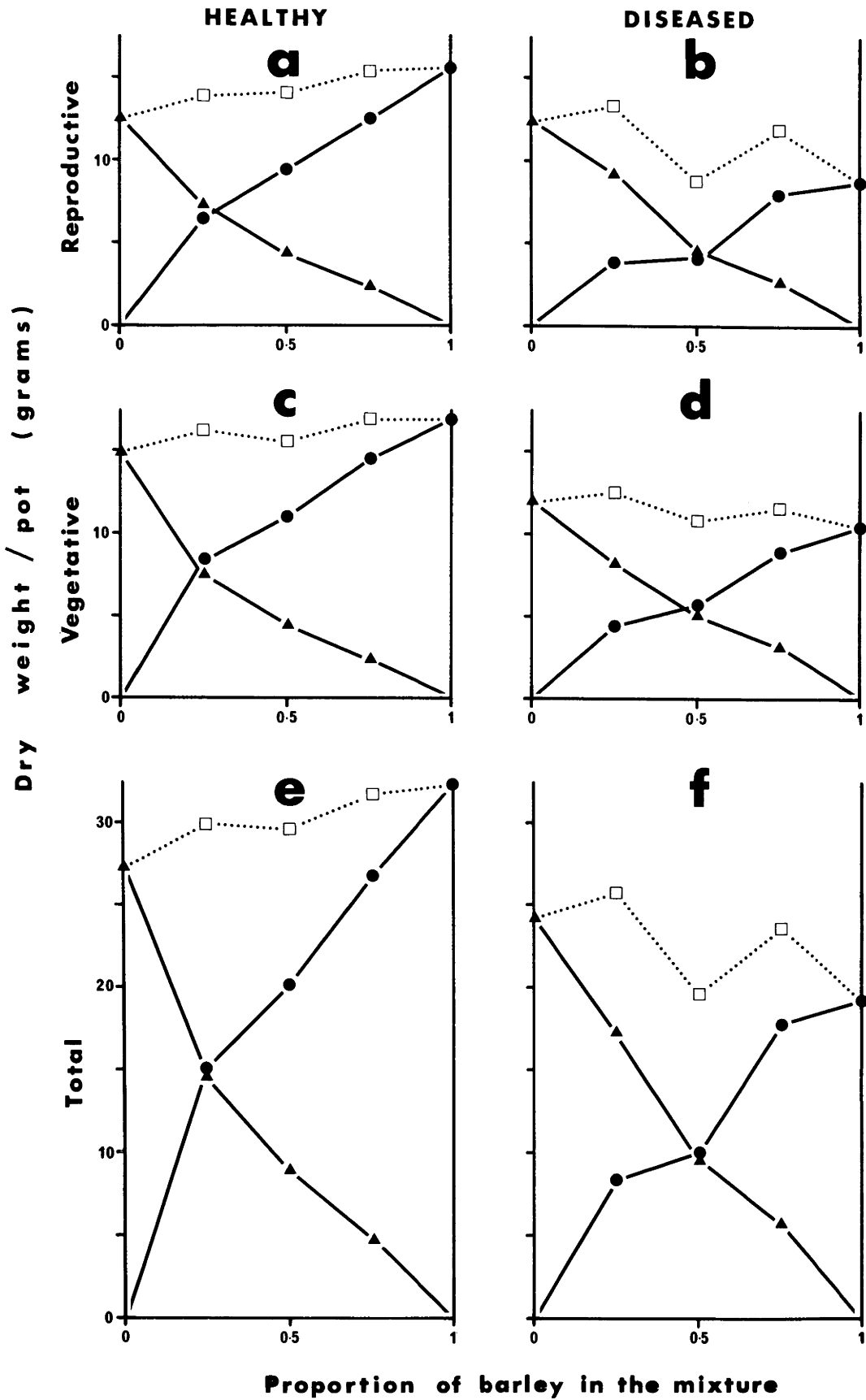
In the replacement diagrams shown in Figure 47 the total yield per pot (47e and f) is sub-divided into its vegetative and

FIGURE 47.

Replacement series diagrams for a competition experiment between barley and wheat showing the effect of the occurrence of powdery mildew of barley on the relative competitive abilities of barley and wheat up to the time of grain filling. (Overall plant density of 2,200 plants per pot).

- a) , b) , dry weights of the reproductive components of barley and wheat plants in the absence and presence of disease.
- c) , d) , dry weights of the vegetative components of barley and wheat plants in the absence and presence of disease.
- e) , f) , dry weights of both reproductive and vegetative components combined in the absence and presence of disease.

- ▲ , yield of wheat (grams per pot);
● , yield of barley (grams per pot);
□ , total yield (grams per pot).



reproductive (all floral structures) components. Comparisons between the yields of the various mixtures was complicated, however, by the obviously poorer growing conditions in the glasshouse housing the infected stand (compare the total yield of wheat in the healthy and disease situations). In Figure 48 this problem was circumvented by transforming gross yield values to relative yields. Once again, RYT values close to unity indicate that these two species are competing (under the given conditions) for the same resources. From the replacement diagrams it is apparent that in a disease free situation the barley retains a competitive advantage over the wheat throughout the life of the mixture. In respect of this, it is interesting to note that a greater proportion of this additional yield appears to be associated with reproductive growth rather than vegetative (the degree of curvature of the barley lines in Figure 48a is greater than that in Figure 48c). The wheat in mixtures did substantially better in the presence than in the absence of disease (compare the nearly straight lines in Figure 48f with the markedly curved one of Figure 48e) while the barley was noticeably worse off.

The tendency of barley mildew to weaken the competitiveness of its host species and thus make the two cereals more evenly matched is further confirmed by the change in the estimated distribution of resources as measured by the relative crowding coefficient k_{bw} . This coefficient expresses in numerical form the degree to which plants of one species occupy more or less than their share of the total 'space' (*sensu* de Wit, 1960). Thus in the present experiment in healthy 50:50 mixtures of barley and wheat plants,

FIGURE 48.

Replacement diagrams for the competition experiment shown in Figure 47 with the gross yield values transformed to relative yields to permit fairer comparisons between the rates of change of yields in the presence and absence of powdery mildew of barley.

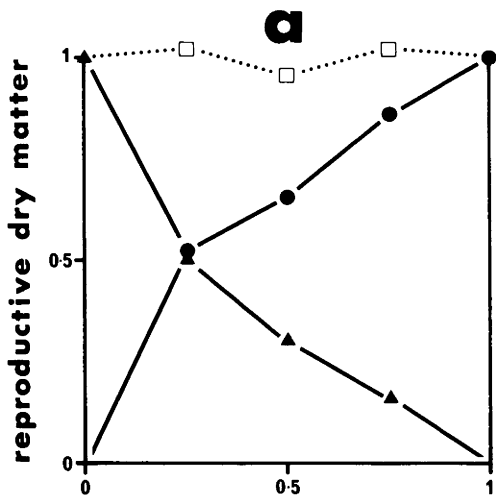
a) , b) , relative yields of the reproductive components of barley and wheat plants in the absence and presence of disease.

c) , d) , relative yields of the vegetative components of barley and wheat plants in the absence and presence of disease.

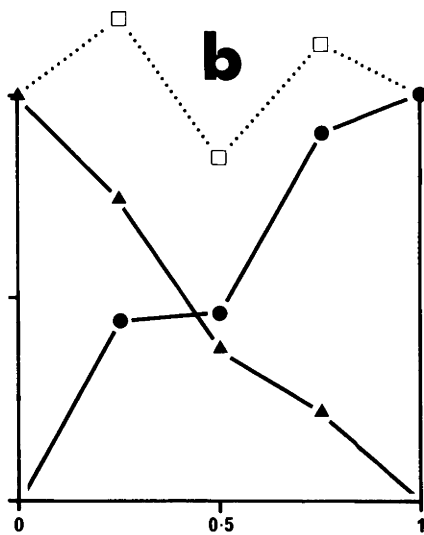
e) , f) , relative yields for both the reproductive and vegetative components combined in the absence and presence of disease.

▲ , relative yield of wheat; ● , relative yield of barley; □ , relative yield total.

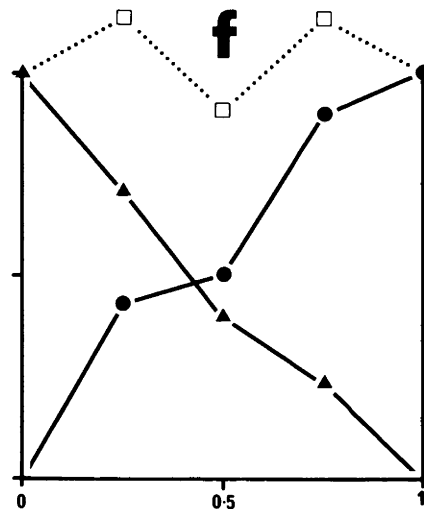
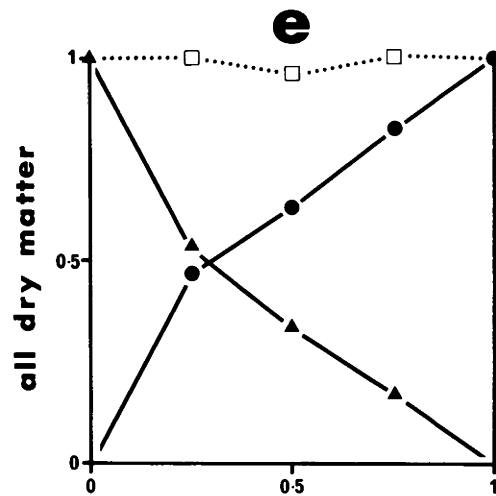
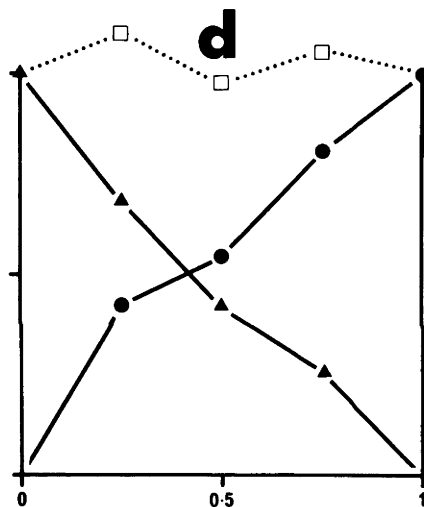
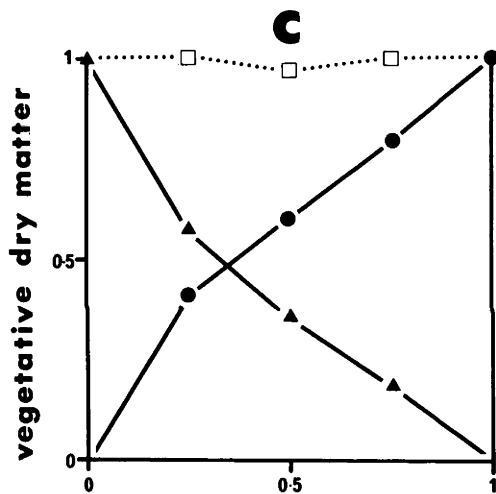
HEALTHY



DISEASED



Relative yield of:



Proportion of barley in the mixture

the barley plants utilized 1.9 times the space that wheat plants used, while in stands subjected to disease, barley plants were restricted to approximately 1.3 times the space used by wheat plants.

Originally, it was hoped to determine the 'relative reproductive rate' of the two species (de Wit, 1970), by comparing the final yield of seed with the original seeding rates. Unfortunately this was not possible as most developing barley seed aborted in the glasshouse conditions (due possibly to excessive watering).

IV. DISCUSSION

In the experiments described here, relative yield total (RYT) values for healthy mixtures of barley and wheat were all close to unity. Such RYT values are indicative of mixtures in which the species present are competing for the same 'space' or growing resources of the environment (de Wit, 1960; van den Bergh and Ennik, 1973) and thus, under the particular experimental conditions provided, may be considered to be occupying the same or very similar ecological niches (Burdon and Pryor, 1975). Obviously in such circumstances if one species has a faster growth rate, or is more competitive than the other species in the mixture, then over a period of time that species will come to dominate and eventually replace the weaker species in all mixtures (regardless of which species is most productive in monoculture).

The degree of curvature of the lines in the replacement diagrams of healthy mixtures provides a measure of the relative competitiveness of the two species in the mixture. Species with replacement series lines which are convex are more aggressive than those species with lines which are straight or concave. Thus in these experiments, barley was more aggressive in all healthy mixtures than the wheat. The greater competitiveness of barley over wheat in healthy mixtures, is reflected in Figures 44 and 45, where the rate at which the barley replaces wheat is plotted against time. The clear advantage of barley over wheat shown by the course lines in these Figures is also supported by the relative crowding coefficient value obtained in the single experiment where the two species in the mixture were allowed to set seed ($k_{bw} = 1.9$). Because of the more aggressive behaviour of barley in these mixtures, it seems likely that a process of, repeatedly sowing, harvesting and resowing the harvested seed, would (under the environmental conditions provided) eventually result in pure stands of the barley. In this respect it is interesting to note that over a thirteen year period Harlan and Martini (1938) found that the composition of an originally 50:50 mixture of the two barley varieties, Deficiens and Hannchen, changed to a ratio of 1:45 respectively.

The relative difference between the competitive abilities of barley and wheat, apparent in the healthy disease-free environments, changed quite markedly as a result of the barley becoming infected with powdery mildew. In situations where disease was present, the competitiveness of barley was reduced and as a result, if disease pressure was maintained for long enough, the distinctly concave

curves of wheat in replacement diagrams are straightened (compare the curvature of the barley and wheat lines in the healthy and disease replacement diagrams of Figure 43). Disease impact on the barley component of mixtures thus caused a change in the relative competitiveness of barley and wheat with the latter doing significantly better than in healthy stands. This is also reflected in changes in the rate at which barley replaced wheat in mixtures (Figures 44 and 45).

The importance of the severity, duration and time of disease occurrence in reducing yield and the competitive ability of the susceptible species in a mixture is well illustrated by the results obtained in these experiments (compare the rapid recovery of the host species freed of disease shortly after infection, Figure 46, with the permanent yield depression suffered by the host component of a continuously diseased system, Figure 43). The importance of the time of disease occurrence is confirmed by de Wit's (1960) consideration of the data of Klages (1936), where leaf rust affecting plants during flowering caused large yield reductions in the susceptible fraction of the crop but produced no compensating yield increase in the resistant fraction.

Although a general relationship between plant density and disease severity was shown in the single experiment conducted at three overall densities, it is apparent from a consideration of all the results obtained in these experiments that an element of artificiality is introduced into these pot trials by the mode of application of disease, the small plot sizes and the intermingling

of mixtures of all proportions. In order to try and overcome this artificiality and at the same time extend this work one step closer to a natural ecosystem, two attempts were made to carry out similar experiments in a field situation. Unfortunately, both of these attempts were thwarted by adverse climatic conditions. In the first case the entire array was successfully grown but had to be abandoned due to the failure of the disease to become established, while in the second case, a prolonged drought shortly after planting reduced mixtures to different density stands of barley only.

SECTION E

GENERAL DISCUSSION

GENERAL DISCUSSION

This discussion is dealt with under four sub-headings. In the first part the main findings of the project are reiterated and related, where possible, to the most similar studies available in the literature. In the second part the significance of the findings for agricultural systems is discussed, partly from the point of view of the possible application of the findings but mainly from the stand point that agricultural systems are in a sense elementary or simplified ecosystems which, in terms of their complexity, stand somewhere between controlled experiments of the type carried out in this project and natural plant communities. The third part deals with the significance of the findings of this project in relation to natural ecosystems especially as they relate to the problems of the creation and maintenance of stability and diversity. The fourth part discusses possibilities for future work.

I. MAIN FINDINGS OF THIS STUDY

1. *Host density per se*

In both the soil-borne and the air-borne systems examined, changing host plant density had significant effects on the epidemiology of the particular fungal pathogen involved.

In the soil-borne system (*Pythium irregulare*-induced damping-off of cress seedlings), the mean rate of advance of the disease front, the apparent infection rate of disease (which largely depends on the rate of advance) and the occurrence of primary infection foci all showed curvi-linear responses to increases in host plant density. Thus at low densities, small increases in host numbers produced large increases in all three disease parameters. As host plant density continued to rise, however, each increase induced a progressively smaller increment in the relevant disease parameter until a limiting value was approached.

In the air-borne system (*Erysiphe graminis* f.sp. *hordei*-induced powdery mildew of barley), on the other hand, the overall rate of increase of disease (r_t) (which seems to be largely a function of the rate of increase of disease due to between-plant transmissions, r_b) approximated to a simple linear relationship with host plant density. Over the range of host densities examined, given increments in host plant numbers produced more or less constant increases in the overall disease rate.

The differences observed in the response of these soil and air-borne diseases to changes in host plant density probably reflect the mode of dispersal of the two ecological groupings of plant pathogens they represent.

Damping-off spreads by means of hyphae radiating out horizontally from diseased plants, at or just below ground level. As the vast bulk of disease transmission is from diseased plants to adjacent healthy individuals, the number of disease sources is relatively unimportant in comparison with the time taken by disease to spread between adjacent host individuals. From a knowledge of the rate of disease advance (and hence the rate of increase) through different density host stands (see Figure 5) and the mean distance between adjacent individuals in such stands, it is possible to estimate the mean time taken for disease to spread between adjacent host individuals. An estimate of this can also be obtained from the direct study of the time taken by damping-off disease to travel different distances (Figure 7). Estimates from both types of experiment are in substantial agreement. In both cases, as the mean distance between adjacent host plants was increased linearly, the time taken to travel between such individuals increased exponentially.

These changes in the growth rate of damping-off are perhaps best explained in terms of the energy supply available to the growing hyphal tips. In very high density stands, where plants are extremely close together, neither the speed of growth nor the capacity of hyphal tips to infect plants appears to be limited by

the energy available, and thus the rate of disease advance (and hence the rate of increase) becomes a constant determined by the speed at which the physical processes of hyphal growth and extension can take place. Although *Pythium* species have been shown to be capable of translocating glucose and other nutrients (Bokhary, 1973) from a food base through an established mycelium to growing hyphal tips, as the distance between adjacent plants increases (that is, plant density decreases), the efficiency of this translocatory process must fall. Additionally, as the distance the fungus grows outwards increases, the number of competing hyphal tips also rises, resulting in a decline in the energy available to each hyphal tip for further growth and infection (that is, the inoculum potential declines; *sensu* Garrett, 1956a; 1956b). Under such conditions the rate of advance of the disease (and hence the rate of increase) is limited by the available energy supply and as the distance between adjacent host individuals continues to increase the fungus takes a disproportionately long period of time to travel between, and infect, adjacent host plants.

A similar correlation between inoculum potential and distance from an infection source has also been shown for *Armillaria mellea* - another translocatory fungus (Garrett, 1970). In a study of the logistics of infection by rhizomorphs of *Armillaria mellea*, Garrett (1956b) found that the speed of infection decreased with increasing distance between inoculum source and host surface and that this could be correlated with a decline, through time, in the growth rate of rhizomorphs due to increasing competition for nutrients from the subordinate apices of rhizomorphs.

The linear relationship found between host plant density and the overall rate of increase of powdery mildew agrees with the theoretical predictions made by Chilvers and Brittain (1972) for an idealized air-borne pathogen system in which both disease pustules and host plants are randomly distributed. These workers argued, that for each particular point in a host plant array receiving inoculum from a range of pustules, the size of each inoculum dose will depend on the distance travelled from the source while the number of doses will depend on the number of existing pustules. If the total number of pustules is increased by random placement of disease plants in the array, then the number of pustules at each distance from the reference point will increase in the same proportion, as will the number of inoculum doses received by the reference point. In general then, the rate at which propagules arrive and cause new infections is directly proportional to the product of the rate at which pustules produce propagules and the number of pustules at each distance from the reference point (which is directly related to plant density). Since the rate at which propagules are produced by pustules is constant at any given time, it appears that the rate of disease increase is directly proportional to plant density - the result obtained here. In contrast to disease increase in the soil-borne pathogen system, the time taken for propagules to be dispersed over quite large distances in the air-borne system is negligible and can therefore be ignored.

Assuming then, that the form of the relationship between the rate of disease increase and host plant density mainly reflects the mode of transmission of the pathogens, it is reasonable to

hypothesize that other soil-borne diseases in which spread and increase is achieved by the growth of hyphae or rhizomorphs through the soil from host to host (for example, *Rhizoctonia* species and the non-sporeing phases of *Phymatotrichum omnivorum* or *Armillaria mellea*; Garrett, 1970), may show similar responses to host plant density as that shown by *Pythium irregulare*-induced damping-off. By way of contrast, most air-borne diseases will probably show similar responses to host plant density as that of powdery mildew.

At the present time this hypothesis cannot be judged against other data because this work appears to be the first of its kind.

2. Pattern in single species stands

In clumped stands of seedlings, the rate of multiplication of damping-off was, in general, slower than in unclumped, evenly spaced stands of the same overall density.

When the number of clumps per unit area was varied, while the proportion of the total area occupied by clumps was kept constant, without changing the overall density of plants, there was no significant change in the rate of advance of the disease between different treatments. The multiplication rate in randomly inoculated plots was higher, however, at 100 clumps per m^2 than at 200 clumps per m^2 due to an unexpected interaction between the multiplication rate and the number of primary disease foci. This was evidently due to changes in the balance between within-clump and between-clump

spread (and hence increase) of disease as a result of the varying numbers of clumps into which the constant number of primary infection foci was partitioned. While recognizing a degree of artificiality inherent in having a random distribution of inoculum in the presence of a clumped distribution of plants, the above result is important as it draws attention to the possibility that under some circumstances the number of primary infection foci or initial level of disease can affect the subsequent apparent infection rate. In epidemiological literature these have previously been considered to be independent variables (van der Plank, 1963; 1968), as indeed they are in the case of randomly or regularly spaced plant arrays.

When the area occupied by clumps was varied, but the number of clumps per unit area was kept constant without changing the overall plant density of the system, both the multiplication rate and the rate of advance of the disease showed a curvi-linear response to increasing clump area, rates in the most condensed clumps (smallest area) being significantly less than those in the least condensed ones (largest area). Altering the area occupied by a fixed number of clumps clearly had a more direct and numerically larger effect on the rate of increase and advance of *Pythium irregulare*-induced damping-off than had alteration of the number of clumps in a fixed proportion of the total area.

Although the data available is insufficient at this stage to seek precise quantitative relationships, a semi-log plot can be used as an effective straightener of either a or r in relation to

the total area occupied (solid graph lines, Figures 49a and b). In a similar way, using data obtained from studies on host density *per se* (mean of values shown in Figures 4 and 5), the epidemic rates α and r may be linearly related to the overall seedling density drawn to a log scale (dotted graph lines, Figures 49a and b). As both these experimental variables are exactly halved for each unit of distance along the horizontal axis, a comparison on each graph between the slopes of the solid and dotted regression lines provides some indication of the relative effectiveness of reducing these factors in order to control epidemic rates. Thus, although varying the proportion of the area occupied by clumps is not as potent as varying the overall density *per se*, it does appear to have some potential as a disease control strategy for a plant species.

So far, no attempt has been made to put ecological terms to the types of pattern which were investigated. Varying the number of clumps while maintaining everything else constant, involved adjusting what Pielou (1969) describes as the 'grain' of a pattern. The grain of a pattern is related to the size of the clumps or patches of the species in question and thus patterns with only 100 clumps per m^2 may be described as 'course-grained', in contrast to those with 1600 clumps per m^2 which are much 'finer-grained'. Pielou contrasts this property of grain with another aspect of pattern which she calls 'intensity' and defines as 'the extent to which density varies from place to place'. When varying grain in the above experiments intensity was held constant at its maximum value. To the extent that the minimum area which would have to be

FIGURE 49.

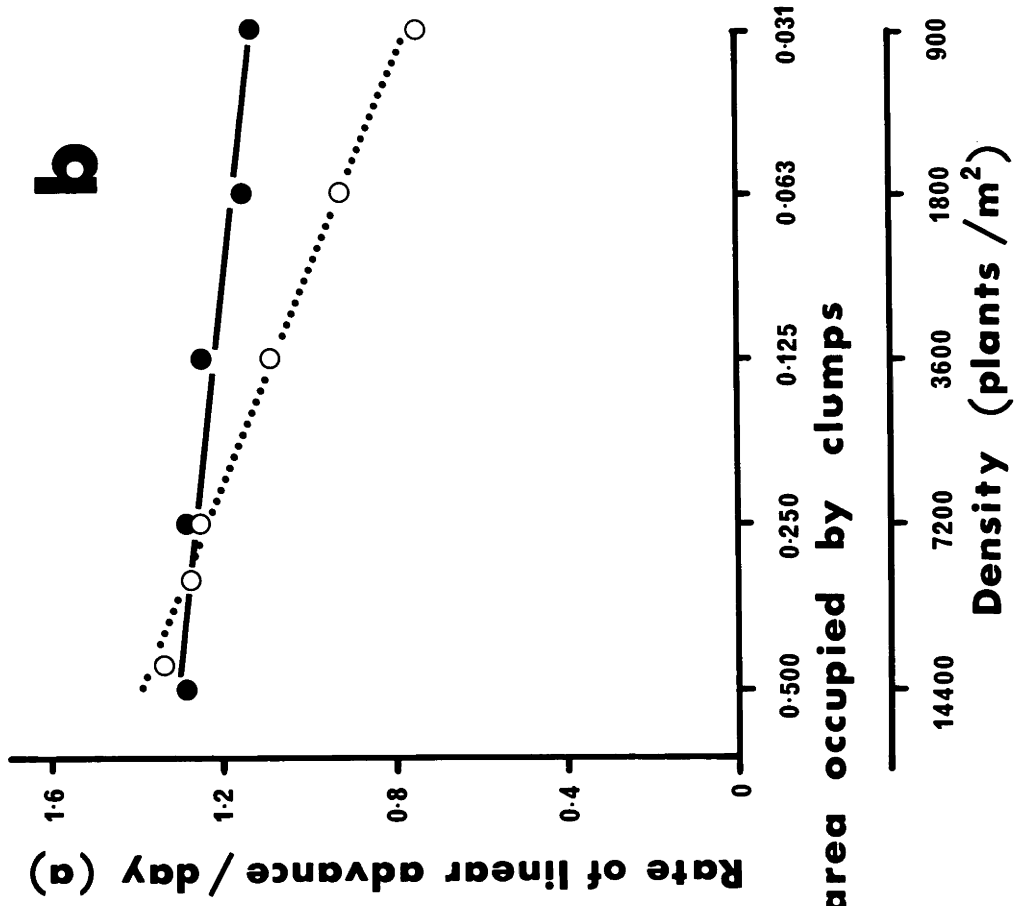
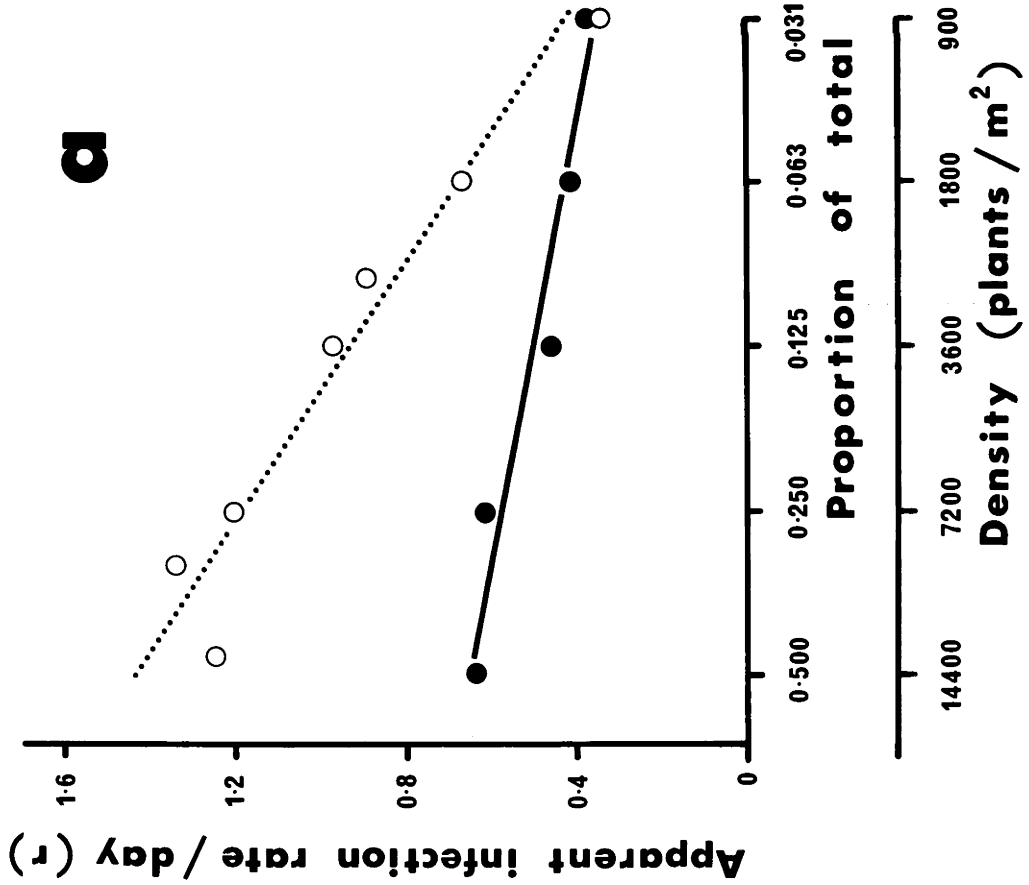
Semi-log plots relating epidemic rates to clumping and density of plants.

a) Apparent infection rate.

b) Rate of advance.

Solid graph lines (●) relate epidemic rates to the proportion of area occupied by clumps drawn to a logarithmic scale (data transformed from Figures 20a and 21, constant plant density of 1840 plants per m^2). Dotted graph lines (○) relate epidemic rates to the overall seedling density drawn to a logarithmic scale (data transformed from Figures 4 and 5).

Both experimental variables, proportion of area occupied by clumps and overall seedling density, are exactly halved for each unit of distance along the horizontal axis. Therefore a comparison on each graph between the slope of the solid and dotted regression lines, provides some indication of the relative effectiveness of reducing these factors in order to control epidemic rates.



sampled in order to encompass a unit of the repeating pattern remained the same, it seems reasonable to say that in experiments in which the number of clumps per m^2 was kept constant, the grain of the pattern, as determined by the differences in plant density within clumps and inter-clump areas ('patch phase' and 'gap phase' respectively of Pielou, 1969), varied simultaneously with the proportion of the total area devoted to clumps.

3. *Mixtures*

Disease rates in both damping-off and powdery mildew were substantially lower in mixtures of host and non-host plants than in host plant monocultures of the same overall density. In both systems, rate factors declined progressively as the proportion of resistant or immune plants increased and the proportion of susceptible individuals decreased. The precise quantitative relationship differed in the two disease systems however, with the rate of decline of the disease parameters becoming progressively greater in the damping-off system as the proportion of susceptibles in the mixture continued to fall, while remaining little changed in the powdery mildew system. These results agree with the generally expressed opinion that mixtures may cause a reduction in disease or pathogen impact (for example, Borlaug, 1959; Simmonds, 1962; Browning and Frey, 1969; Adams, Ellingboe and Rossman, 1971; Cherrett *et al*, 1971) although they disagree with a number of other studies in which mixtures have been found more susceptible than monocultures

(Sandfaer, 1970; Chamblee, 1958; Intern. Rice Res. Inst., 1974).

For the damping-off system, by comparison with equivalent monocultures, it was shown that disease rates in mixtures of susceptible and resistant plants were largely determined by the net density of the susceptible plants present. The presence of resistant plants *per se* did not alter disease rates much, although the resistance and survival of these individuals is of significance to the final disease impact on the crop as a whole. (In certain limited circumstances, resistant plants also interfered with the transmission of inoculum and thus reduced rates of disease increase slightly).

In mixtures of susceptible and immune plants inoculated with powdery mildew, overall rates of disease increase were also found to be substantially related to the net density of the susceptible plants present. However, as the number of immune plants in the array rises these have an increasing impact on the overall rate of disease increase due to the interception, and hence wastage, of inoculum. In mixtures containing a high proportion of immune plants, inoculum interception by these plants reduced overall rates of disease increase by more than 10%. Variations in the pattern of placement of host and non-host individuals had no effect on the epidemic rates of this disease.

One obvious criticism that can be levelled at this work is its artificial setting, epidemics being generated in controlled environment chambers rather than in a field situation. The only work of a comparable nature is that of Leonard (1969) which was carried out in a field setting and it is pertinent therefore, to

an equation identical in form with that obtained from empirical results. Furthermore, in a theoretical evaluation of the effect of mixing susceptible and resistant varieties on plant disease, Kiyosawa and Shiyomi (1972) found that, at least in pathogens with shallow spore dispersal gradients, Leonard's model fitted their theoretical findings. Additionally, van der Plank (1963), in one of the exercises appending a chapter of his book, points out '.. that diluting susceptible plants with immune plants in a varietal mixture does not proportionately reduce the logarithmic infection rate..' although he had little evidence to support this statement. Overall it would appear, therefore, that a direct relationship between the rate of disease increase and \log_e of the proportion of susceptible plants in the mixture is to be expected on both theoretical and experimental grounds.

4. *Competition in mixtures*

Competing mixtures of barley and wheat were found, (under the particular experimental conditions provided), to compete for the same 'space' (*sensu de Wit, 1960*) or growing resources of the environment. In healthy mixtures, the barley component was considerably more competitive than the wheat component, occupying more than its share of the available space and thus tending to replace the wheat component in the mixture. In mixtures in which the barley component was infected with powdery mildew, on the other hand, the competitiveness of the barley was reduced while the relative

competitiveness of the wheat was increased, thus allowing the two species to remain together in semi-equilibrium.

The replacement series approach has been used extensively to study the interaction of species in healthy mixtures (for example, de Wit and van den Bergh, 1965; de Wit, Tow and Ennik, 1966; Baeumer and de Wit, 1968; England, 1968; van den Bergh and Elberse, 1970; Hall, 1974) but little attempt has been made to extend its use to the study of interactions between mixtures of healthy and diseased species. An exception to this is the work of Sibma, Kort and de Wit (1964) who studied the effect of nematodes in a mixture of susceptible and resistant host plant species. These workers found similar results to those recorded here, with nematode infestations reducing the competitive advantage of the host species and thus allowing the normally less competitive non-host to return a higher yield. Like the experiments described here however, these trials did not allow for a density-dependent relationship between host and pathogen as the nematodes were evenly spread through the soil prior to experimentation. The few other interactions between healthy and diseased plant species or varieties which have been studied are generally special cases of viral diseases (for example, Sandfaer, 1971; de Wit's (1960) re-examination of the data of Reestman, 1946), which throw no further light on the results obtained here.

The design of the experiments carried out to investigate the effect of disease impact on the competitive interaction between two species did not allow full development of a density-dependent relationship between the pathogen and the density of host plants in

each experimental pot. Despite this, the results did show how disease impact might reduce the competitive ability of a host species in comparison with that of an unaffected non-host species. If this finding is considered together with the density-dependent relationship which has been shown to exist between host plant and pathogen, it would seem that for the pathogen to have a truly stabilizing effect on mixtures of host and non-host species the replacement series lines of such an interaction would have to be similar in general shape to those shown in Figure 50.

In the absence of disease the host species, being the more competitive, gains a greater than fair share of the available resources in all mixtures regardless of the relative proportions of the two species present (note the convex host replacement series lines in Figure 50a). Such a mixture is unstable as the more competitive species will, through time, slowly replace the less competitive one until only the more competitive species remains.

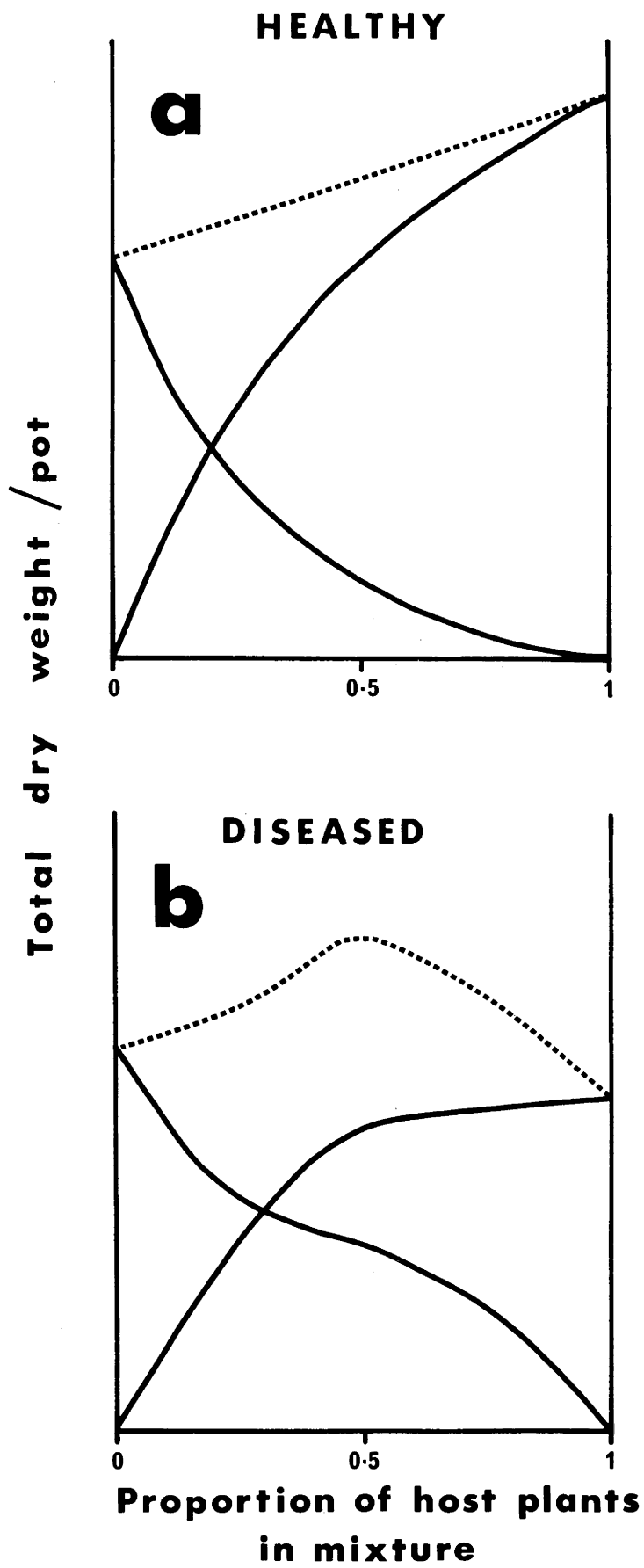
In the presence of disease, host plants in mixtures containing a low proportion of host individuals would probably retain much of their competitive advantage, as the very low *host* plant density of such mixtures, combined with the inoculum intercepting potential of large numbers of resistant or immune plants, would result in very low rates of disease increase. In mixtures containing a high proportion of host plants, however, the high *host* plant density, coupled with the presence of relatively few resistant individuals, would result in comparatively high disease rates and thus the competitiveness of the species would be severely reduced. If such

FIGURE 50.

A graphical illustration of the hypothesized equilibrium attainable in mixtures of a host and a non-host species in the presence of a pathogen in contrast to the unstable competition interaction between the two plant species in the absence of the pathogen.

- a) Competitive interaction between host and non-host species in the absence of the pathogen.
- b) Competitive interaction between host and non-host species in the presence of the pathogen.

Solid lines indicate the general shape of the yield response of host and non-host plants to changing mixture proportions; broken lines indicate total dry weights.



changes in the relative competitive ability of the host species are sufficiently pronounced then there will be a range of intermediate situations in which 'over-yielding' (Trenbath, 1974) mixtures will occur. That is, the total yield of both host and non-host components in the mixture will exceed the yield of either component in monocultures inoculated with the pathogen (Figure 50b). Moreover, within this range of over-yielding mixtures there will be a particular combination of host and non-host species which will produce a maximum yield. (Considered from a different point of view over-yielding mixtures are equivalent to the 'monoculture deficit' of Chilvers and Brittain, 1972).

II. SIGNIFICANCE FOR AGRICULTURAL SYSTEMS

1. *Monocultures*

From an agricultural or horticultural point of view, low planting density *per se* is unattractive as a disease control measure because the consequent inefficient utilization of environmental resources, reducing yield per unit area, will normally outweigh any productive advantage to be gained from moderation of disease impact on the crop.

Similarly, considered in isolation, variations in the pattern of placement of a single species (degree of clumping etc.) are unlikely to achieve sufficient disease moderation to outweigh yield reductions

which would result from the inefficient utilization of environmental resources and the intensification of inter-plant competition within clumps.

2. *Mixtures*

In agricultural situations the yield reduction limitations associated with reducing host density *per se* as a disease control measure may be circumvented by growing a number of plant species, or more commonly a number of plant varieties possessing different patterns of resistance to parasites, in association with one another. Such mixtures provide a step in the direction of increasing diversity and are very useful in developing ideas concerning the influence of reduced host density and the presence of non-hosts on disease rates.

Perhaps the most complex of agricultural mixtures are the recently developed 'multilines' where blends of more than ten (Rockefeller Foundation, 1963) different isogenic lines of the same species are grown together in order to reduce disease impact. The lowering of host density through the mutual dilution of different plant varieties appears, from the results of the work in this thesis, to be intrinsic to the disease control achieved by such mixtures, although the proponents of this approach usually do not discuss their strategy in these terms (Browning, 1957; Borlaug, 1959; Suneson, 1960; Browning and Frey, 1969; Leonard, 1969; Sumner and Littrell, 1974).

In general, where a mixed planting proves healthier and consequently more productive than a comparable monoculture in a disease situation, (that is, the mixture over-yields) four possible pathological explanations should be considered. Firstly, there is almost certain to be a greater proportion of plants in the mixture showing resistance to some or all of the prevailing pathogens than in the monoculture. Secondly, to the extent that these resistant plants have substituted for susceptible plants, the density of susceptible plants in the mixed stand will be less than that in the monoculture and this factor may affect inoculum transmission. Thirdly, inoculum passing between susceptible host plants in a mixed stand may be collected by, or suffer some other kind of interference from, non-host plants located *en route*. Finally, in a mixed plant stand subjected to various host-specific parasites, it is possible that cross protection, the triggering of plant defences by an avirulent parasite producing enhanced resistance towards subsequent attack by virulent parasites, may play some role.

Of the four possibilities, the direct contribution of plant resistance is the most obvious and important component of disease control in mixtures. Thus, in the model systems used here, the deliberate insertion of say 25% *Lolium rigidum* or *Triticum aestivum* plants into a stand immediately ensured that that proportion of plants would survive the epidemic. In the more complicated agricultural field situation, where it is not certain in advance which races of parasite will be important during the growth of a crop, the planting of multilines provides the best chance that a reasonable proportion of the plants will prove immune to such pathogens as appear. Not

surprisingly then, most attention has been directed towards this component of disease control in mixtures (for example, Jensen, 1952; Browning, 1957; Borojevic and Mistic, 1962), but it is surprising that Sumner and Littrell (1974), working with southern corn leaf blight in mixtures of maize lines, were able to conclude that the presence of resistant plants was the *only* factor operating to control disease. Certainly in the two systems examined here, the resistance *per se* of *Lolium rigidum* and *Triticum aestivum* to disease cannot account directly for the decrease in epidemic rates within the susceptible component of the respective mixtures.

The contribution made by decreases in the density of susceptible plants to reducing disease rates has been largely ignored by previous workers. In the present studies the contribution of this factor was ascertained by the use of appropriate controls. In the soil-borne system the rate of disease advance through mixed stands was found to be exclusively determined by the net density of the susceptible species. This was true regardless of the overall density or composition of the mixture being examined. Similarly, the rate of disease increase in low to medium density stands in the soil-borne system and in mixtures containing a high proportion of susceptible individuals in the air-borne system also appeared to be determined solely by the net density of susceptible plants in the mixture. Rates of disease increase in high density mixtures (soil-borne system) or in mixtures containing a high proportion of resistant individuals (air-borne system) were, on the other hand, found to be less than that expected from comparison with equivalent susceptible monocultures. Even in these cases, however, the

contribution of host density reductions to disease moderation was still very important. Thus in the air-borne disease system, although disease rates in 30% susceptible mixtures were 53% below those in pure stands of the same overall density, it was estimated that 80% of this reduction in disease resulted from lowered host density and only 20% through the operation of other factors.

Obviously in single pathogen experiments there is no possibility of cross-protection phenomena operating. Consequently, in situations where disease rates are lower in mixtures than in equivalent monocultures of the susceptible species, it seems most likely that the resistant plants are interfering in some way with the transmission of inoculum. In the soil-borne disease system this depression of epidemic rates is thought to be due to healthy resistant plants supporting diseased susceptible individuals and thus preventing them from acting as transmission bridges. In the air-borne system, resistant individuals interfered with and reduced the amount of inoculum passing between susceptible plants by acting as passive traps on which conidia became impacted.

In summary, it appears that while the factor which provides the main limitation to the ultimate level of disease impact in mixed species stands or multilines is likely to be the survival of unaffected resistant plants, the associated reduction in susceptible plant density makes significant contributions to disease control through a lowering of the rate at which infection develops. Interference with the transmission of inoculum by resistant plants occurs, but is of much less significance than the other two

components of disease control achieved through mixed plantings. Comparing the results obtained here with previous work on multilines (for example, reviews of van der Plank, 1968; and Browning and Frey, 1969; and the important experimental paper of Leonard, 1969), it is clear that there is a general equivalence between the results obtained. However, nowhere in the multiline work do the effects of mixtures on epidemic rates appear to be related explicitly to changes in the net density of susceptible plants. In fact, it is usually implied that in substituting resistant for susceptible plants it is the insertion of resistant plants rather than the removal of susceptible plants which is responsible for reduction of epidemic rates. Thus Browning and Frey (1969) state that 'resistant components apparently act as barriers to the spread of the pathogen'. Similarly, Trenbath (1975) states that each pathogen race 'will face a strong fly-paper effect which will limit its multiplication' and even Leonard (1969) prefers to conclude that 'resistant plants in host mixtures were found to reduce the rates of increase of stem rust by reducing the number of effective dispersals', although elsewhere in his paper he relates rates of increase of disease to the proportion of susceptible plants present. These workers seem to have been led to misinterpret this point because rate data from adequate monocultural controls was not available to compare with that from mixtures.

III. SIGNIFICANCE FOR NATURAL ECOSYSTEMS

As Chilvers (1972) pointed out, the study of the effect of plant parasites on natural communities is largely unexplored. Natural plant communities are often extremely diverse and complex and as a consequence, the very factors which make them interesting are also the factors which provide the greatest impediment to experimentation. This problem may be minimized, however, by experimentation with less complex agricultural or laboratory systems which, by providing a graded increase in complexity and diversity, may give valuable insight into the problems of the maintenance of diversity and stability in natural ecosystems.

1. *Host density per se*

Although low planting density *per se* is unattractive as a disease control measure in agricultural systems, such a mechanism of disease control has special interest in considerations of natural plant communities since it contains certain features of a self-regulatory feedback system. Thus, because of its faster rate of spread and increase at high plant densities, the pathogen is likely to kill more plants at high than at low plant densities; this death of plants reduces plant density; and, as shown in this thesis, lowered plant density in turn tends to curb the pathogen through its effect on transmission from plant to plant. In addition, if

hosts are sparsely distributed, the number of primary infection foci developing from a given density of inoculum has also been shown to be much less than within a dense host stand, and from the standpoint of the pathogen this represents a lowering of its birthrate following transmission through time. Disease control through reduced host density thus has special advantages, because, unlike reductions in inoculum density, which can normally only affect the number of initial foci, reducing host density has also been shown to lower the rate of transmission of disease through space. This may be particularly important if the disease situation is one in which secondary spread out from primary infection foci is a large factor in determining the number of host plants which ultimately become infected (for example, most air-borne pathogens). In such circumstances, reducing the number of primary infection foci *per se* is not very effective in controlling disease; the most effective control being one which moderates the infection rate (van der Plank, 1963).

2. *Mixtures*

a) *Mixtures in general*

Density-dependent interactions may permit host plants to achieve some kind of stable equilibrium with their pathogen, although the density at which this would occur, is likely to be much lower than that at which the plant species can utilize all resources available

within the particular environment. It has been hypothesized that in such a situation this incomplete utilization of environmental resources would lead to invasion by other species which are resistant to the particular pathogen (Harper, 1969). In a disease free situation, such species, because of their very similar or identical resource requirements but inferior competitive abilities, may be excluded from the habitat by the former species. In the event of specific parasite attack on the dominant species (with the effect of either killing individuals or reducing their relative competitive ability) however, the less aggressive species would be able to compete more effectively, and thus enter into and increase in the community.

In natural ecosystems such changes in the relative competitive ability of plant species, due to the action of parasites, may act as negative feedback mechanisms operating to maintain the mixture in a dynamic equilibrium close to that mixture composition which produces the maximum yield (see Figure 50b). Thus in Figure 50b, if the amount of disease in the mixture declines, the competitiveness of the host species will increase and, through time, the proportion of host plants in the mixture will also increase. At higher host plant proportions (and thus high *host* plant densities) disease rates increase and individual host plants gain little 'disease escape' advantage. The competitiveness of the host species is consequently reduced. Under these circumstances the non-host species is favoured and, with time, will increase in the mixture. This in turn will cause a reduction in the proportion of the host species in the mixture, thus reducing the impact of the pathogen and increasing the competitiveness of the host species. In this

way a dynamic equilibrium between the host and non-host species could be achieved in the presence of disease, compared to the unstable competitive situation existing between the two species in the absence of parasites.

Such an equilibrium could occur when any two species are competing for the same environmental resources or limiting resource, regardless of whether the success of competition in a disease free situation is determined by one species possessing a faster growth rate *per se* (RYT equal to unity) or by one species producing allelopathic substances against the other (RYT less than unity). However, the more aggressive species must be more severely affected by disease than the less aggressive one. The actual proportions of host and non-host in the equilibrium would depend on a wide range of variables including any factors which influenced the relative competitive ability of the two species in the mixture or the rate of increase of the pathogen.

While the example illustrated in Figure 50 describes the interaction of two different plant species and a single host-specific parasite, it can be readily appreciated that the non-host species may, in turn, be subject to control by other density-dependent parasites. Eventually, the density of each species in a mixture may be adjusted to a level which provides useful control of its parasites, while the total productivity of the system is maintained by adjustments of the number of plant species occupying the habitat. In complex natural communities then, in which a variety of plant parasites will be more or less continuously available, it is usual

for there to be a diversity of plant species having different patterns of resistance, thus ensuring that each plant is not subject to exploitation by all of the parasites present (for example, Burdon and Chilvers, 1974a). In this way, continuous interactions between plants and their respective parasites provides a possible rationale for the vegetational diversity which characterizes most natural plant communities (Harper, 1969; Janzen, 1970; Chilvers and Brittain, 1972).

The disease control advantages obtained in natural plant communities are essentially the same as those obtained in agricultural multilines. In both fields, (natural disease control through vegetational diversity and agricultural multilines), the presence of resistant plants and reductions in host plant density are the prime factors in reducing disease rates, although other factors like cross-protection and interference to inoculum transmission play some role. This moderation of the impact of parasites on host plants mixed with non-hosts has been noted on a number of occasions in natural ecosystems. Thus the damage caused by weevils to white pines (Graham, 1915; MacAloney, 1930), insects in general to Swedish forests (Trägårdh, 1925) and spruce budworm to fir forests (Balch, 1946; Mott, 1963; Fauss and Pierce, 1969) was always lower in situations where the host species was growing in association with one or more non-host species. Unfortunately, no similar data is available concerning the effect of fungal pathogens.

b) *Pattern of host and non-host placement in mixtures*

In natural ecosystems, regular or evenly spaced arrays rarely

occur (Greig-Smith, 1964), most species showing aggregated or clumped patterns of distribution (for example, Phillips, 1954; Kershaw, 1957; Mott and McComb, 1974).

Varying the pattern of clumping of a plant species, while not as potent as changing host density *per se*, appears to have potential as a disease control strategy in some circumstances. Of course, disease control is only one factor in the success of a species and the effects of pattern are not confined to this. For example, on the one hand, tight clumping of members of the same species aggravates problems caused by competition for identical resources within a restricted area, while on the other, clumping of individuals will favour enhanced reproductive output in out-crossing species, over that which would occur if the species was widely dispersed at low densities. Moreover, clumped or aggregated distributions are the natural outcome of successive generations of seed production or asexual reproduction in most species, whereas diffuse distributions are maintained only in response to some continuing influence (for example, allelopathy). For these reasons it will not be easy to judge the contribution to disease control of clumping in natural plant stands. A minimum experimental requirement would be that different patterned arrays existing in similar environmental conditions are exposed to the same inoculum density of parasites.

Experiments investigating the effect of plant clumping on disease rates in the soil-borne pathogen system were restricted to a single species for reasons of experimental simplicity. It seems reasonable to say, however, that in natural situations such

patterns might still play a role in the amelioration of soil-borne diseases, if all or most intervening species were non-hosts.

The influence of host spatial distribution on the disease rates of air-borne pathogens is somewhat more complicated, but van der Plank (1960) has suggested (for agricultural systems) that making fields larger and correspondingly fewer (thus increasing the average distance between 'clumps' of fields) may reduce the chance of a general epidemic. If this argument still applies on a reduced scale in natural ecosystems, then species which exist in well spaced clumps surrounded by a 'sea' of non-hosts should suffer less disease than species which are more evenly distributed through the ecosystem. No data is available to test this possibility for air-borne fungal pathogens, but it is interesting to note that in at least one case involving insects the clumping of host plant individuals may be enhanced by seed predation (Platt, Hill and Clark, 1974).

IV. PROSPECTS FOR FUTURE WORK

While the present study has demonstrated the occurrence of density-dependent interactions between a soil-borne fungal pathogen and its host plant and an air-borne fungal pathogen and its host plant, there is a considerable need to extend these studies to at least a few more pathogen - host plant pairs in order to show whether the results obtained here reflect general phenomena common

to most fungal pathogens. The use of controlled environment conditions would seem to offer numerous advantages for such studies.

Additionally, although attempts to examine individually the various factors contributing to disease increase did not form part of the objectives of the present project, there is a need to combine a more detailed examination of such factors with a simple modelling approach in order to try and provide a more detailed explanation for the observed empirical relationships.

To study the full effects of parasite damage on plant-plant competitive interactions it will be necessary to extend such studies to field situations so that the pathogens used may 'behave' as naturally as possible. This is particularly important for airborne diseases. At first this approach may be restricted to a single host-pathogen pair and one non-host, but as better understanding of the processes involved is achieved, increases in the total number of plant species and the number of host-pathogen pairs would provide very useful information on the effect of diversity of both plant and pathogen species on disease rates in natural communities.

There is also a need to approach the problem at a community level. Surveys of the various parasites present in a particular community, their host ranges, the relative abundance of these hosts and the amount of damage inflicted, could be combined with deliberate actions to perturb the community away from its equilibrium position. Such actions might include protecting host species from parasite damage or artificially altering the relative

proportions of the species under consideration. A careful monitoring of the response of the community to such actions should provide much useful information. In this respect the near clear-felling (for wood chips) of eucalypt forests on the south coast of New South Wales on a 500-1000 acre block system seems to offer the opportunity to manipulate the relative balance of the regenerating species while retaining an adult reference stand close by. Plant communities dominated by introduced weedy species which are the target of biological control programs are also potential sources of information concerning host plant - parasite and host plant - non-host plant interactions. This is particularly the case where the control agent is a fungal pathogen, as these have been far less widely used than insects.

SECTION F

BIBLIOGRAPHY

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SECTION G

APPENDIX I -

SOME PUBLISHED PAPERS RELEVANT TO THIS THESIS

Fungal and Insect Parasites Contributing to Niche Differentiation in Mixed Species Stands of Eucalypt Saplings

J. J. Burdon and G. A. Chilvers

Botany Department, School of General Studies, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

Abstract

Parasitic damage to the leading shoot of young regrowth eucalypts was found to vary considerably between trees and between different stands but it averaged greater than 20% 'effective leaf area loss' overall. Many fungal parasites and some of the phytophagous insects responsible for this damage exhibited host specificity or host preference towards trees of a particular subgenus of *Eucalyptus*. These findings are discussed in relation to the hypothesis that parasites play an important role in the maintenance of stable associations between codominant species of *Eucalyptus*.

1. Introduction

In the forests and woodlands of south-eastern Australia, native eucalypts are normally associated together in mixed stands composed of two or more codominant species. Pryor (1953) showed that as many as 12 different species associations may be represented within a single square mile of forest and that under natural conditions these were delineated quite sharply from one another to form a complex mosaic. Pryor later (1959) reviewed some of the more important ecological implications of such a distribution pattern. He concluded that the location of boundaries between adjacent associations was keyed to local changes in environmental factors such as altitude, aspect and soil drainage, and this viewpoint has been reinforced by most subsequent findings (Florence 1971). In particular, a number of workers have detected consistent differences in soil chemistry between adjacent associations (Moore 1961; Winterhalter 1963; Parsons and Specht 1967; Parsons 1968). Pryor also drew attention to a second problem posed by the very existence of stable associations between species of the same genus. Codominant eucalypts appear to exploit the same physical and chemical resources of their shared habitat and to show similar adaptations to its physical extremes, often to the point of showing strong morphological convergence. Superficially, such a situation appears to contradict the 'competitive exclusion principle' as stated by Slobodkin (1962): '... no two species can indefinitely continue to occupy the same ecological niche'. Different species coming to occupy the same niche would be expected to merge into a single species by hybridization, or the species having superior competitive ability, however slight, should eventually eliminate the other. Pryor was able to exclude the first of these two possibilities in the present case by demonstrating that *Eucalyptus* species within an association were normally drawn from different subgeneric groups which could not interbreed, but the problem of their continuing coexistence remained.

Recently it has been suggested that this type of dilemma may be resolved if proper account is taken of the power of host-specific parasites* to differentiate the ecological niches of plant species which are otherwise homologous in their habitat requirements. For instance, Harper (1969) pointed out that a reduction in the density of a plant species as a result of parasitic activity could provide room in the same niche for a further species to establish itself. Following this, Janzen (1970) used static graphical models to explain how the high species diversity and the long distance between conspecific adult trees in lowland tropical forests might result from the activity of host-specific seed predators which prevent seedlings from becoming established near a pre-existing tree. Chilvers and Brittain (1972) later devised a simple continuous systems model to illustrate how two competing plant species could be maintained in dynamic equilibrium with one another as a result of density-dependent feedback from their respective host-specific parasites.

The two main premises underlying such host-parasite models appear to be generally valid. The existence of density-dependent interactions is well known from the field of biological pest control (e.g. Nicholson 1958), although precise data from controlled experiments like that of Huffaker (1958) are admittedly still rare. The other requirement, for host-specific parasites, would be readily satisfied in most ecosystems, since many fungi and cecidiogenic insects are known to have restricted host ranges. However, it is doubtful if many parasites are confined entirely to single host species, which raises the question of how much specificity can be expected in forests composed exclusively of species of the single genus *Eucalyptus*. The literature is of only limited help in judging this point. Although it is probable that more than 1000 species of parasite have been recorded on *Eucalyptus*, the host range of these parasites within the genus has rarely been recorded adequately. However, there are a few exceptions to this general rule, five of which are of particular interest. Heather (1971) reported that the fungi *Phaeoseptoria eucalypti* and *Septoria normae* were only to be found on eucalypts within the subgenus *Symphyomyrtus*† while *Aulographina eucalypti* appeared to be restricted to the subgenus *Monocalyptus*. Walker and Bertus (1971) have also shown that *Ramularia piterika* is similarly specific to the subgenus *Corymbia*. In the case of insects, Moore (1970) recently divided the psyllid genus *Glycaspis* into two subgenera, each of which parasitizes a different *Eucalyptus* subgenus. Since associated *Eucalyptus* species are usually from different subgenera, such a pattern of parasite specificity, if common, would reduce the overlap between their respective ecological niches and be consistent with the requirements of a negative feedback system.

The present paper describes a preliminary attempt to assess the degree to which the niches of some associated *Eucalyptus* species are differentiated by parasitic attack. The Brindabella Mountains of the Australian Capital Territory provide a convenient 'laboratory' for such a study because the associations there all contain one species from the subgenus *Monocalyptus* (*M*) and one species from the subgenus *Symphyomyrtus* (*S*). Following earlier disturbances of the natural forest, young regrowth trees have become established in a number of places. Four dense stands of saplings,

* The term parasite is used throughout this paper in a broad sense, intended to include phytophagous insects with those organisms, such as fungi and cecidiogenic (gall-forming) insects, to which the term is usually applied.

† *Eucalyptus* nomenclature follows the recent classification of Pryor and Johnson (1971).

representing a strongly competitive stage of development toward a mature stand, were selected for this study, as follows.

Site 1, at an altitude of 730 m, supported a mixture of *E. radiata* (*M*) and *E. viminalis* (*S*);

Site 2 (1130 m), *E. dives* (*M*) and *E. dalrympleana* (*S*);

Site 3 (1280 m), *E. delegatensis* (*M*) and *E. dalrympleana* (*S*);

Site 4 (1220 m), *E. pauciflora* (*M*) and *E. dalrympleana* (*S*).

Throughout one growing season, a comprehensive collection of fungal and insect leaf parasites was made from each *Eucalyptus* species at each of these sites. During the same season, an attempt was made to assess the impact of all these organisms in terms of 'effective leaf area' lost by each *Eucalyptus* species. Where possible the species of parasite responsible for each type of damage was traced, in order that some judgment could be made about the degree to which damage was specific to one or other host plant.

2. Methods

(a) Evaluating Parasite Distribution

(i) Collection

(1) *Fungi*. Whole leaves exhibiting different types of fungal lesion were sampled from living trees during the period from December 1971 to October 1972. The more common lesions were scored in the field according to a simple scale of abundance.

(2) *Insects*. Systematic collections of adult beetles and leafhoppers were made periodically between December 1971 and April 1972 (after April they became scarce until the following season). At each site, comparable numbers of saplings of both host species (generally 30–50 of each, within an area of 0.1–0.2 ha) were searched systematically for leaf and stem insects, which were knocked into plastic collecting bags containing a small quantity of alcoholic fixative. Collections were made at different times of day under a variety of weather conditions.

The more prominent dipterous and lepidopterous larvae were also collected individually during the above period. Sessile insects were assessed *in situ*.

(ii) Identification

Emphasis was placed on effecting reliable distinctions between different species of parasite rather than making formal identifications, and expert advice was sought to achieve this. Nevertheless approximately one-quarter of all parasites collected, including many of the most common ones, were identified to the specific level, and more than half the remainder were placed in a genus. In the case of the fungi, which possess fewer taxonomically useful attributes than the insects, a number of problems were encountered which will require more detailed studies, involving cross-inoculation tests, to resolve satisfactorily. For example, a *Mycosphaerella*, which on anatomical grounds appeared to be a single species, was found to have an unusual host distribution, being very common on the *Monocalyptus* species and absent from the *Symphyomyrtus* species at the lowest site, but reversing this pattern at the highest site. This fungus was readily cultured on a number of occasions and it was found

(b) *Damage Assessment*

At each site, four saplings of each species were selected on their general appearance to be representative of the stand, and the leading shoot cut off at the point where it attained a diameter of 1 cm. This standard was adopted after field examinations showed that it coincided closely with the lower limit of foliage. These shoots were bagged separately and returned to the laboratory for detailed damage assessment. Since the damage took various forms depending on the type of parasite responsible, each category had to be measured in a different way and an overall estimate of damage calculated by summing the separate contributions expressed in terms of the percentage of 'effective leaf area' lost to the plant. Practically all leaves examined had been formed during the current season, since very little of the previous season's foliage had survived at these altitudes.

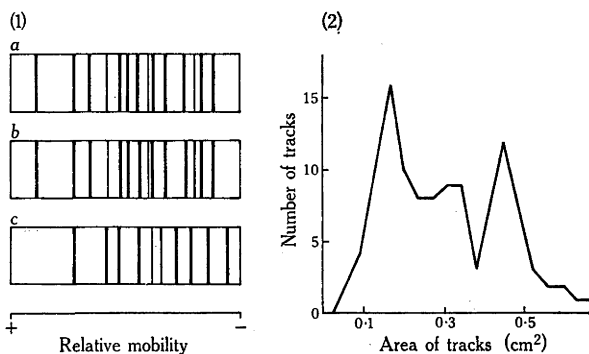


Fig. 1. Comparison of electrophoretic protein patterns of three different *Mycosphaerella* isolates.

(a) Isolate from *E. radiata* (*Monocalyptus*). (b) Isolate from *E. dives* (*Monocalyptus*). (c) Isolate from *E. dalrympleana* (*Symphyomyrtus*).

Fig. 2. Frequency size-class distribution of lepidopterous larval tracks on eucalypt leaves.

(i) 'Total' Leaf Area

Initially, the total leaf area of each shoot as sampled was measured by removing all leaves and passing them through a calibrated Hayashi Denko automatic area meter.

(ii) *Missing Leaf Area*

(1) *Mutilated leaves*. Slots and indentations in the leaf lamina, caused by biting insects, were traced out on paper and paper templates were constructed for area determination in the automatic meter.

(2) *Missing leaves*. In the few cases where there was clear evidence of leaves missing as a result of insect attack, leaves from similar but undamaged shoots were substituted at those points and a conservative estimate made of the lost leaf area.

(iii) *Dead Leaf Area*

(1) *Spreading necrotic lesions*. Large dead areas of leaf, mostly due to necrogenic fungi, were cut out carefully and measured in the area meter.

(2) *Local necrotic lesions*. Local lesions varied from small discrete spots caused by the fungus *Hendersonia*, to long scribbly tracks of various lepidopterous larvae,

but they all posed the same problem in that it was impractical to measure the area of every lesion. A sample of lesions of each type was therefore measured carefully and used to determine the size class frequency distribution. In some cases, for example the *Hendersonia* spots, this approximated closely to a natural distribution curve, so that thereafter it was only necessary to count the number of spots and multiply by the spot area mean to obtain a reasonable estimate of the total leaf area loss. In other cases, for example the lepidopterous larval tracks (Fig. 2), the size class frequency distribution was bi- or trimodal, which meant that when counting the tracks they had to be classified into two or three appropriate size classes.

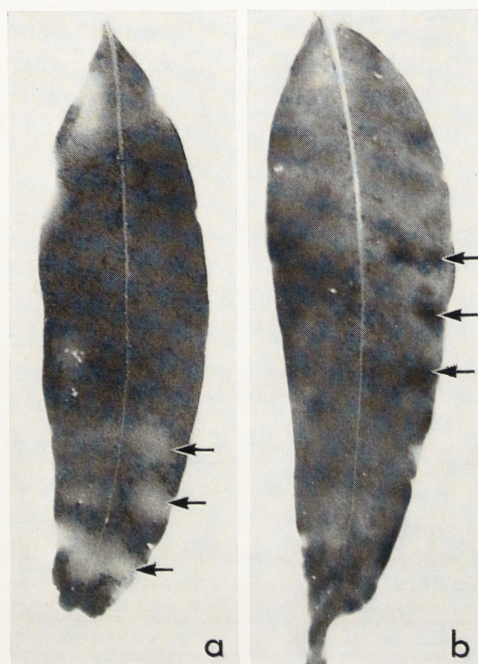


Fig. 3. Autoradiographs of leaves of *E. dalrympleana*, infected with *Septoria normae*, fed for 3 min with $^{14}\text{CO}_2$ at a concentration of $0.16 \mu\text{Ci/ml}$ of gas space.

(a) Leaf heat-dried between aluminium plates immediately following treatment.

(b) Leaf left in illuminated photosynthesis chamber for 60 min after feeding and replacement of $^{14}\text{CO}_2$ with fresh air before drying.

Lesions arrowed; both $\frac{2}{3}$ natural size.

(iv) Diseased Leaf Area

A special problem was posed by certain fungi, in particular *Septoria normae*, where host tissue remained alive for a long time after infection. It seemed that the productivity of such diseased tissue would be reduced as a result of disturbance of the photosynthetic machinery, but it could not be assumed to have failed altogether. Furthermore, there was the possibility that the diseased tissue might accumulate photosynthates from adjacent healthy tissues (Smith *et al.* 1969). Both of these possibilities were investigated by use of radioactive tracers.

For instance, *c.* 3-month-old excised whole leaves of *E. dalrympleana* with lesions caused by *Septoria normae* were incubated at 25°C and given short pulses of $^{14}\text{CO}_2$ under light at 42 lumens/m^2 , then both diseased and healthy portions of the same leaves were immediately sampled and extracted in 30% ethanol to remove soluble carbon compounds. Aliquots of these extracts were then mixed with Bray's solution and assayed for radioactivity in a Beckman liquid scintillation system. It was found

that the diseased tissue produced only 8% of the count rate detected in the healthy tissue, which showed that photosynthesis had been all but suppressed in the former. Visual evidence of this phenomenon of photosynthetic depression is shown in Fig. 3a. However, when similar leaves, after being given the same short pulses of $^{14}\text{CO}_2$, were left for a while in an ordinary unlabelled atmosphere before sampling, there was clear evidence of redistribution of the labelled photosynthates involving a net accumulation in the disease lesions. Thus the mean activity of diseased samples was 38% that of healthy samples after 15 min, and after 60 min had risen to 66%. By this time some of the individual lesions had in fact accumulated radioactivity to a higher level than the surrounding healthy tissues, as shown in Fig. 3b. Taking into account the net loss of radioactivity from the healthy portion of this closed translocatory system, it was calculated that each unit area of disease lesion reduced the photosynthates which would have been available for export from the leaf by an amount equivalent to the productivity of at least 1.29 units* of healthy tissue. Accordingly, the actual area of all disease lesions due to *Septoria normae* were multiplied by this factor to arrive at an estimate of 'effective leaf area loss' for this disease.

Table 1. Host distribution of the most common fungal species

M, *Monocalyptus* species: M1, *E. radiata*; M2, *E. dives*; M3, *E. delegatensis*; M4, *E. pauciflora*
S, *Symphyomyrtus* species: S1, *E. viminalis*; S2, S3, S4, *E. dalrympleana*

Frequency of fungal spp.: +++ abundant; ++ common; + present; - absent

Fungus	Distribution of fungal species							
	Site 1		Site 2		Site 3		Site 4	
	M1	S1	M2	S2	M3	S3	M4	S4
<i>Hendersonia</i> sp. 1	+	+	++	++	+	++	+++	++
<i>Aulographina eucalypti</i>	+	-	++	-	++	-	++	-
<i>Mycosphaerella</i> sp. 1	-	-	-	+++	-	+++	-	+++
<i>Mycosphaerella</i> sp. 2	+++	-	+++	-	-	-	-	-
<i>Septoria normae</i>	-	+++	-	++	-	-	-	+
<i>Seynesia</i> sp. 1	-	-	+	-	+	-	+	-
<i>Stagonospora</i> sp. 1	-	-	+	-	++	-	-	-

3. Results

(a) Host Distribution of Parasite Species

(i) Fungi

A total of 23 different fungal species were distinguished from leaves collected at the four sites. The site and host distribution of the seven most common species are given in Table 1. With the single exception of *Hendersonia*, these species exhibit field specificity towards one or other subgenus of *Eucalyptus*.

$$* \text{ Lesion factor} = 1 - (D_b/C_b) + \frac{D_a - D_b}{C_a + (D_a - D_b)(A_a/A_c)},$$

where D_b and D_a are the activities per unit area of diseased lesion tissues, before and after redistribution of the tracer had been allowed to take place within the leaves; C_b and C_a are the activities per unit area of healthy control tissues, before and after redistribution of the tracer; A_c and A_a are the areas of healthy and diseased tissue of leaves counted after redistribution of the tracer.

(ii) *Insects*

A total of 99 different species were distinguished from systematic collections of adult beetles and leafhoppers made at the four sites. Table 2 summarizes the host distribution of the 17 species which contributed 1% or more of the total catch. Most of these were collected from two or more sites. Four of these species (*Eurymeloides punctata*, *Eurymela* sp. 1, *Chrysophtharta fuscum* and *Gonipterus suturalis*) were found exclusively on eucalypts of one or other subgenus. In three other species (*Chrysophtharta agricola* and *Pentatomidae* spp. 1 and 2) more than 90% of the individuals were found on one or other eucalypt subgenus. In view of the potential mobility of these seven species and the close proximity of the different host subgenera

Table 2. Host distribution of the most common insect species

Insect species	Percentage of total collected	Found at sites	Total no. of individuals caught on:		Host preference of insect
			<i>Monocalyptus</i>	<i>Symphyomyrtus</i>	
<i>Chrysophtharta agricola</i>	25	1, 2, 3, 4	30	292	S
<i>Eurymeloides punctata</i>	13	1, 2, 4	—	173	S
<i>Pentatomidae</i> sp. 1	9	1, 3, 4	108	10	M
<i>Gonipterus scutellus</i>	5	1, 2, 3, 4	28	32	—
<i>Chrysophtharta aurea</i>	4	1, 2, 3, 4	13	37	—
<i>Tartessus fulvus</i>	3	2, 3, 4	28	11	—
<i>Automolus</i> sp. 1	3	2, 4	23	15	—
<i>Heteronyx striatipennis</i>	3	3, 4	10	25	—
<i>Eurymela</i> sp. 1	3	2	34	—	M
<i>Chrysophtharta fuscum</i>	2	3, 4	—	31	S
<i>Edusella</i> sp. 1	2	2, 4	23	8	—
<i>Pentatomidae</i> sp. 2	2	1, 2, 4	2	25	S
<i>Cadmus litegrosus</i>	2	2, 3, 4	7	17	—
<i>Eurymela rubrolimbata</i>	2	1, 3, 4	11	11	—
<i>Gonipterus suturalis</i>	2	2, 4	24	—	M
<i>Paropsis lutea</i>	1	1, 2, 3, 4	9	10	—
<i>Lagria grandis</i>	1	1, 2, 4	4	14	—

(in many cases the crowns were touching or overlapping), such a pattern of distribution indicates that these insects have a strong preference or perhaps a specific requirement for a particular subgenus of *Eucalyptus*. Six of the remaining insect species were distributed with a host bias greater than 70%, leaving only four of the 17 major species more or less equally distributed between the two eucalypt subgenera.

Of larvae which were collected, those of *Perga* and *Pseudoperga* were the most abundant, totalling between 500–600 individuals. These larvae could not be identified to species level, so any host bias which might exist could not be detected. Approximately 20 other larval species (mainly Lepidoptera) were distinguished in the collections but these were present at low frequencies only. In addition, certain very small lepidopterous larvae (*Incurvariodea*?) were estimated during leaf damage assessment. On the basis of the number of tracks counted (c. 15 per sq metre of foliage), several thousand individuals must have been present over the four sites. All of these were found on the one host species—*E. dalrympleana*.

From field assessments of sessile insects, scale insects (*Ericoccus* sp.) were found to be moderately abundant, being distributed as a limited number of compact colonies on species of both *Eucalyptus* subgenera. Seventeen different types of insect-caused leaf and stem galls were distributed throughout the sites at low frequency. Only two types were found on both host subgenera, which indicated a high degree of host specificity among this group.

(b) *Damage to Leading Shoots*

(i) *Overall Leaf Losses*

The sampling method provided *c.* 1 sq m of leaf area for damage assessment from each species. In Table 3, damage measurements are expressed in terms of the percentage of the leaf area sampled, to permit ready comparisons between the 'effective

Table 3. Different classes of leaf damage on the leading shoots

4a and 4b refer to the same site sampled in March and June respectively

M indicates *Monocalyptus* species: *M1*, *E. radiata*; *M2*, *E. dives*;

M3, *E. delegatensis*; *M4*, *E. pauciflora*

S indicates *Symphyomyrtus* species: *S1*, *E. viminalis*; *S2*, *S3*, *S4*, *E. dalrympleana*

Site	Species	Mean leaf area (m ²) sampled	Percentage effective leaf area loss (mean of four samples)			
			Missing	Dead	Diseased	Total ± SEM
1	<i>M1</i>	0.29	2.7	15.1	—	17.8 ± 9.3
	<i>S1</i>	0.29	3.6	0.3	9.1	13.0 ± 1.6
2	<i>M2</i>	0.26	11.2	11.9	—	23.1 ± 8.8
	<i>S2</i>	0.21	13.8	25.6	4.5	43.9 ± 8.8
3	<i>M3</i>	0.43	5.1	1.9	—	7.0 ± 2.1
	<i>S3</i>	0.33	9.1	19.0	—	28.1 ± 8.5
4a	<i>M4</i>	0.17	20.2	1.0	—	21.2 ± 6.9
	<i>S4</i>	0.18	27.5	4.8	—	32.3 ± 9.7
4b	<i>M4</i>	0.29	12.7	2.4	—	15.1 ± 8.5
	<i>S4</i>	0.34	9.1	20.6	0.3	30.0 ± 10.7

leaf area' lost by different *Eucalyptus* species. Damage varied considerably between sites, between species, and between individual trees of the same species. The site with the greatest damage (2) had double the percentage effective leaf area loss of the site with the least (1). Most of this discrepancy was due to the heavy losses sustained by *E. dalrympleana* at site 2. *E. dalrympleana* in fact showed consistently more damage than its various *Monocalyptus* associates, and at two sites (2 and 3) this difference was statistically significant. Samples taken from site 4 at two different times of the year exhibited very similar total percentage effective leaf area losses.

(ii) *Different Classes of Damage*

Missing and dead tissues accounted for most of the effective leaf area lost; diseased tissue was insignificant on all but two species (Table 3). Although the percentage of missing leaf tissue varied considerably between sites, it was more or less similar for the two *Eucalyptus* species at any one site. By contrast, the percentage of dead tissue differed greatly between the two species at each site. Thus, *E. radiata* (*M*) had *c.* 50 times the dead tissue shown by its associate *E. viminalis* (*S*), and *E. dalrympleana*

(S) had 10 times the dead tissue borne by *E. delegatensis* (M). At site 4, while the total loss of effective leaf area remained fairly constant between March and June, the relative proportions of missing and dead tissue changed markedly, particularly on *E. dalrympleana*, where the percentage of missing tissue declined by one-third and the percentage of dead tissue increased fivefold.

(iii) Proportion of Damage due to Host-specific Parasites

It was clear that, in general, most of the missing leaf area resulted from the attacks of biting insects, and the greater part of dead and diseased tissues was caused by parasitic fungi. The presence of fruiting structures on most of the fungal lesions made it possible to distinguish between their causal agents. A few of the more distinctive lesions caused by insects were also distinguished. These data permitted some of the damage to be classified according to whether it was caused by host-specific or host-non-specific parasites. Fig. 4 shows the proportion of all damage, measured on each species, which was identified as host-specific within the particular association.

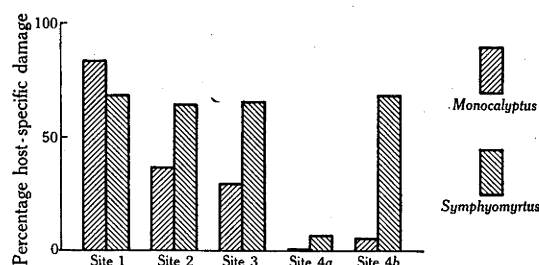


Fig. 4. Percentage of the total leaf damage on each eucalypt species which was identified as host-specific within the association. 4a and 4b refer to the same site sampled in March and June respectively.

Monocalyptus species: site 1, *E. radiata*; site 2, *E. dives*; site 3, *E. delegatensis*; site 4, *E. pauciflora*. Symphyomyrtus species: site 1, *E. viminalis*; sites 2, 3 and 4, *E. dalrympleana*.

4. Discussion

In this brief survey, parasite damage to the leading shoot of regrowth *Eucalyptus* saplings was found to vary considerably among individual trees and between different species, but taken overall it averaged more than 20% effective leaf area loss. Although most measurements were taken at one time only, from general observations throughout the year and the results of the one repeat assessment, it appeared that the overall level of damage at each site remained fairly constant, as overlapping waves of parasites attained successive optima. Preliminary experiments involving artificial defoliation of eucalypt seedlings (not described), suggested that damage of the above order can bring about a marked depression in the overall growth rates of the plants, which is in agreement with many other reported findings. Kulman (1971) reviewed a total of 174 cases of natural or artificial defoliation and concluded that all but seven showed a direct correlation between the severity of the defoliation and the consequent reduction in growth. It therefore seems reasonable to envisage an ecologically significant role for eucalypt leaf parasites in these sapling stands.

Of the 27 leaf parasite species most commonly represented, 11 were collected exclusively from one or the other subgenus of *Eucalyptus*. This finding lends considerable support to the original proposition that host specificity should be sought predominantly at the subgeneric level, although there was evidence to suggest that some parasites may be restricted to an even narrower range of hosts. For instance, *Mycosphaerella* sp. 1 was found on both *E. dives* and *E. radiata* of the series *Piperitae*

but not on *E. delegatensis* or *E. pauciflora* from other series within the same subgenus. Approximately half of all the leaf damage recorded at the four sites could be recognized as the result of parasites exhibiting host specificity of the above types. This figure is certain to be an underestimate, because much of the damage observed could not be related to any particular parasite species.

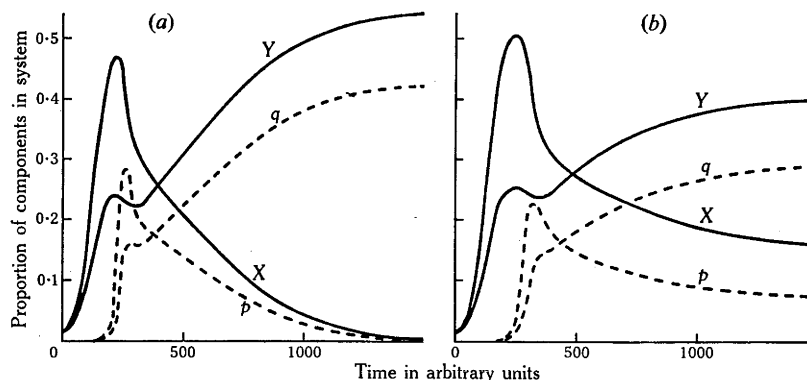


Fig. 5. Computed illustrations of the effect of a non-specific parasite on two competing host species. (a) A system in which the parasite moves freely between the two host species. (b) A system in which the parasite exhibits a 'food-conditioned' form of host preference.

Computations are based on the simple model:

$$\frac{dX}{dt} = R_X X(1 - X - Y) - D_n X - D_p p,$$

$$\frac{dY}{dt} = R_Y Y(1 - X - Y) - D_n Y - D_q q,$$

$$\frac{dp}{dt} = R_p(p + B_q q)(X - p) - D_n p - D_p p,$$

$$\frac{dq}{dt} = R_q(q + B_p p)(Y - q) - D_n q - D_q q.$$

X and Y represent the proportion of the two hosts in the environment; $p + q$ represents the proportion of a non-specific parasite in the environment, p being the proportion on host X and q the proportion on host Y ; R_X , R_Y , R_p and R_q represent growth rate constants for the four components; D_n is a death rate constant due to random non-biotic factors; D_p and D_q are the death rate constants of parasitized tissues; B_p and B_q are bias constants describing the success rate of inoculum transfer between host species relative to the success rate of transfer within host species.

To compute Fig. 5a, both bias constants were set at 1.0 to permit inoculum to flow as freely between host species as within host species. In Fig. 5b both bias constants were set at 0.33 to reduce the flow of inoculum between species. All other parameters and starting conditions were given identical values in both computations.

In addition to the host-specific parasites there were many non-specific phytophagous insects which nevertheless showed distinct host preferences, both in terms of their distribution in the field and in simple food choice experiments in the laboratory. The proposals for density-dependent feedback mechanisms to control species diversity (Janzen 1970; Chilvers and Brittain 1972) have specified the need for host-specific parasites, but it is worth considering the possibility that host preference may have similar potential. With simple modifications to the Chilvers and Brittain (1972) continuous systems model, host preference was therefore examined theoretically (Fig. 5).

Fig. 5a illustrates a simple ecosystem with two hosts X and Y and a non-specific parasite pq . Parasite pq actually exhibits 'preference' for the faster-growing host X to the extent that it multiplies more rapidly on this host and causes more damage to it. This is why X rapidly loses its initial advantage over the slower-growing Y . However, computations show that this type of parasite has no potential for bringing the competitive host system into equilibrium, and host X can be seen declining towards extinction in the example given. Fig. 5b is an illustration of the same components, which have been given the same initial properties as in Fig. 5a, but in this case the parasite pq is 'food-conditioned' (Jermy *et al.* 1968), such that parasite produced on host X (p) can transfer to host Y with only one-third of the efficiency with which it reinfects host species X , and parasite produced on host Y (q) will be similarly biased against transfer to host X . As the illustration shows, such a parasite generates sufficient feedback damage to the more plentiful host species to bring the system into equilibrium. It is less efficient in this regard than two completely host-specific parasites would be, and it will only sustain equilibria within certain limits of the parameters supplied, but it does indicate that non-specific parasites which show a food-conditioning type of host preference could make a contribution to an ecological feedback system.

Taking all the above factors into consideration, there appears to be considerable potential within these young eucalypt associations for the operation of density-dependent stabilizing mechanisms based on the impact of parasites. This conclusion receives some support from a comparison which was made between contiguous sapling and mature stands of *E. dalrympleana* plus *E. pauciflora*. The mature stand was found to contain 33 *E. dalrympleana* per hectare compared with 87 *E. pauciflora*, a ratio of 0.38 to 1. In the dense young sapling stand there were 4119 *E. dalrympleana* per hectare compared with 3684 *E. pauciflora*, a ratio of 1.12 to 1. If the mature stand is considered as the 'normal' situation arrived at after many years of equilibration, then the sapling stand has a preponderance of *E. dalrympleana* plants. It is perhaps significant, therefore, that the *E. dalrympleana* saplings were found to be subject to a very much more severe parasite impact than the *E. pauciflora* saplings (29.9% compared to 15.1% effective leaf area loss) while adults of these two species in the mature stand were found to suffer more or less similar parasite impact (9.1% compared to 7.3% effective leaf area loss). The overall reduction in damage from sapling to adult stages is also consistent with the operation of density-dependent parasitism. However, it must be acknowledged that the reduced impact of parasites upon mature trees will also be due in part to genetic selection processes. Parasite attack was seen to be distributed unevenly between saplings of the same species (cf. standard errors of means in Table 3), and it is inevitable, during the intense competition which would accompany such an enormous reduction in host density, that the more susceptible host individuals will become preferentially eliminated.

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Leaf Parasites on Altitudinal Populations of *Eucalyptus pauciflora* Sieb. ex Spreng.

J. J. Burdon and G. A. Chilvers

Botany Department, School of General Studies,
Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

Abstract

Preliminary studies of fungal parasites and phytophagous insects on saplings of *Eucalyptus pauciflora* Sieb. ex Spreng. growing at different altitudes, suggest that the impact of these organisms is inversely related to the rigour of the physical environment.

Introduction

Eucalyptus pauciflora Sieb. ex Spreng. grows in the Brindabella Mountains of the Australian Capital Territory within the altitudinal range 1100-1830 metres. Through most of this range it occurs alone, but at lower altitudes it forms mixed stands with *E. dalrympleana* Maiden.

Pryor (1956) studied variation in *E. pauciflora* between different altitudes within the Australian Capital Territory-Kosciusko region and found that tree height, leaf length and bark thickness decreased with increasing altitude; while by contrast, fruit diameter showed a progressive increase with altitude. In addition, limited tests of the capacity of seedlings to withstand frost showed that those derived from seed from high elevations were much more resistant to low temperatures than seedlings from plants at lower elevations. Green (1967) made a more detailed study of the problem of temperature effects upon plants derived from seed collected at different altitudes and came to the conclusion that low winter temperatures play a major role in the maintenance of the clinal variation. More recently Paton (1972) suggested that other environmental factors may prove to be important in determining the altitudinal distribution of *E. pauciflora*. So far, there appear to be no reports on the role of other organisms within this ecosystem.

The present paper describes a preliminary survey of leaf parasites* and their effect on regrowth saplings of *E. pauciflora* at different altitudes in the Brindabella range. Four sites, having similar aspect and slope, were selected at altitudes of 1220, 1480, 1650 and 1750 metres. At the lowest site (1220 m), *E. dalrympleana* occurred mixed with *E. pauciflora* and this species was consequently included in the study. Several collections of insects and fungi were made during the period January to April 1972, and in late March 1972 the leading shoots were sampled from four representative saplings at each site and measurements made of the 'effective leaf area' lost to insect

* The term parasite is used throughout this paper in a broad sense, intended to include phytophagous insects with those organisms, such as fungi and cecidiogenic (gall-forming) insects, to which the term is usually applied.

and fungal attack. The methods used to collect parasites and to assess leaf damage have been detailed elsewhere (Burdon and Chilvers 1974).

Results

The main findings are summarized in Fig. 1.

Fig. 1a shows the mean production of new leaf area (spring and summer growth) per leading shoot for each altitude. Leaf area growth was significantly greater at 1220 m altitude than at 1750 m ($P < 0.05$), and exhibited a progressive decline in between. Leaf growth of *E. pauciflora* was very similar to that of *E. dalrympleana* at the lowest site.

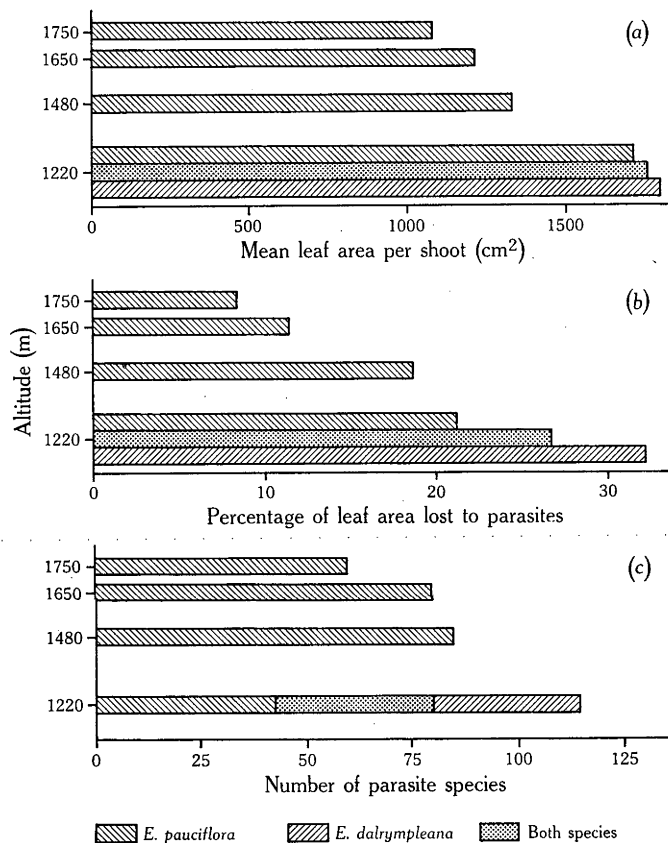


Fig. 1. Altitudinal changes in *E. pauciflora*. (a) Mean production of new leaf area on the leading shoot of saplings growing at different altitudes. (b) Mean percentage loss of new leaf area to insect and fungal attack in the same samples. (c) Total number of parasite species collected at each altitude.

Fig. 1b shows the effects of parasitic attacks at each altitude, in terms of the mean loss of 'effective leaf area' expressed as a percentage of the total leaf area produced.

Differences between altitudes were not very significant owing to the high variance between trees (a comparison between the 1220 m and 1750 m sites gave $0.2 < P < 0.1$), but the mean values show a consistent trend towards decreased damage at higher altitudes. In the mixed stand at the lowest site, *E. dalrympleana* had lost relatively more 'effective leaf area' to parasites than had *E. pauciflora*.

Fig. 1c shows the number of different parasite species (Appendix 1) collected from trees at the various altitudes. The greatest number of species occurred at the lowest altitude and progressively fewer species were represented as the altitude increased. This altitudinal change in species numbers occurred throughout all the insect orders but was most marked in the case of Coleoptera, which declined from 53 to 23 species between 1220 and 1750 m altitude. By contrast, there was practically no change in the number of fungal species recognized. In the mixed stand, approximately one-third of the parasite species collected were found on *E. dalrympleana* as well as *E. pauciflora*, and another third were only found on *E. dalrympleana*. Only three of the latter group were represented at any of the higher altitudes populated by *E. pauciflora* alone.

In addition to the above results, two other general observations were made. At the lowest site, for reasons which are not yet understood, hardly any of the previous season's leaves remained on the *E. pauciflora* saplings at the time of sampling. By contrast, at the highest site most of the previous season's leaves had persisted, and a few leaves appeared to have survived from the season before that. These older leaves supported a large number of fungal leaf spots, with the result that fungi were among the most prominent parasites at the higher altitudes. Insect galls were also more common at the higher sites than lower down. By contrast, the migratory phytophagous insects, particularly adult chrysomelids and scarab beetles, were most numerous at the lowest site, where they were responsible for the greater part of the leaf damage measured.

Discussion

In *Eucalyptus pauciflora* sapling stands the relationship between increased leaf surface area growth and decrease in altitude is consistent with the results of earlier studies (e.g. Pryor 1956; Green 1967, 1969), which showed similar altitudinal trends in other growth parameters. These authors demonstrated, in pot experiments carried out under standardized conditions, that growth rates of seedling progeny also varied with the altitude of the parent trees. This indicates that the rates of growth at different altitudes are to a large extent genetically determined, and the above authors discuss the significance of this in terms of adaptation to physical stresses imposed by the environment. No doubt physical factors are paramount determinants of growth rate at the higher altitudes, and they probably define the uppermost limit to which *E. pauciflora* can grow (c. 2000 m). However, it seems likely that biotic factors are more important at the lower altitudes. For instance, from 1370 m downwards *E. pauciflora* is subjected to progressively increased competition for space in the habitat from *E. dalrympleana*, which was found to represent 5% of tree boles at 1370 m, increasing to 17.5% at 1310 m and eventually reaching 27.5% at 1220 m (the lowest site). A little distance below this altitude *E. pauciflora* is replaced quite abruptly in the mixed stand by *E. dives* (on the ridges) or *E. delegatensis* (in the heads of the more sheltered gullies). It may reasonably be concluded that competition from these larger tree species is the main factor preventing the spread of *E. pauciflora* to lower altitudes.

in the range. However, the downhill trend towards greater diversity of parasites and increased leaf damage on *E. pauciflora* raises the possibility that these organisms may play a role in tree population changes at lower altitudes.

A 20% reduction in the current season's leaf area must have a considerable impact on growth, reducing it below the theoretical growth potential which should result from interaction between the plant genome and the physical environment alone. Perhaps this is sufficient to prevent *E. pauciflora* from fully exploiting the environmental resources at lower altitudes, leaving space for the entry of another tree species having a somewhat different range of parasites, in the manner suggested by Harper (1969). Certainly the reduction in net growth must have an adverse effect on the overall competitive ability of the affected trees.

In view of the possible ecological implications, further work, aimed at obtaining a more precise definition of the altitudinal distribution and impact of leaf parasites on *E. pauciflora*, appears to be justified. It would also be valuable to know whether the observed trends are directly attributable to the physical environmental gradient or whether it is a response to the increasing density of plant tissue at the lower altitudes. Reduction in the number and diversity of free-living insects at the higher altitudes, and the relative increase in truly parasitic forms living within the protection of the host tissues, suggest that the environment exerts a direct selective effect on the parasites. On the other hand, the fact that there is relatively little difference in damage and diversity of parasites between *E. pauciflora* at the lowest two sites (compare Figs. 1b and 1c), suggests that the dilution of the 1220 m *E. pauciflora* stand with *E. dalrympleana* might have counteracted the general downhill trend towards increased leaf damage and diversity of parasites on *E. pauciflora*. Burdon and Chilvers (1974) provide evidence for the existence of a considerable degree of host-specificity among the parasites, and Chilvers and Brittain (1972) describe a density-dependent mechanism based on host-specific parasites which could produce such an effect.

Acknowledgments

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Appendix 1. Taxonomic Affinities of Parasite Species Collected

Parasite species marked with asterisks (*) were collected only from *Eucalyptus dalrympleana*.

Coleoptera. CERAMBYCINAE: *Phoracantha* sp.* CHRYSOMELINAE: *Chrysophtharta agricola*, *C. aurea*, *C. fuscum**, *Chrysophtharta* 4 spp., *Chrysophtharta* 2 spp.*, *Paropsis augusta*, *P. augustata*, *P. lutea*, *Paropsis* 3 spp., *Paropsisterna nucea**, *Paropsisterna* 2 spp., *Trachymela chapuisi*?, *T. regularis*, *T. nr rosea*, *Trachymela* sp.*, *Trachymela* 3 spp. CRYPTOCEPHALINAE: *Cadmus crucicollis*, *C. flavocirnotus*, *C. gigas*, *C. litegrosus*, *Cadmus* 6 spp., *Cryptocephalus haematoides*, *C. subfasciatus*, *Cryptocephalus* 4 spp., *Edusella multicolor*, *Edusella* 3 spp., *Haltica* ? sp., *Ishiocephala* 3 spp., 1 unidentified sp. LAMINAE: 1 unidentified sp.* LAGRIA: *Lagria grandis*. APIONIDAE: *Euryrochus* sp.*, *Euryrochus* sp., 3 unidentified sp. ATERPINAE: *Rhinaria* sp.*, *Rhinaria* sp. BEASHYDARINAE: *Prypmus angustus*. CLEONINAE: 1 unidentified sp. CYLLORHAMPHINAE: *Cyllorhamphus* sp. ERIRRHININAE: *Rachiodes multidentatus*, *Rachiodes* sp.*, *Rachiodes* 2 spp. GONIPTERINAE: *Gonipterus scutellatus*, *G. suturalis*. HAPLONYCHINAE: *Haplonyx schonherri*, *Haplonyx* sp. RHADINOSOMINAE: *Rhadinosomus larcordairai*. MELOLONTHINAE: *Heteronyx striatipennis*, *Heteronyx* 2 spp., *Eurychelus mannoratus*, *Automolus funereus*, *Automolus* 2 spp., *Automolus* sp.* RUTELINAE: *Anoplognathus suturalis*; 4 unidentified species of larvae.

Hemiptera. AUSTROGALLOIDINAE: *Austroagalloides rosea*. EURYMELIDINAE: *Eurymela rubrolimbata*, *E. tonnoiri*, *Eurymeloides bicincta*, *E. punctata**, *Katipo* sp., 1 unidentified nymph sp., 3 unidentified nymph spp.* IDIOCERINAE: *Idiocerus* sp. TARTESSINAE: *Tartessus fulvus*. COCCOIDEA: 2 unidentified spp. COREOIDEA: 4 unidentified spp. PENTATOMINAE: *Hypogomphus* 2 spp., *Hypogomphus* sp.*, 3 unidentified spp., 1 unidentified sp.* SPONDYLIASPINAE: *Glycaspis* sp.

Hymenoptera. PERGINAE: *Pseudoperga* sp. (larvae).

Lepidoptera. 18 unidentified species of larvae.

Orthoptera. ACRIDOIDEA: 2 unidentified spp., 1 unidentified sp.*

Phasmatodea. PHASMATIDAE: 1 unidentified sp.

Insect galls. 25 distinctive types of insect galls were recorded (9*).

Ascomycetes. HYSTERIACEAE: *Aulographina eucalypti*, 2 unidentified spp. (1*). SPHAERIACEAE: *Mycosphaerella* sp.*, *Seynesia* sp.

Deuteromycetes. PHOMACEAE: *Dichomera* sp., *Hendersonia* sp., *Septoria normae**; 2 unidentified spp.

Epidemiology of damping-off disease (*Pythium irregulare*) in relation to density of *Lepidium sativum* seedlings

BY J. J. BURDON AND G. A. CHILVERS

Botany Department, School of General Studies,
Australian National University, P.O. Box 4, Canberra, Australia

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SUMMARY

Controlled experiments on post-emergence damping-off, using small populations of garden cress seedlings (*Lepidium sativum*) inoculated with *Pythium irregulare*, demonstrate that planting density of the host population plays an important role in determining the rate of multiplication and the rate of advance of the disease. At high seedling densities the disease is transmitted readily between host plants, but at lower densities the greater distance between adjacent plants reduces the probability of successful transmissions, and this is reflected in the parameters of multiplication and advance. A simple negative relationship was found between the mean distance separating adjacent plants and both rate of advance of disease front and rate of multiplication of disease in a randomly inoculated seedling stand.

INTRODUCTION

In studies on the epidemiology of damping-off diseases, considerable attention has been given to the role of environmental factors such as soil moisture, soil aeration, soil pH, temperature, incident light, plant nutrient status and the incidence of other micro-organisms (Garrett, 1970; Hendrix & Campbell, 1973). Since the high level of susceptibility of very young seedlings to damping-off pathogens is lost quite rapidly as the plant tissues mature, disease incidence in a given environment can be viewed as a consequence of the interaction between the rate of seedling development and the rate at which the pathogen mobilizes for the attack (Garrett, 1970; Webster *et al.* 1970). Thus Leach (1947) showed that, at any particular temperature, the proportion of plants killed prior to emergence could be predicted from the ratio of the linear growth rate of the pathogen to the rate of seedling emergence. This may not have meant, however, that the linear growth rate *per se* was necessarily the only property of the pathogen that was causative in this instance because correlates of the linear growth rate, such as increases in the number of hyphal tips per unit area of host surface and the level of metabolic activity of the pathogen, could have made important contributions to its overall inoculum potential (*sensu* Garrett, 1956). Nevertheless Leach's work is especially notable for the stress it placed on time as a dimension of critical importance in this disease syndrome.

In addition to the need for appropriate stages of host and parasite to coincide in time, it is also necessary for the two organisms to be juxtaposed in space. While

this latter statement is self-evident, its implications, in terms of the spatial distribution of the two organisms, have not attracted a great deal of attention, due perhaps to a tendency for the main areas of concern with damping-off to emphasize extreme situations. Thus horticultural practice, especially that involved in growing nursery seedlings, seeks to prevent contact between host and parasite entirely, while many of the controlled experiments designed to study the effect of environmental variables are set up with contact between host and parasite deliberately maximized. In more natural situations such clear-cut divisions rarely occur since graded differences in the frequency and distribution of host plants and inoculum of the parasite will affect the probability of their cohabiting the same point in space. Considered over the long term, spatial distribution of the organisms is of course largely determined by the prevailing environmental conditions, but in the short term it is profitable to consider the disposition of host plants and inoculum as primary variables in the system. For instance, Baker and his co-workers (Baker, Maurer & Maurer, 1967; Baker, 1968, 1971) have recently investigated, by means of theoretical models, the proportion of primary infection events to be expected from different levels of inoculum intensity applied to a constant array of plant hosts.

Van der Plank (1963) notes the two main parameters which are required to determine the course of an epidemic: the initial level of disease and the subsequent rate at which disease multiplies within the system. In damping-off diseases the former can be equated with the number of primary infection loci and the latter with the multiplication of secondary infections. The multiplication of disease by secondary infections involves spread from the primary foci and is hence a phenomenon in which both space and time are of importance. The authors' interest in this question stems from suggestions (Harper, 1969; Janzen, 1970; Chilvers & Brittain, 1972) that density-dependent interactions between plants and their parasites are of great significance in natural ecosystems, where not only inoculum intensity but also plant density is a significant variable. More specifically, changes in plant density are seen as a form of adaptation to environmental circumstances (predominantly biotic), and in this sense should be equated with phenotypic plasticity and genetic selection. Damping-off diseases provide particularly suitable experimental systems for the study of density effects because, as Garrett (1970) states, they represent 'true epidemics in microcosm'.

In the study described here, different density populations of *Lepidium sativum* L. were inoculated with a standard quantity of *Pythium irregulare* Buisman (IMI 183522) in order to evaluate the effects of host density on epidemic rates within the system. Experiments were carried out under conditions of low light and high humidity intended to prolong the susceptible state in the host plants and thereby minimize the effect of increased resistance with time. Two different experimental arrangements were employed to measure: (i) rates of multiplication of disease within randomly inoculated seedling stands, and (ii) rates of advance of disease fronts moving across seedling stands from a line of inoculum placed along one edge.

METHODS

Multiplication of disease in a randomly inoculated stand

Preparation of inoculum. Particulate inoculum was prepared according to the method described by Chilvers (1962). Conical flasks (250 ml) containing 100 ml of sterile vermiculite (Grade 3; Neuchatel Asphalt Co.) moistened to maximum water-holding capacity with 60 ml of a nutrient solution containing 20 g of dextrose/l of carrot-potato extract (Dade & Ginnell, 1966), were inoculated with *P. irregulare* and incubated for 8 to 10 days at 25 °C. The resulting concentrated inoculum was washed six times in 200 ml lots of sterile distilled water to remove most of the residual nutrients and to separate the granules of vermiculite. The concentrated inoculum was then diluted by mixing with sterile vermiculite in a ratio of 1:400.

Inoculation of seed boxes. Medium-coarse washed river sand (1000 g), with the water content adjusted to 14% by weight, was distributed evenly in plastic food containers measuring 25 × 25 cm and 7 cm deep. *L. sativum* seed, spaced to represent appropriate densities, was then sown evenly in an area 21 × 17 cm in each container and covered with 150 ml of dilute inoculum. The containers were sealed with plastic film (sandwich wrapping) to maintain a high humidity and incubated in L.B. type phytotron cabinets (Morse & Evans, 1962) at 21 °C using a 14.5 h day with a light intensity of 48 lm/m². In each experiment, four to six planting densities were replicated four or more times.

Measuring the rate of multiplication of disease. After 3 days incubation the numbers of seedlings appearing were compared with the known seeding rates for evidence of pre-emergence damping-off. In all cases the majority of seedlings emerged successfully, indicating that pre-emergence damping-off was negligible. At the same time, and at every subsequent 24 h interval up to the tenth day after inoculation, the number of diseased plants in each container was counted. Plants within 2 cm of the edge of the plots were ignored.

Advance of the disease front

Preparation of inoculum. Young *L. sativum* seedlings were cut off at ground level and laid upon the surface of *P. irregulare* mycelium growing on potato dextrose agar. The seedlings became rapidly infected and were used as inoculum on the following day.

Inoculation of seed boxes. The experimental system was set up as described above except that, after sowing, the *L. sativum* seeds were covered with sterile vermiculite. Five diseased seedlings were laid along one edge of the plot. Each separate experiment examined several different host densities using a minimum of four replicates of each density.

Measuring the rate of advance of the disease. On the third day after inoculation and at 24 h intervals thereafter until the tenth day from inoculation, five measurements were made in each container of the distance that the disease front had advanced from the inoculated edge of the plot. The measurements were spaced evenly across the plot excluding the outermost 2 cm on each side.

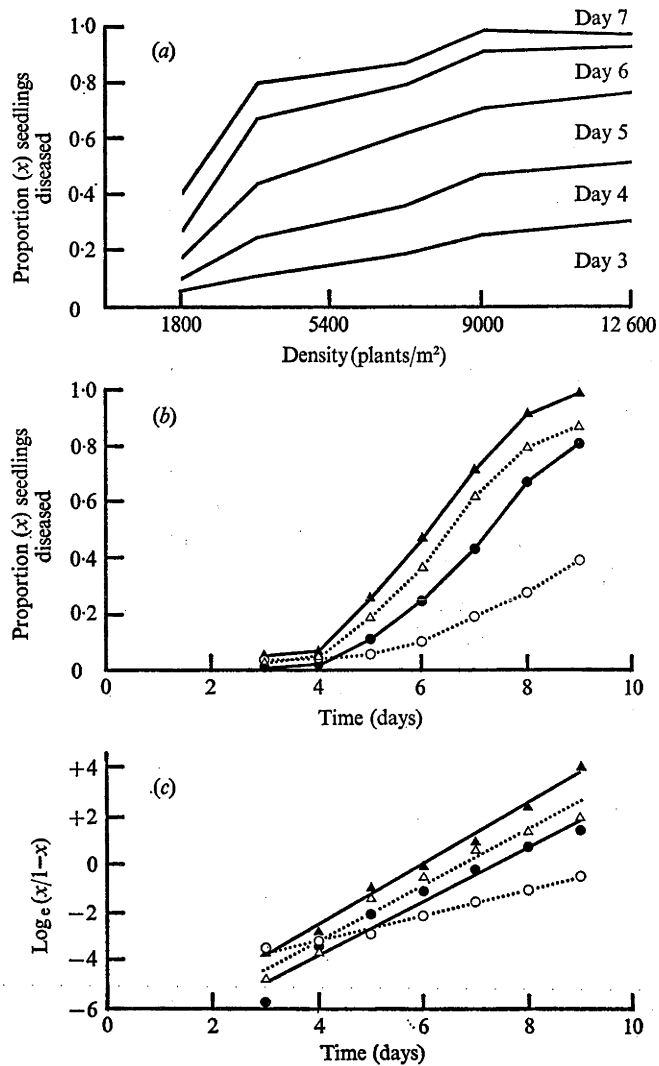


Fig. 1. Detailed results of a single experiment of damping-off in randomly inoculated plots. (a) Proportion of diseased seedlings (x) in relation to planting density, plotted for five consecutive days. (b) Changes in proportion of diseased seedlings (x) with time. Symbols as for Fig. 1 c. (c) $\text{Log}_e (x/1-x)$ plotted against time and fitted by linear regression. ○, 1800 plants/m²; ●, 3600 plants/m²; △, 7200 plants/m²; ▲, 9000 plants/m².

RESULTS

Multiplication of disease in randomly inoculated seedling stands

The results of one experimental run are expressed as the proportion of damped-off seedlings (x) plotted against host density (Fig. 1 a). It can be seen that early in the epidemic there is a more or less linear relationship between the proportion diseased and host density, but as time progresses this relationship is lost. In Fig. 1 b, x is plotted against time (days) in order to illustrate the progress of the epidemic in host popula-

tions of different densities (mean of five replicates). From those curves which go close to completion it can be seen that the epidemic follows a sigmoidal type of progress curve. These curves were 'straightened' by transforming x to $\log_e(x/l-x)$ (van der Plank, 1963), and are shown together with the lines of best fit determined by regression analysis (Fig. 1c). The gradient of these lines provides the best estimate of the multiplication rate or 'apparent infection rate' (r) of the disease (van der Plank, 1963) for each host density. The highest apparent infection rates occur in the denser host stands and the lowest rate in the least dense stand.

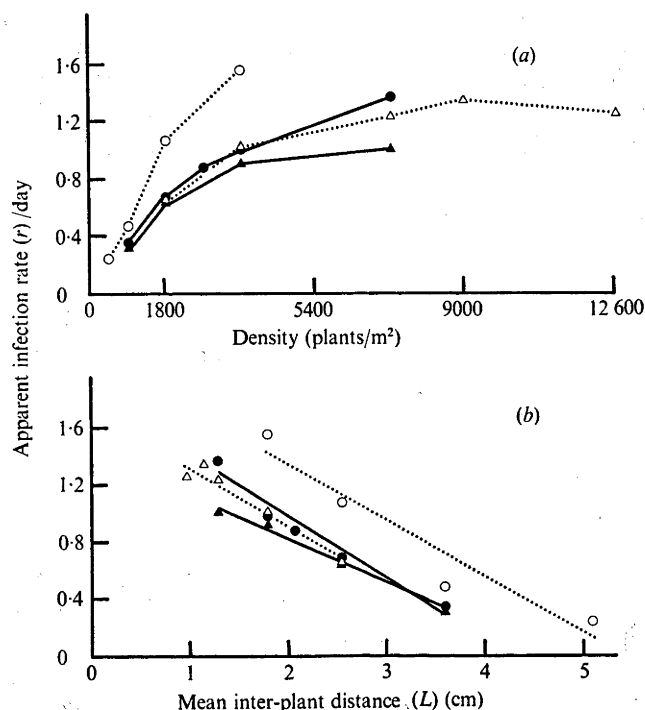


Fig. 2. Combined results of four experiments on damping-off in randomly inoculated plots. (a) Index (r) of rate of multiplication of disease (rate of change of $\log_e(x/l-x)$, per day) plotted against seedling density. (b) Index (r) of rate of multiplication of disease plotted against the mean distance (L) separating adjacent seedlings, fitted by linear regression. ○, ●, △, ▲, results of different experiments.

The r values derived (in the manner above) from the results of four separate experiments are plotted against the density of plants in the stand (Fig. 2a). Despite variation between the separate experiments a relationship can be clearly discerned. Increases in host density within the range 0–2000 seedlings/m² produce large increases in the apparent infection rate, but increases in host density above that range have progressively less effect on r until a maximum rate is approached in the region of 10000 seedlings/m².

Changes in plant density (D) affect the mean distance (L) between adjacent plants ($L \propto 1/\sqrt{D}$), and when r values were plotted against this parameter they appeared to

be related in a simple linear manner with negative slope. The slopes of the four linear regression lines fitted to such plots representing the four experiments (Fig. 2*b*) are statistically significant ($P < 0.05$ and in two cases $P < 0.01$).

Advance of the disease front

Mean values for the daily advance of damping-off (a), derived from more than 100 measurements within each treatment (five measures in each of four replicate containers on all 7 days), are plotted against seedling density (Fig. 3*a*). The four graphs show better agreement than those for rates of multiplication (Fig. 2*a*), which is due in large part to the greater number of measurements available in this case. The general form of the relationship between the rate of advance of the disease and host density is similar to that for the apparent infection rate. Increasing plant density within the range 0–2000 seedlings/m² produces large increases in a , but further increases in density have progressively less effect on a until a maximum rate is approached in the region of 20000 seedlings/m². When a values were plotted against the mean distance

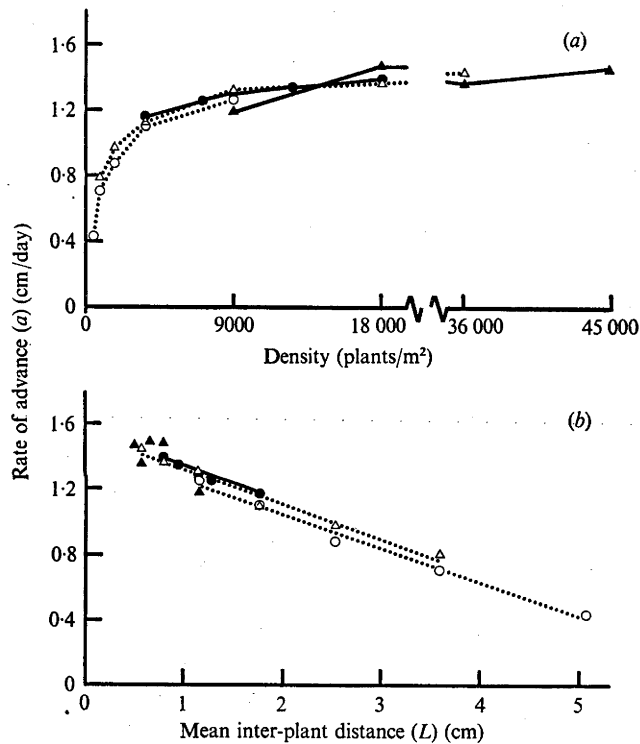


Fig. 3. Combined results of four separate experiments on damping-off in plots inoculated along one side. (a) Rate of advance of disease per day in relation to seedling density. (b) Rate of advance of disease per day in relation to the mean distance separating adjacent seedlings (L), fitted by linear regression. \circ , \bullet , \triangle , \blacktriangle , results of different experiments.

between adjacent plants (L) a close approximation to a simple linear relationship with negative slope was obtained (Fig. 3*b*).

DISCUSSION

Previous experimental studies of host density effects on damping-off disease have all been confined to measuring disease incidence at one particular time after inoculation (Johnson, 1914; Hartley, 1921; Gibson, 1956). Gibson (1956) concluded, from his own and previous studies, that there was a direct relationship between seedling density and the incidence of damping-off, although some of his graphs are distinctly curved. The inadequacy of experiments which seek to establish the relationship between disease incidence and host density at a single arbitrary time after inoculation is demonstrated by the results presented in Fig. 1*a*. From this one can select a linear relationship, or relationships with varying degrees of non-linearity, depending on the time after inoculation at which the disease incidence is evaluated. The linear relationship at the beginning of the epidemic is therefore a substantiation of Gibson's (1956) earlier conclusion, but in only a limited sense. This early relationship possibly reflects a simple proportionality between host density and the number of successful encounters with initial inoculum which result in a primary infection. As the epidemic progresses the rate of spread outwards from these primary foci comes increasingly to dominate the relationship, which is also affected by the declining proportion of susceptible plants available for infection.

The most interesting result to emerge from this study is that over a wide range of host densities the rate of advance of the disease front, and the apparent infection rate of damping-off in a randomly inoculated system (which largely depends on the rate of advance), both apparently showed a negative linear relationship with the mean distance between adjacent plants in the host stand. The relationship can be stated as: r (or a) = $i(1 - L/j)$, where r is the apparent infection rate (van der Plank, 1963), a is the rate of advance, L is the mean distance between adjacent seedlings in the host stand, and i and j are constants.

i and j , the intercepts obtained by extrapolating the linear relationship to the r (or a) and L axes respectively, define the epidemic rate limits within the system. Thus, from the i intercepts it appears unlikely, however much the seedling density is increased, that a would exceed 1.6 cm/day or that r , the rate of change of $\log_e(x/1-x)$, per day, would greatly exceed 1.8 for the given conditions. Similarly, from the j intercepts, it appears that compound interest epidemics involving spread by secondary infections would be unlikely where the mean distance between adjacent plants exceeds 5-7 cm.

A simple inverse relationship between inter-plant distance and the rate of multiplication or advance of disease does not appear to have been noted before. The only work that specifically mentions inter-plant distance, of which the authors are aware, is that published by Scott (1956). In studying the epidemiology of white rot of onions caused by *Sclerotium cepivorum*, Scott found that the percentage of infected plants in experimental plots was approximately inversely proportional to their spacing, and he attributed this to the effect of spacing on the transmission of the parasite. Transmission

of this soil-borne disease is essentially similar to that of damping-off and it seems probable that other disease systems of this general pattern, e.g. Panama wilt disease of bananas (Rishbeth, 1955), could exhibit the same type of relationship. Such a relationship, if established for field conditions, could have significance for disease control and for this reason alone merits further study.

If this type of relationship should prove to have widespread validity, the finding would be extremely important for studies on problems of ecosystem stability and the regulation of natural plant populations. It is increasingly clear that an understanding of such phenomena can only come through an examination of rates of change in the ecosystem, and there is reason to believe that such interactions as those between plants and their parasites are one of the more important components in the ecological equation (Chilvers, Brittain & Burdon, 1973; Burdon & Chilvers, 1974).

This work was carried out while one of us (J.J.B.) held a C.S.I.R.O. Postgraduate Studentship. We are grateful to Dr D. J. Stamps of the Commonwealth Mycological Institute for authentication of the *Pythium* isolate.

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