

**MICROBIAL PLANT PATHOGENS:
NATURAL SPREAD, AND POSSIBLE RISKS IN THEIR INDUSTRIAL USE**

A study of the necessity, content and management principles
of a possible Community action

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This work has been carried out under the study contract
No 724-ECI UK with the Commission of the European Communities

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CHAPTER I

SUMMARY OF CONCLUSIONS

**Proposed
Action**

Conclusion

Chapter

List to
be comm-
issioned

1. Plant diseases can be caused by fungi, bacteria, mycoplasmas, rickettsia-like organisms, viruses and other pests; of these, only some fungi and bacteria are likely to be used industrially. IV
2. There is no evidence that the industrial use of plant pathogens has so far caused the spread of plant disease. V, VI, VIII, IX
3. Economic losses caused by natural infections of crops are not precisely documented, but are thought to be about 15% of overall production. V
4. The spread of plant disease is largely by the aerial route, and a consequence of man's cultivation of crops. IV, V, VI
5. Many plant pathogens have a limited geographic distribution and countries impose strict regulations to prevent their introduction. IV, V, X
6. Microorganisms including plant pathogens are being grown increasingly on an industrial scale. VIII
7. There is a need for biotechnologists to be aware of plant pathogens and able to check whether an organism might be pathogenic. XII
8. Any risk that biotechnology might cause the spread of plant disease could be eliminated by the use of saprophytes or non-pathogenic strains. VIII, IX, XII
9. If a plant pathogen has to be used, plant health regulations must be respected; but even for indigenous pathogens, there should be consultation with the relevant plant protection authorities at the development stage. IX, XII

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|---------------------|-----|---|---------------|
| | 10. | At present, not more than a few thousand litres of a fully virulent pathogen can be handled under contained conditions. | VII, IX |
| | 11. | Before a plant pathogen is used on a (large) industrial scale, tests should be made of its pathogenicity when grown under the proposed conditions. | IX, XII |
| | 12. | If, when tested under the conditions described in conclusion 11, the organism is still pathogenic further assessment of the risks involved in its use should be made. | VI, VII, IX |
| | 13. | Existing regulations control the introduction of non-indigenous organisms, but are not designed to control industrial use of plant pathogens. | IX, X, XI |
| | 14. | There may ultimately be a need to modify existing regulations to cover industrial use of plant pathogens. | IX, X, XI |
| List to be prepared | 15. | There is a need for plant pathologists to know of and to be involved in the current industrial uses of plant pathogens. | XII |
| | 16. | There is a continuing need to gather information on the economic losses caused by fungal plant diseases; there is less knowledge about those caused by bacteria. | V |
| | 17. | There is a need for research on the epidemic spread of bacterial plant disease from localised sources. | IV, V, VI, IX |
| | 18. | There is a continuing need for basic research on the nature of pathogenesis at the molecular, biochemical and biophysical levels. | IV, VI, IX |

CHAPTER II

TERMS OF REFERENCE PROVIDED BY THE COMMISSION

Introduction

In a report on hazards involved in the industrial use of microorganisms (EUR 6349 EN) Sargeant and Evans concluded that "with the exception of a few that affect plants, it is very unlikely that dangerous pathogens will be used on a large industrial scale". They therefore recommended that the Commission should sponsor a report on the natural spread of plant diseases which should "include consideration of whether industrial production and use of plant pathogens is likely to spread the diseases they cause". This present report follows from that recommendation.

Terms of reference

To prepare a protocol concerning the natural spread of plant pathogens and the hazards associated with the industrial use of plant pathogens. This protocol will have to include:

- A. A review of the natural spread of plant pathogens with specific reference to the situation in the European Community,
- B. An evaluation of the hazards associated with the industrial use of plant pathogens,
- C. Recommendations for actions (setting of Community standards, improvement of methods for assessing the spread of microbial plant diseases and for assessing hazards associated with the industrial use of plant pathogens, development of protection procedures) which ought to be carried out at Community level.

CHAPTER III

FORMAT OF THE REPORT

In Chapter I we present a summary of our main conclusions.

Chapter II contains the terms of reference provided by the Commission, with a brief explanation of the raison d'etre of the report.

Chapter IV gives a general introduction to microbial plant pathogens, to the types of disease that they cause and how they are spread; this is for the general reader who is not very familiar with the subject.

Chapter V describes the economic consequences of plant infections, emphasizing those infections caused by fungi and bacteria.

Chapter VI is a general introduction to the subject of aerobiology, summarising the main factors involved in the generation of aerosols, the factors that influence survival of microorganisms in air, and their movement in the atmosphere. A brief survey is given of the methods available for detecting and identifying airborne particles with some comments on their use.

Chapter VII describes how aerosols can be created and released during the manufacture of industrial products derived from plant pathogens.

In Chapter VIII we review the present uses of plant pathogens and speculate about possible uses that could follow the growth of Biotechnology.

Chapter IX considers the possible hazards that might be involved in the industrial exploitation of plant pathogens.

Chapter X summarises some of the current international policies of the member states towards plant pathogens and considers their relevance to the industrial use of plant pathogens.

CHAPTER IV

GENERAL INTRODUCTION TO MICROBIAL PLANT PATHOGENS; HOW THEY ARE SPREAD AND INFECT PLANTS; THE DISEASE PROCESS.

Plant pathogens can be viroids, viruses, mycoplasmas, rickettsia-like organisms, bacteria and fungi which are able to live in plants as parasites, causing the plants to "suffer" in some way. This suffering ranges from invisible molecular and biochemical changes, through more obvious symptoms of disease, whether of form or function, to the rapid death of plant parts or of whole plants. European examples of a disease caused by each of these groups of plant pathogens are given in the following table:

<u>Group of organisms</u>	<u>Name of organism</u>	<u>Plant and effect</u>
viroid	ChSV	Chrysanthemum stunt
virus	BYDV	Barley yellow dwarf
mycoplasma		Aster yellows
rickettsia-like organism		Clover club leaf
bacterium	Corynebacterium michiganense	Bacterial canker of tomato
fungus	Venturia inaequalis	Apple scab

Damage to plants is often caused by a variety of non-living ("abiotic") agencies such as: extremes of the weather, deficiencies of essential plant nutrients, pests, air pollution, and by weed-killers and insecticides applied for purposes other than disease control. However, it is with living ("biotic") agents that the scientific discipline of plant pathology is concerned. In some countries the list of these living agents includes nematode worms and flowering plants that parasitize other plants. This is not generally so in Europe and usually it is only with diseases caused by the groups of microbes mentioned at the start of this Chapter that the plant pathologist is concerned. Some of the fungi may produce quite large structures as part of their life cycles, for example the toadstools and brackets most often seen on trees.

Although from its derivation "plant pathology" might be taken to be an account of plant diseases, the best idea of what it is about can be gained from what plant pathologists do. They are concerned with, and about, the control of microbial plant diseases. The focus of their effort is the control of plant disease in field, orchard, forest, market garden, glasshouse and mushroom house. They are also concerned with plants grown for pleasure in the home, small garden or park. Plant pathology involves recognising diseases (diagnosis), understanding them, and controlling them. To this end plant pathogens are handled and studied in various ways by a wide range of people: by farmers and growers, by advisory ("extension") scientists

employed by governments, by commercial firms that develop or sell control materials (at present mainly chemical fungicides), by teachers and students at various institutes of agriculture, horticulture and forestry, and by research workers of many kinds in universities, research stations and commercial firms, concerned with understanding and controlling plant disease.

Wild plants, that is, those not sown and tended by man, also have serious diseases caused by pathogenic microbes. A few disease organisms are known, such as the rusts, because they have "caught the eye" of field naturalists who have seen the spore-forming pustules with the naked eye; others, such as Puccinia graminis on Barberry bushes, have been studied because it was realised that they are reservoirs of infection for important crop plants. Otherwise our knowledge of disease in "natural" environments is scanty.

There is a remarkably complacent ignorance about plant diseases outside the small circle of people who are knowledgeable about them. A very much greater amount of effort is devoted to the control of human diseases, but it should not be forgotten that plants provide us with food, and that the majority of people have not enough food to eat. Plant pathogens are therefore a threat to our existence.

The effects of plant diseases seem to have been felt as long as man has grown plants as crops. Although our knowledge of the evolution of plant pathogens is virtually non-existent, it is evident from recorded history that it is man's act of growing many genetically similar plants together which is a prime cause of the loss of food plants to disease. As man's numbers increased so more food became necessary and man invented the idea of crops. This enabled his numbers to increase even more, but disturbed the balance of the natural environment.

As so often happens, nature sought to restore the balance. Crop predators ranging from herbivores to microbes were able to increase their numbers in competition with man, and at man's expense. Those predators that could be seen could be dealt with, by fences or by scarecrows; but the unseen microbes were a different proposition. The result was war with nature; the cost: famine; the counter strategy: first rotation of crops, followed much later, by insecticides/fungicides, and then by resistant varieties of crop plants. But still the battle continues with the balance held, marginally now, in man's favour by research, but still with the occasional defeat with consequent famine, and continuing economic loss (see Chapter V). Plant pathogens know no frontiers, and our measures to stop them spreading can never be completely successful.

Of all plants of use to man the most important are the grasses. Wheat, rye, barley, oats, maize, rice and sorghum are grasses as well as those "ordinary" grasses eaten by grazing animals. We cannot over-emphasize this central role of grasses in either the development of agriculture, which made everything we know as civilisation possible, or our continuing survival as an animal species on earth (Wittwer, 1980). Potatoes, sugar cane, sugar beet, vegetables, fruit, grapes, coffee, tea, cotton, for example, matter, but are of lesser importance as food at the present time. It seems unlikely that this situation will change in the 21st century, and hence plant pathogens affecting members of the grass family or Gramineae must be of central concern to plant pathology and plant pathologists. Any changes in agriculture or technology will be attempts to find substitutes for or to improve production from what is largely the grass-

derived food of man.

However, man does not live by bread alone, and a large number of non-food plants, for example commercial and ornamental trees, shrubs, bulbs, cut flowers etc. are also important to him. Man's intervention has made changes in their genetic character and in the intensity of their cultivation, which also make them more vulnerable to epidemic disease. The wider influence of man's activities on the world's stock of wild plants is mentioned in Chapter V.

There is no reason to suppose that we have discovered the causes of all existing plant diseases (rickettsia-like organisms and mycoplasmas have only been discovered and studied as plant pathogens in the last two decades), but at present diseases caused by fungi, viruses and bacteria are the main microbial "living-agent" threats to crop production.

Plant diseases caused by viruses are different in many ways from those caused by fungi and bacteria. The viruses cannot be grown artificially without the living plant; only relatively recently have they been "seen" in plant cells as they are between 10 and 100 times smaller than bacteria. Lying on the border of living things, they have no "metabolism" of their own; they do not respire, break down carbon compounds, fix CO₂ or otherwise behave like "normal" microbes (Postgate, 1969). When they invade a plant they use materials from within the plant cell to synthesize more of themselves. As will be seen in Chapter VIII, biotechnological interest in plant pathogens is confined to some fungi and some bacteria. Thus, while viruses, mycoplasmas and rickettsia-like organisms can cause severe, and sometimes unsuspected, losses of crop plants, in the rest of this Chapter we shall consider only fungal and bacterial diseases. However, it must be realised that not all plant pathogenic fungi can grow in the absence of (away from) the host plant, and that this is especially true of the rusts and mildews, to some of which we shall refer in the next Chapter. Such organisms will not be usable by Biotechnology.

In recent years, students of biology have developed what is known as the "ecosystem" approach, (Odum 1959), to looking at and understanding both semi-natural and artificial (farming and cropping) systems. The idea is not a new one, but as it is a useful concept it is mentioned here. In a sense (eg, in some dictionaries) ecology = biology, hence the ecosystem concept is a very wide one. There are considered to be four parts to ecosystems:

- (i) abiotic substances - inorganic and organic materials
- (ii) producer organisms - plants on land, phytoplankton in the sea
- (iii) consumer organisms - animals
- (iv) decomposer organisms - saprophytes which re-cycle the elements used by (ii) and (iii) back to (i).

The concept is suitable for quantitative research, and much is now known about form and function of ecosystems. Most fungi and bacteria are saprophytes which decompose dead remains; the few that are parasites have the exceptional ability to decompose living plants. Indeed, some plant pathogens are taxonomically very closely related to decomposer organisms; most of the known species of Fusarium, a fungal genus, and of Pseudomonas, a bacterial genus, are saprophytic decomposers. However each genus

includes pathogens that can cause severe crop losses: for example, *F. solani* f. *cucurbitae* which caused great problems in the Dutch cucumber trade (Zadoks, 1967), and *P. syringae* pv. *phaseolicola* which continues to cause losses of beans. Thus, plant pathogens are very unusual organisms amongst masses of others, very like them, that are able to perform almost all kinds of chemical and biochemical transformations, thereby maintaining ecosystems of many kinds and sizes.

Amongst these microbes there are some fungi (Ainsworth, 1971), mostly saprophytes, which cause crop losses by producing mycotoxins. These mycotoxins are secondary metabolites that seem to have no value to the fungus, but make the crop poisonous to man and animals; for example, *Aspergillus flavus* can produce a very powerful carcinogenic hepatotoxin (Sargeant, et al., 1961), and some *Fusarium* spp. can produce oestrogen-like materials which, when eaten by animals, cause serious reproductive disorders with consequent further "crop" loss.

What regulates plant pathogens and the occurrence of the diseases that they cause? Why are they sometimes so important that the resultant damage to a crop is severe, while at other times disease does not even appear? Over the years a hypothesis has evolved to relate several interacting factors and this seems useful in explaining and forecasting the occurrence and severity of most economically important plant diseases; this despite the lack of knowledge of the detail of individual plant diseases. Although it is possible to see the overall pattern by considering the interaction of "plant pathogen - host plant - environment" the effects of many variables on these interactions are unknown. Some, if discovered, are of unknown significance in relation to the "disease triangle" (Fig. 1); for example, the importance of the ecology of leaf surface organisms (Preece & Dickinson 1971) is, at present, little understood (Zadoks & Schein, 1979).

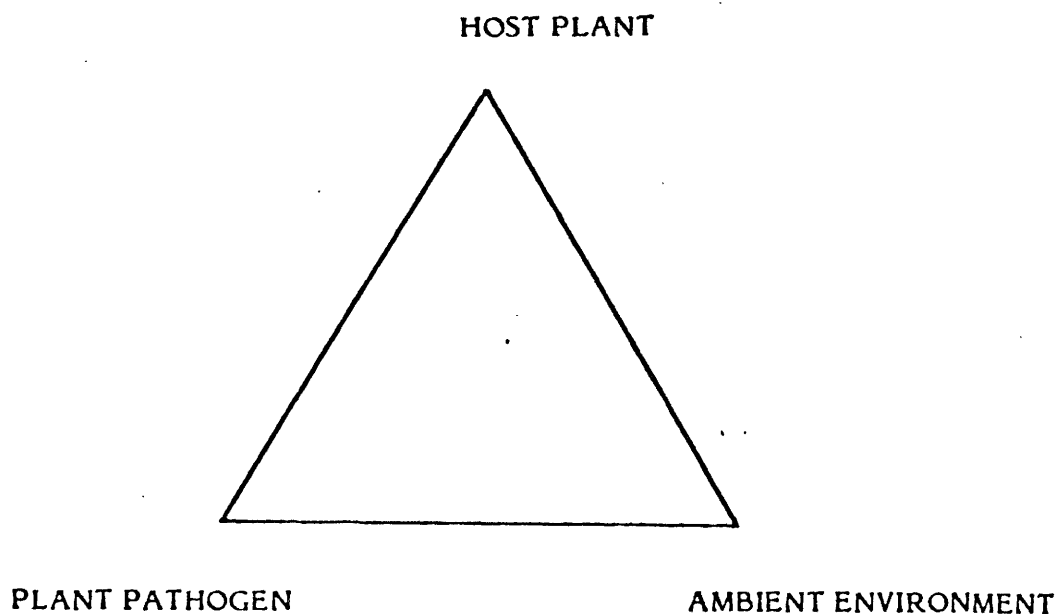


Figure 1. "Triangle" of factors which must interact to give plant disease

In order that infection can occur it is necessary that the factors at all three corners of the disease triangle are suitable. For example:

- the disease organism: must find a suitable host,
must arrive at the right time,
must be able to invade the host;
- the host plant: must be susceptible to infection by the
pathogen,
must, in some cases, be at the right stage of
development (Populer, 1978);
- the environment: the humidity must be suitable,
the temperature must be suitable.

Only if all the conditions are fulfilled can the pathogen, whether it be the residuum of a previous infection in that field or a new arrival from afar, become established in the crop and the invisible disease processes begin. For these processes to continue and produce serious crop losses, environmental conditions must remain suitable for a sufficient length of time. Like every other dynamic relationship between living things, there are many processes and sub-processes involved in the initial infection and the development of disease. This time a circle model (Fig. 2) helps understanding, especially as the processes of infection (growth in the host plant and dissemination of further infective entities known as propagules) often occur over and over again, even before disease symptoms become visible. Disease attack is thus a "cyclic" process.

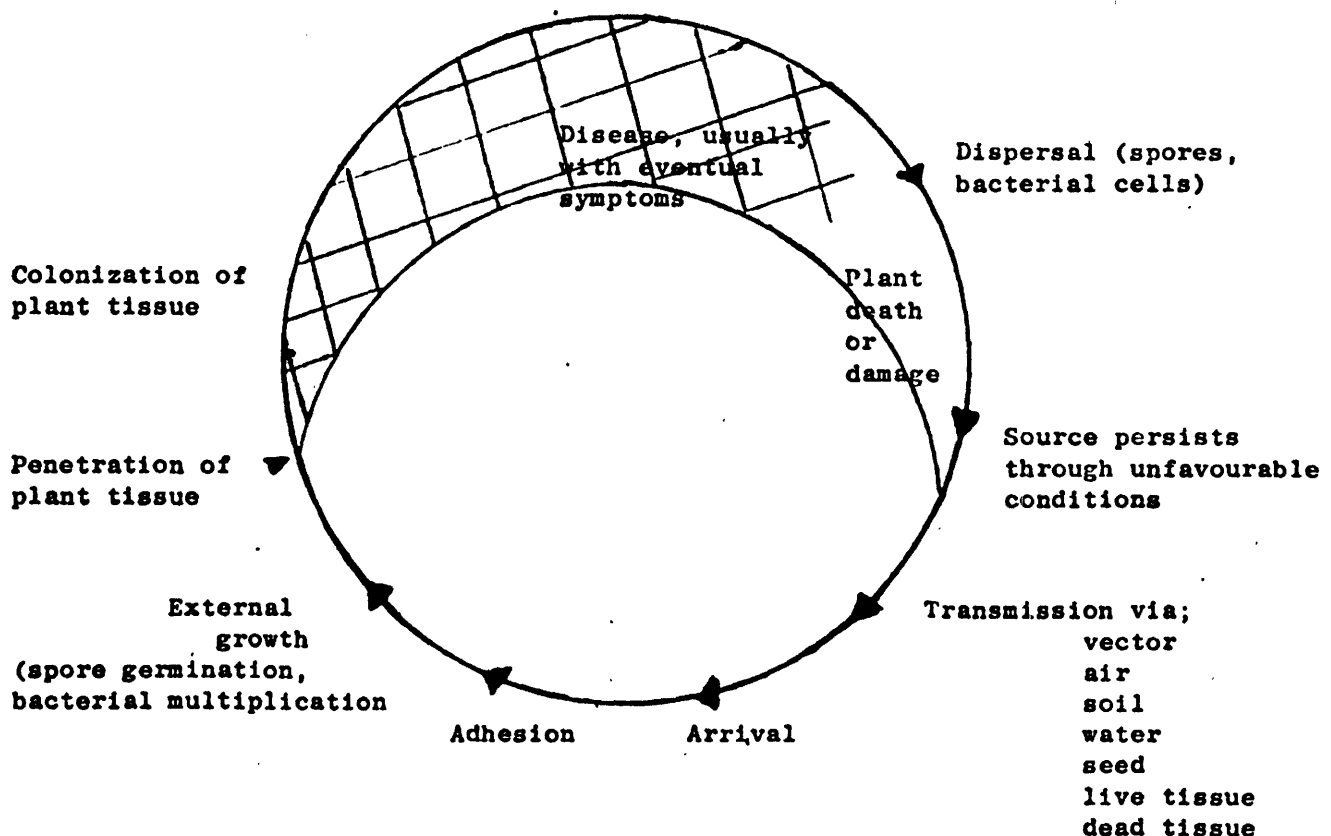


Figure 2. The disease cycle

For a plant to become infected a source of the pathogen is necessary. In the case of a fungal infection, the inoculum may already be in the plant if the plant is systemically infected, or it may arrive as a spore from a neighbouring leaf, or from afar. Some fungi are able to produce a large, thick-walled, resistant structure of some kind that persists in the soil until conditions are suitable for it to grow out and produce spores. These can rise in air currents and infect nearby plants or those further away, but fungal mycelia also can survive airborne travel and produce infection of suitable host plants (see Chapter VI). The idea that aerosols might play a part in the spread of bacterial plant disease, as they do for human and animal disease, "has only recently been considered a possibility by plant pathologists" (Harrison, 1980). Unlike fungi, bacteria that infect plants do not produce spores; they may have to rely for protection, during airborne travel, on the production of polysaccharides derived from plant sugars (see Chapter VIII) or on the closer proximity of a host plant. Seeds and pollens have been implicated in the spread of fungal and bacterial diseases (Phatak, 1980) and airborne pollen has been shown to exacerbate fungal plant diseases of several kinds (Preece, 1976). Insects, surface water, irrigation, and the introduction of infected material into the field or glasshouse are also factors that contribute to the spread of disease.

If the pathogen has arrived by the airborne route, it must land on the surface of a susceptible host plant; this can involve impaction of the propagule, by wind, onto the surface (so breaking through the quiescent boundary layer that surrounds the plant) or, in quiescent conditions, by sedimentation of large particles onto the surface of the plant. Also of importance may be adhesion of the propagule which would otherwise move away from the surface or be washed off by rain water. Persistence of surface wetness for defined periods (Preece & Smith, 1961), and very precise temperatures may be necessary for spore germination, with subsequent outgrowth of a hypha, or for bacteria to be able to multiply on the surface of the plant.

Fungi and bacteria differ markedly in their modes of penetration: fungi penetrate actively, often through the intact surface of the plant by use of enzymic or physical processes; the germinating spores of many fungi produce swellings, called appressoria, on the ends of their germ tubes which assist penetration. Bacteria are unable to penetrate an intact plant surface; they usually enter at wounds, but may do so through natural openings of the plant such as stomata and lenticels. Once inside the plant, the pathogen is protected from adverse influences, such as radiation and changes in humidity and, if compatible with the plant tissues, begins to colonize the plant. In fungal and bacterial diseases, dispersal from the first infection site on a leaf commonly begins before the disease symptoms become visible. Many cycles of infection and dispersal may occur before the grower notices that his plants are infected, that is, sees symptoms of disease, such as brown spots, etc. In the case of fungi and some bacteria there may be a dormant phase in the life cycle by means of which they may "over-summer" or "over-winter" depending on the geographical location.

Plant disease control measures are based on procedures intended to disrupt the "plant pathogen-host plant-environment" triangle or prevent or slow down one or several of the processes in the disease development cycle. Thus drainage may be improved to eliminate waterlogged conditions which

favour infections of roots (excessive application of water, often at relatively low temperatures, is part of the problem of "damping off" diseases of seedlings). The pH of the soil may be adjusted to favour crop growth rather than infection. Crop rotation may be used to prevent the build-up of infected debris in the soil. The production by plant breeders of resistant or partly resistant host plants, and the protection of plants against infection by chemical fungicides, are vital parts of disease control programmes (we now have virtually no useful bactericides for use on plants since the use of mercury seed dressings and streptomycin has been disallowed).

If conditions remain suitable for long enough an epidemic can occur. As disease spreads from plant to plant in a crop, the process can be represented by a sigmoidal progress curve (Large 1952) - similar to the microbial growth curve of laboratory batch cultures.

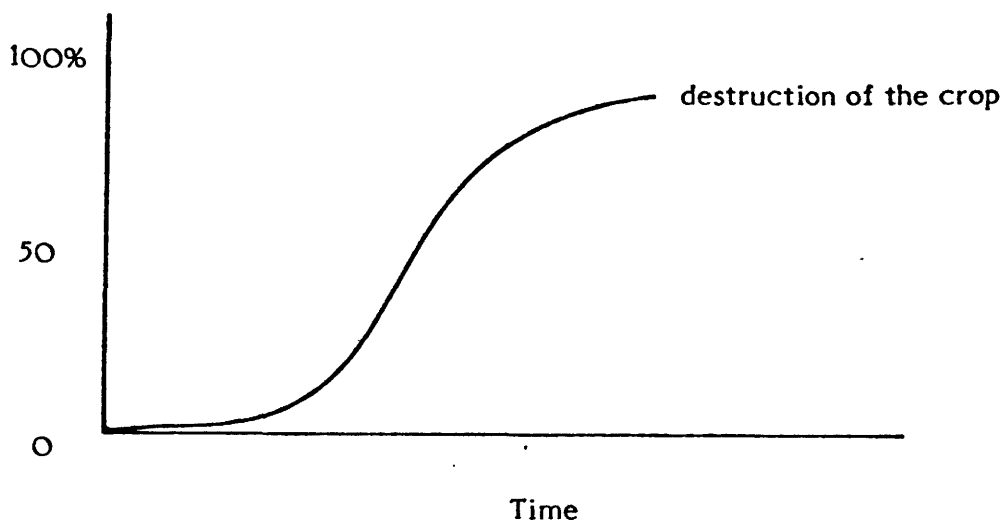


Figure 3. Progress curve, showing the development of disease with time within a crop. Sigmoidal in shape, it represents the summation of countless disease development cycles on, for example, leaves or roots.

Clearly neither the disease triangle, the disease circle nor the progress curve can apply without the plant pathogen, and one way of trying to simplify disease control is to exclude or eradicate the pathogen from a geographical area or from entire countries. This is attempted by legislation in many countries, as is discussed in greater detail in Chapter X.

This brief account of plant disease has inevitably been very superficial. We have emphasised the importance of the spread of pathogens by the aerial route because we believe that for crop plants this is the most important. However other mechanisms of spread do exist, for example via animal, bird and insect vectors. In ecosystems the situation is very complex, and among the 350,000 species of plant which are estimated to exist (Wittwer, 1980) there must be many, still unsuspected, parasitic relationships.

The progress of plant disease is also a complex process, probably

affected by many factors as yet unknown. For example, only very recently was it realised that the spread of Dutch elm disease, caused by a fungus carried by a beetle, is naturally controlled under some circumstances by a second fungus. This grows in the bark of some races of elm, and discourages colonisation by the beetle larvae (Webber, 1981).

We return to the main message. Crop plants present special opportunities for pathogens. Some of the economic consequences of fungal and bacterial diseases are discussed in the next chapter.

CHAPTER V

ECONOMIC CONSEQUENCES OF FUNGAL AND BACTERIAL PLANT DISEASES

The emergence of plant diseases

To fungi and bacteria, living plants are a potential source of food. In a natural ecosystem plants usually grow in mixed populations, with many different types of plant taking whatever opportunities are available to use the sun's energy. Natural selection pressures cause the plants to evolve in a variety of ways in response to the wide range of local habitats offered. Always, the plants which succeed develop a strategy for survival which is a compromise between their need to combat the activities of competitors (including pathogenic fungi and bacteria) and their need to grow.

Although, in this Chapter, we refer mainly to losses of annual crops, it must be recognised that the effects of diseases of perennial plants such as trees and shrubs will be felt for much longer. An annual crop may be lost completely, but it can be replaced within a year; it may take several years to replace a shrub, or generations to replace trees such as elms and oaks that have both economic and environmental value.

Crop plants are protected by man and relieved of many competitive pressures. They are specially bred to exhibit features, such as high yield, uniform quality, and resistance to disease, which appear desirable to man. They are often grown intensively with very large areas devoted to a single crop, a crop which is genetically homogeneous. To those pathogens that can penetrate the plants' defences such crops are a vast store of food that can be used to further their spread.

It is a challenge: the selective pressures are so great that a previously unknown race of pathogen can emerge in response. The results of this were recognised from early times and led to the practice of crop rotation which reduced the selective pressures.

At this point we would mention that changes in cultivation methods can encourage the appearance of plant disease, with consequent crop loss. Here we give just two recent examples:

No tilling Because it was observed (Brooks & Dawson, 1968) to reduce the incidence of "take-all", winter wheat in England is now often sown with little or no preparatory ploughing. This practice has recently been blamed (George, 1981) for the appearance, this year, of ergots which have increased the rejection rate of new grain. Deep ploughing, last year, would have prevented germination of the ergot sclerotia by means of which the disease organism over-winters.

Crop rotation Although this is an ancient practice, the reasons for its success in reducing the incidence of plant diseases are complex and, even now, not fully understood. The decline of its use has been implicated also in the recent increased incidence of ergots (George, 1981).

All experience now suggests that plant pathogens emerge, as

described, in response to the growing of a crop. When a known plant pathogen is grown in an industrial fermenter it will tend to lose its pathogenic properties. In a fermenter the ability to infect a crop plant offers no selective advantage (see Chapter VIII).

Plant breeding has, increasingly, to take into account the need to build disease resistance into crops, and in this connection the importance of having a wide genetic base for such breeding is now better recognised than in the past. Too often resistance resides in a single gene and confers resistance to a restricted number of pathogenic races. Wittwer (1980) has stated that the average half-life of useful cultivation for new resistant crop varieties is only five years. The longer term possibility of retaining the initiative in plant breeding is being jeopardised by the continuing rapid spread of uniform agriculture. This is causing great loss of wild plants. According to Raven (1981) over one third of the world's plant species face extinction before the end of the century, and the most important task of museums and botanic gardens is to preserve the genetic material of as many of these species as possible.

Economic losses caused by plant pathogens

It can be shown by referring to well documented examples of catastrophes which periodically overtake agriculture that fungal and bacterial plant diseases can be of overwhelming economic importance. The next section of this chapter gives a number of examples. It is more difficult to assess the importance of the smaller losses which continue, at varying levels, from year to year. Horsfall and Cowling (1978) give many reasons why so little is known about these smaller losses. They nevertheless imply that much would be gained if measurement of loss were better. This is really obvious, because measurement is an essential prerequisite for adequate control. The final section of this chapter briefly discusses these less spectacular, but persistent losses.

Examples of losses caused by catastrophes

There have been many epidemics affecting crops, but we shall discuss only a few examples selected to illustrate specific details. Further examples can be found in the literature (eg, see Carefoot & Sprout, 1967; Zadoks, 1967; Horsfall & Cowling, 1978).

The Irish potato blight epidemic of 1845/6 This was undoubtedly the worst European epidemic of recent times. As pointed out by Horsfall and Cowling (1978): until early in the eighteenth century, the population of Ireland had been in reasonable equilibrium with its food supply: oats, wheat and barley. With the introduction of the potato the yield of food per hectare could be increased three-fold; the population doubled. The crop area was increased, and between 1800 and 1845 the population doubled again to 8 million, and the people had become utterly dependent upon a single food crop: potatoes. As Large (1940) put it, "from the substance of the potato alone was built the stuff of human bone, muscle, sperm, and milk for the young." The potato crop, Solanum tuberosum, was probably of one genetic type, a variety called "Lumper". Certainly the propagation of the crop via tubers would have guaranteed a limited genetic base. The virulent fungus, Phytophthora infestans, which causes the disease potato blight, does not appear to have been troublesome prior to the 1840s. Its origin is unknown, but it may have been introduced from South America, the natural home of the potato. Certainly, by 1845 it was widely present in Ireland.

The decisive event which started the epidemic was environmental. The weather in Ireland during the summer of 1845 was cold and wet. A contemporary writer described six weeks of rains and fog, with temperatures well below average. Such conditions favoured the infective stages of the disease cycle, which require a film of water on the plant leaves. Thus the factors of the "disease triangle" (Chapter IV, fig. 1) interacted, many disease cycles (Chapter IV fig. 2) took place, and the crop was a total failure. In subsequent years the, by now universal, presence of Phytophthora infestans continued to spoil the crop. Nothing could be done because nothing was known about potato pathogens, nothing about genetically resistant wild potatoes, and nothing about their use in breeding new, resistant varieties. A million Irish died, a million and a half emigrated, and the population of Ireland today is still lower than it was in 1845.

Today potato blight is still a problem. Its control depends on the use of more resistant potato strains and on sprays which contain chemicals toxic to the fungus. If this treatment is withheld, and the environment is "right", infection can still cause catastrophe. This happened in Germany in 1916 when copper for Bordeaux mixture was not available; some of the civilian population starved (Carefoot & Spratt, 1967).

The United States maize leaf blight epidemic of 1970 This disastrous epidemic, which was the subject of a study by the National Academy of Sciences, led to the destruction of 15% of the US maize (corn) crop (Horsfall, 1972).

The epidemic started in Florida and spread in one season throughout the country. The host plant, maize, Zea mays, was genetically very similar everywhere because, since the 1950s, maize had been bred from a female line with sterile pollen, to avoid the expense of "detasseling".

The pathogen, a fungus called Helminthosporium maydis, became adapted to growing on the male-sterile cytoplasm, and the new race was able to spread rapidly because of the unusually wet weather of 1970.

In retrospect it was clear that the price paid in genetic uniformity for this new low cost plant breeding technology, using the male-sterile strain, was too high.

Ceylon coffee rust of the late 19th century This is mentioned because it led to the elimination of the coffee industry in Ceylon (now Sri Lanka), and because the disease may yet have an important impact elsewhere. An account of the disease is given by Horsfall and Cowling (1978).

Coffee was first grown in Ceylon after seven live coffee beans had been stolen from Africa and taken to the island. During the 19th century the British used plants derived from this narrow genetic base to break the Arab coffee monopoly. By 1870 about 200,000 ha of Ceylonese plantations supported the export of 50 million kg of beans per year.

In 1870 the fungus Hemileia vastatrix made its appearance in Ceylon. It killed the coffee trees so rapidly that in less than 20 years the coffee industry was completely destroyed.

The disease has recently appeared in South America. Waller (1979) has suggested that the epidemiological evidence is consistent with airborne spread of the disease from West Africa, but there is still some doubt about this because the airborne route has not previously been thought to be

important in the spread of coffee rust. However, the realisation that certain human and animal diseases can be spread in this way is relatively recent.

Mildew of vines In 1847 the mould Uncinula necator appeared, probably from the UK, in a greenhouse in Paris, and next year was found generally in the area. Within six years it had spread to the whole vine-growing area around the Mediterranean (Zadoks, 1967). Like potato blight this is now held in check by chemical countermeasures. The mould was probably introduced into England on vines imported from America.

This example illustrates the susceptibility of an European crop to a "new" pathogen, and the need for quarantine regulations.

Tobacco blue mould In 1958, Peronospora tabacina was imported, under licence from the UK Plant Health authorities, for use in fungicide experiments. In that same year the mould appeared on tobacco plants grown for virus research at four research institutes (three in England, one in the Netherlands) and in a commercial tobacco crop in England. The following year the disease appeared in the tobacco fields of Belgium and the Netherlands. Thereafter, inoculum was so plentiful that the mould was able to spread over Europe, the disease advancing in Germany at a speed of 5-20 km per week (Zadoks, 1967).

After more than 400 years of cultivation in the absence of the pathogen, it is not surprising that the crop was so susceptible. Subsequently, by natural selection and careful breeding, resistance to the mould was increased to a point where the disease was no longer completely destructive and tobacco crops could again be grown. Like the vine mildew epidemic cited above, P. tabacina arose from a point source; but the fact that the source was a licenced laboratory emphasises the risks involved in handling, even knowingly, a non-indigenous pathogen capable of infecting an indigenous crop.

Yellow rust of the wheat cultivar, "Heines VII" Partly because it was resistant to all known races of yellow rust, Puccinia striiformis f. sp. tritici, the wheat cultivar, "Heines VII" of Triticum vulgare, was introduced into the Netherlands in 1950. Three years later a small area of crop was found to have been attacked by what appeared to be a new race of the rust. By 1956 "Heines VII" growing 800 km from the original source was affected following the spread of spores on the wind (Zadoks, 1967).

This is just one of many examples that could be given of the problem, so important to plant breeders, that new races of pathogens appear in response to the development and widespread use of cultivars bred for resistance to the known pathogenic races. Other more recent examples exist in wheat and in other crops. As we have already stated, the half-life of useful cultivation for a new variety is limited!

Bacterial canker of Tomato So far we have discussed fungal diseases of field crops. Bacteria can also cause economically important disease, and this can occur in horticultural crops. Tomato canker, caused by Corynebacterium michiganense is one example. Substantial numbers of plants have to be destroyed in attempts to control the spread of this disease.

Fireblight of apples and pears This second bacterial disease, caused by Erwinia amylovora, led to the abandonment, early in this century, of commercial pear growing in parts of the western side of the USA. It was

found in the UK in 1957 on pear trees (Eden-Green & Billing, 1974). From there it spread to the mainland of Europe and causes losses in many countries including Poland, the Netherlands, Germany, France and Belgium. In 1974, Eden-Green and Billing predicted that if it should spread to the warmer fruit growing centres of Southern Europe and the Mediterranean area, the results could be serious: the disease has now been present in south-west France since 1979 and it is unlikely that it will be eradicated. As will be mentioned further in Chapter VI, it is known that spread of this organism can be by the aerial route. (See also Eden-Green & Billing, 1972).

Persistent losses caused by plant disease

While it is the catastrophic epidemics that are remembered and rightly feared, it is the continuing toll of crops taken by commonly occurring diseases that constitute the greater long term loss. Plant pathologists are often asked whether a disease causes significant loss of a crop, and, if it does, what the value of that loss may be. Like many apparently simple questions, these are not easy to answer. However, anyone who has walked through fields of crops, or even grown fruits or vegetables in the garden, must have noticed that crop losses do occur. These losses can be from a variety of causes such as: lack of water, lack of nutrients, wind damage, a poor season, depredations of birds, mammals, pests or simply competition with other plants. With such a variety of causes it is not surprising if little information seems to exist on crop losses due to microbial infections alone. The problem is increased by the difficulty of knowing what the yield might have been had none of these adverse factors operated. Moreover, when assessing the monetary value of crop losses, the cost of the diverse measures taken to attempt prevention or control of plant disease should be included.

In spite of these problems, there are methods available for measuring crop losses caused by microbes (see Preece 1971), but although such estimates are of obvious interest and importance, it is difficult to persuade biologists to make the necessary, rather mundane, long term surveys of a given area; see, for instance, the problems encountered by Kennedy and Alcorn (1980) in their survey referred to below. However, some estimates have been made and we give a few examples of these in the following tables.

Table 1. Crop losses due to infectious plant diseases in the United States (from LeClerg, 1964)

Mean annual crop losses due to diseases alone			
Barley	14%	Beans	22%
Maize	15%	Fruits & Nuts	21%
Oats	21%	Ornamentals	15%
Wheat	28%	Potatoes	23%
		Tomatoes	23%

It has been estimated that in the UK overall losses of barley from leaf diseases are about 12% of the potential yield. The cost of cereal mildew,

comm.).

A comparison has been made (Cramer, 1967) of world losses caused by disease with those caused by pests and weeds. This is shown in Table 2.

Table 2. Annual world losses (tons $\times 10^{-6}$) to diseases, compared with those to pests and weeds (from Cramer, 1967). Approximate per cent losses are given in brackets.

Commodity	Potential Production	Losses due to			Actual Production
		Diseases	Pests	Weeds	
Wheat	351	33 (9)	18	35	266
Rice	439	39 (9)	121	47	232
Maize	339	33 (10)	44	44	219
Potatoes	400	89 (22)	24	17	271
Sugar (beet & cane)	1330	232 (17)	228	175	695
Vegetables	280	31 (11)	23	24	202
Fruit	197	33 (17)	11	12	142

Thus, although the occasional catastrophic losses are more spectacular, the continuing, insidious annual losses of, say 15% are greater in the longer term.

More recent estimates of US crop losses caused by procaryotic organisms have been given by Kennedy and Alcorn (1980). These are shown in Table 3 which also indicates those organisms that were of most economic importance during the three years 1975-1977.

Table 3. Estimated dollar-losses ($\$ \times 10^{-6}$) in the USA from major diseases caused by bacteria, rickettsia-like organisms(*) and a mycoplasma(+) (from Kennedy & Alcorn, 1980).

	Year		
	1977	1976	1975
Pathogens or diseases			
<i>Pseudomonas glycinea</i>	59	64	63
<i>Agrobacterium tumefaciens</i>	2	23	21
<i>Corynebacterium insidiosum</i>	16	17	16
<i>P. syringae</i>	8	18	29
Phony peach*	1	20	17
<i>Erwinia soft rot</i>	11	14	11
Ratoon stunt*	NA	10	14
<i>P. solanacearum</i>	10	9	8
<i>Xanthomonas malvacearum</i>	11	5	5
Pierce's disease*	10	3	1
<i>X. phaseoli</i>	4	5	5
<i>Corynebacterium nebraskense</i>	4	3	4
<i>Erwinia amylovora</i>	2	5	4
Lethal yellowing+	3	3	3

Kennedy and Alcorn (1980) also include figures for some lesser losses caused by a number of other bacteria, some of which are mentioned elsewhere in this report. For instance: *E. chrysanthemi* (tobacco), \$2.3M; *X. juglandis* (walnut, \$2.2M; *Ps. phaseolicola* (beans), \$2M; *X. campestris* (crucifers), \$1M and *C. michiganense* (tomato), \$0.2M.

Conclusion

This chapter has inevitably been somewhat sketchy and anecdotal. The principal messages are that plant diseases are ever present, that they can sometimes be of overwhelming economic importance, and that plant pathogens are constantly evolving in response to the introduction of new crops and new cultivars.

CHAPTER VI

GENERATION, SURVIVAL, TRANSPORT AND DETECTION OF VIABLE AIRBORNE PARTICLES

Earlier chapters have established that a few fungi and bacteria can cause economically important diseases of plants, and that these pathogenic agents are often spread by the aerial route. In this Chapter we discuss ways in which they can become airborne, factors which affect their transport, survival, and infectivity, and conclude with a brief review of methods for sampling airborne particles.

Generation of airborne particles

The large-scale growth of microorganisms is generally in liquid culture (see Chapter VII), and it is, therefore, the mechanisms of aerosol generation from liquids that are most relevant to this report. One of the first publications to stress the importance of aerosols as vehicles for the spread of disease to humans was the report of Huddleson and Munger (1940) on an epidemic of brucellosis at the Michigan State College in 1938/9. This had been caused by the release, throughout a large part of the building, of an aerosol of *Brucella melitensis* from "an improperly used Sharples centrifuge" (Phillips, 1965) located in the basement. It resulted in 45 clinical cases of brucellosis, one of whom died. Subsequently a very considerable literature has accumulated on all aspects of aerosols and their part in the spread of disease (see for example Johansson & Ferris, 1946, Darlow, 1972). Aerosols have also been implicated in the spread of diseases previously thought to be spread only by contagion: for example, Winkler *et al.* (1973) described a fatal rabies infection, acquired in the laboratory, by inhalation of the aerosol produced by a "kitchen type" blender used to homogenise rabid goat brain.

In a classic report on laboratory infections, Sulkin (1961) had concluded that 80% of those infections that could not be related to an overt accident were attributable to the unsuspected generation and release of aerosols of infectious particles. Kenny and Sabel (1968) demonstrated that several common laboratory procedures, such as using a tabletop centrifuge, a Waring blender, a mechanical Vortex mixer, a sonic oscillator, mixing cultures with a pipette, opening or dropping lyophilized cultures, dropping a flask of culture or spilling culture from a pipette, all generated aerosols of various densities of particles, but that in all cases there were particles in the 1 - 5 μ m diameter range that most readily penetrates the alveoli of the lungs. Tomlinson (1957) showed that a screw-capped bottle with an infected rim liberates an aerosol when the cap is unscrewed, and that again some of these particles are in the 1 - 5 μ m range. It is thus safe to say that any rupture of a liquid film will generate an aerosol. This fact, so long unrecognised, should have been obvious from a study of the drawings, first published in 1894, and the photographs of 1908 by A.M. Worthington in his study of splashes (Worthington, 1963).

Because all the operations directly involved in the large-scale growth and harvesting of microorganisms (and in the extraction of their products) inevitably involve the rupture of liquid films - stirring, bubbling, pouring, centrifuging, spraying etc. (see Chapter VII) - it must follow that such processes will generate aerosols. It is noteworthy in this connection that many devices used for the deliberate generation of aerosols make use of systems similar to those used in biotechnology: the "spinning disc" or "spinning top", long used for crop spraying and for spray drying, is similar to

centrifuges which have been shown (eg, see Rutter & Evans, 1972; Evans *et al.* 1974) to produce persistent aerosols of viable particles; the spinning disc (Walton & Prewett, 1949) has been modified (May, 1966a) to produce nearly homogeneous aerosols with any desired initial droplet size from about 10 - 200 μm ; ultrasonic vibrations can produce aerosols in the 4-5 μm range (McCubbin, 1953, Muromtsev & Nenashev, 1960). Aerosols can also be formed hydraulically by forcing liquid through an orifice; this has been used, in a refined form, in the swirl chamber atomizer used in agricultural spraying equipment (Green & Lane, 1964). Sparging liquid with a gas generates an unhomogeneous aerosol; the best known atomizer based on this principle is the Collison spray (Collison, 1935), especially as modified by May (1973), the MRE design, for the production of monodisperse (single particle) aerosols.

The action of waves at sea produces aerosols, particularly by entrapping air bubbles which then burst at the surface. Blanchard and Woodcock (1957) showed that as the sea roughens the bubble frequency can increase to 30/cm²/second with an average bubble size of 100 μm , and Dyrssen (1972) has estimated the annual fall out of sodium chloride onto the world's land from the sea to be about 10⁹ tons. Wilson (1959) showed that nitrogenous materials and potassium derived from marine organisms may be deposited up to 10 miles inland from the sea, and more recently it has been found that Scottish pine forests growing on poor soil, receive much of their nutrients from airborne particles and gases derived from the sea (Anon, 1981). Darlow and Bale (1959) showed that even the flushing of WCs could produce a persistent aerosol in a similar way.

When droplets are released from a suspension of microorganisms some will consist of a solid nucleus of microorganism(s) suspended in liquid medium. This medium can be "pure" water or may have materials dissolved in it, as when the medium is culture fluid in which the cells have been grown. The nature of those dissolved materials can affect the subsequent viability of the microorganisms in the aerosol. It is not possible to be dogmatic about the effects of these other materials present in the aerosol particle because, as we shall show later, there are so many factors that can affect aerostability; but it has generally been found that amino-acids, inositol and saccharides (especially di- and tri-saccharides) and, not surprisingly therefore, culture medium, often afford protection to organisms.

As soon as the droplet has been released from the body of the liquid, the medium surrounding the microorganism will start to evaporate. This evaporation is extremely rapid; by calculation (Green & Lane, 1964) it can be shown that even in air that is fully saturated with water vapour (RH = 100%), as might be found in the centre of a cloud of newly released droplets, isothermal distillation in an ambient temperature of 20^o will cause all the surrounding liquid, typically water, to evaporate: and that even if the liquid has a vapour pressure as low as 5 x 10⁻⁴ mm of mercury, a 4 μm particle will evaporate in 4.5 seconds; a 2 μm particle in less than 0.6 seconds. In practice these times are likely to be slightly increased by the very slight cooling produced by the evaporation.

Whether or not an aerodynamically stable aerosol particle is produced will depend much upon the size of the particle; the larger the particle the greater will be its rate of fall (sedimentation rate) and its terminal velocity. Thus, if we assume that the particle is of unit density, a 100 μm sphere has a terminal velocity of 24.7cm/second, whereas a 2 μm particle can only reach 0.013cm/second. So, the smaller the particles the slower will be their

sedimentation rates and, consequently, the longer will they remain airborne. As a large droplet takes longer to evaporate and falls more rapidly than a small one, it follows that, usually, only droplets of not more than about 20µm will be able to evaporate quickly enough to remain airborne, unless maintained by turbulent air currents.

Unless they are hygroscopic, the larger particles that fall upon a surface can dry out, fragment, and give rise to secondary aerosols. An example of the potency of aerosols from such dried material is the laboratory epidemic of Soviet haemorrhagic fever reported by Kulagin *et al.*, (1962): 113 people acquired the disease which was spread as an aerosol of virus-bearing particles from dried urine and faeces in the cage litter of 357 wild mice and voles; only three of the cases had had direct contact with the animals. That deliberately dried material, such as lyophilised cultures, can be similarly infective was shown by the accidental dropping of an ampoule of Venezuelan Equine Encephalitis virus on the landing of the fourth floor of a laboratory; Slepshkin (1959) reported that 24 workers, distributed about the third, fourth and fifth floors, were infected by the resultant aerosol, although Kenny and Sabel (1968) showed that 80% of the number of bacterial particles released by a similar simulated accident were larger than 15µm.

Survival of airborne organisms

So far we have considered mainly the generation of aerosols from liquid milieu. It is worth noting in passing that aerosols of bacteria generated from the dry state, as powders, have different survival properties from those generated from liquids; for example, such bacterial aerosols are less sensitive to ultra violet irradiation and, unlike aerosols from liquids, are able, when held in the dark, to repair damage done by irradiation (Dimmick, 1960). Such effects could result from the different method of aerosol generation producing a different distribution of particle sizes, and consequently, in the way in which the aerosol equilibrates with the surrounding atmosphere (eg, see Anderson & Cox, 1967). These effects could be important in downstream processing where the product required is a dried powder of the cells themselves. The human body is a copious generator of dry, viable particles, of which it produces in the order of thousands per minute (May & Pomeroy, 1973); many important plant pathogenic fungi disperse their spores in a dry state.

An initially viable organism in an aerosol is subjected to a variety of stresses which can kill or render it so fragile that its viability can only be demonstrated by careful choice of collecting apparatus and of recovery medium. The complexity of the interacting stresses has been symbolised by Larson (1973) who suggested the following equation:

$$R = a - bx - cx_2 - dx_3 - ex_4 - fx_5 - gx_6 - h_1x_1x_2\cdots - h_1x_1x_2\cdots - E$$

where R = response of the organism to aerosol stress, including the ability to infect a host

x_1 = Relative Humidity (RH)

x_2 = Temperature

x_3 = Chemical toxicity

x_4 = Radiation (solar)

- x_5 = Particle size
- x_6 = Microbial resistance factor (a time factor is implied)
- E = Inherent variability - statistical error.

To this should have been added a very important term representing the conditions of growth.

However, survival of the organism in a condition allowing infection of a suitable host will depend upon four main factors:

- a) the type of organism,
- b) culture conditions when the aerosol is generated,
- c) duration and conditions in the aerosol,
- d) the eventual growth environment.

The method and duration of sampling can also have an effect, as discussed later.

We will now consider these in sequence.

a) the type of organism

Since so many factors influence survival, it is not possible to make a "league table" of aerostability; but it is certain that some organisms are more aerostable than others. The spores of many moulds are very tough, and even the mycelia, the growth filaments from which those spores arise, have been found to survive in natural aerosols (Kramer & Pady, 1968). The effects of environmental conditions upon moulds have not, however, been studied in anything like the detail that they have been for bacteria. Amongst the bacteria, spores of Bacilli are regarded as tough, Escherichia coli is recognised as a typical, rather labile Gm -ve organism, Pasteurella tularensis as a fragile organism. In the case of E. coli it is safe to say that different strains will probably show a similar pattern of behaviour (Cox, 1966); but the nature of that pattern will depend upon the other factors that we shall next consider.

b) culture conditions when the aerosol is generated

Relatively little work has been reported on this subject and that which has been published has been done with only a few genera and species of organisms; but it does seem that the most important factor at this stage is the physiological state of the organism. Physiological state is often equated with culture age, and it is certainly true that an older culture will often survive aerosolisation better than a young, actively growing, culture. This was demonstrated, for example, by Brown (1954) who had previously shown (Brown, 1953) that, for E. coli, survival in aerosols is lowest at the transition from "lag" to "log" phases of a batch culture.

In a batch culture it is not easy to control the rate of growth of an organism, but in continuous culture it is possible to control this merely by the rate at which fresh medium is supplied to the culture. Using the continuous culture technique, Dark and Callow (1973) showed that fast growing cells are less aerostable than slow growing cells, but that even so

their aerostability was much less than that of batch grown cells. In a series of experiments, using defined and complex media in batch and continuous culture, they showed the effects of different media, of harvesting times and growth rates, demonstrating that with a complex medium aerosol survival is better in batch than in continuous culture (90 - 100% compared with 1 - 4%), but that in defined media the difference is less marked. Changing the growth medium can also affect other properties of organisms; for instance, Phillips (1965) showed that conidia of Fusarium roseum produced on a rich medium were more infective than those produced on a poor medium. Thus, physiological state refers to more than the age of the culture.

Organisms are very adaptable and exhibit quite different properties when grown under different conditions. In a previous report Sargeant and Evans (1979) drew attention to the changes that can be induced by the use of different growth-limiting nutrients and cited the change that occurs in the cell wall of Gm+ve organisms, the phosphorus-containing polymer teichoic acid being replaced by the phosphorus-free polymer teichuronic acid following a change from phosphorus excess to phosphorus limitation. Indeed, Dark and Callow (1973) showed, by their experiments with chemically defined media in continuous culture, that survival of E. coli in the aerosol state is improved about two fold, at slow growth rates, by using phosphorus or sulphur as the growth limiting nutrient. Now, although the cell walls of E. coli do not contain teichoic acid, it were not unreasonable to anticipate that such fundamentally important nutrients could change, for better or for worse, the aerostability of an organism. The effect of aerosol stress upon the cell envelope - wall or membrane - has been well demonstrated by Hambleton and Benbough (1973) who showed that organisms sensitive to aerosol stress became sensitive to lysozyme, RNase, DNase, trypsin and actinomycin D after even one second in the aerosol state, although previously insensitive to these agents. They found moreover (Benbough & Hambleton, 1973) that as much as 20% of the dry weight of an organism could be lost in the aerosol; a further indication of the loss of integrity of the cell envelope. Nevertheless, there is some laboratory evidence, using fluorescent antibody staining, that flagella (usually thought of as "delicate") are not necessarily lost from aerosolized bacteria (K.P. Norris, pers. comm.). Unfortunately there are no quantitative data on this point which might have an important bearing on the maintenance of the infectivity of a bacterial plant pathogen.

The composition of the medium could have another effect: as mentioned earlier, the presence of some sugars can enhance aerostability; Cox (1966), for example, showed that raffinose can increase the survival of E. coli held as an aerosol at a low relative humidity (20 - 30%); conditions that are generally liable to diminish survival. The production of polysaccharides usually requires the presence in the medium of some excess sugar (Evans, Yeo & Ellwood, 1979); but the presence of polysaccharide may itself be protective against desiccation in the aerosol (Hedges, 1926). Leach et al. (1957) found that the polysaccharide produced by Xanthomonas campestris pv. phaseoli provided protection against desiccation and, in diluted culture, against ultraviolet irradiation; but Southey and Harper (1971) were not able to show any increased survival of Erwinia amylovora cells aerosolised from 0.05% polysaccharide (plant exudate) in daylight, although, when held in the airborne state in the dark the cells' survival was improved by its presence. The concentration used by Southey and Harper however, in no way matched the concentration of the exudate from an infected plant which has been implicated in increasing dispersal and survival (eg, see Eden-Green & Billing, 1972).

c) duration and conditions in the aerosol

These two effects, duration and conditions, are not easily separable since the effect of a deleterious agent is affected by the time for which an organism is exposed to that agent. In laboratory investigations comparisons of different conditions are usually made using identical exposure times and then, often, the logarithm of survivors is plotted against the age of the aerosol. This can give the impression that death in the aerosol is a first order reaction. However, if the percentage of survivors is similarly plotted, it can often be seen that although there is a rapid initial decay to, say, 2% in the first 15 minutes, after even 3 hours there may still be 1% surviving. This is common to all processes of disinfection, including the effect of household disinfectants upon suspensions of "germs".

We shall now consider the principal causes of microbial death in the aerosol.

One of the most studied variables is relative humidity which has been shown to have a marked effect upon aerosol survival. In general it may be expected that death rates will be highest at the extremes of RH, 90 - 100%, and 20% or less; but even amongst the relatively few organisms that have been tested, there are plenty of exceptions. For example Anderson, Dark and Peto (1968) showed that for Aerobacter aerogenes, RH 95% and 50% were most lethal, but for Serratia marcescens strain UK8, survival was greatest at these humidities; Staphylococcus epidermidis however was able to survive well at all RH from 95% to 40% which was the lowest tested. However, Teltsch and Katzenelson (1978), who sampled air up to 350m downwind of a sewage wastewater spray used to irrigate a field, found that the recovery of enteric organisms was greatest at RH above 45%. They also showed that about 50% of the number of particles recovered was larger than 7 μ m.

The effect of RH, itself a complicated subject, is made more complicated by the way in which the organisms are handled after collection from the aerosol. Whereas the initial rate of evaporation of water from the bacterial particle has been shown to have no effect upon survival (Cox, 1968) it has been shown (eg, see Bateman *et al.* 1962) that the water content of cells is related to the RH of the aerosol, and by Goldberg and Ford (1973) that it is rather the rate at which the cells are rehydrated which affects survival; thus apparent survival is greatly improved by transferring the cells to an atmosphere with a RH of 90% before sampling. It is such points as these that may make difficult the extrapolation of laboratory results to outdoor practice; indeed Voinov and Khor'kov (1968) found that foot-and-mouth disease virus (FMD) survived in aerosols for only 24 hours, and yet, in the field, Hurst (1968) was able to produce "strong evidence" that the virus can be transmitted up to 1,000km.

A contrary effect is exerted by the so called Open Air Factor (Druett & May, 1969) which is probably the result of a reaction between ozone and olefines. This has a markedly lethal effect at night upon many of the bacteria and viruses that have been tested; even upon those that have been shown to be remarkably aerostable in the laboratory. It has even been suggested, in retrospect, that OAF may have been responsible for the prevention of the appearance of FMD and Newcastle Disease Virus in certain areas of the UK during the epizootics of those diseases in 1967/8 (Hugh-Jones, 1973). However OAF cannot be relied upon to kill airborne organisms since it seems only to be present, in lethal concentration, in those bodies of air that have passed over suitable generating areas, such as

towns and oil refineries.

OAF is only important in the dark because during daylight hours the sunlight can itself have an effect both by raising the temperature of the aerosol cloud and increasing the death rate, as well as through its ultraviolet component which, as has been shown by many workers (see Anderson & Cox, 1967 for a brief review) can be lethal to organisms in the air. However, it is probable that solar radiation can have a further effect by producing compounds photochemically (including ozone) that reduce the viability of airborne organisms or their competence to infect the host plant. The presence of contaminant materials dissolved in raindrops or by the rain water as it flows over the soil, might stress even further, or relieve, organisms already stressed by airborne travel.

Again it is not certain that the results of laboratory experiments will necessarily represent the effect of the environment upon naturally generated aerosols; those laboratory experiments that have been concerned with the airborne spread of human or animal disease have necessarily concentrated upon particles that can be inhaled deep into the lung, 1 - 5 μ m. Experiments designed to establish the effects of environmental factors upon the viability of aerosolised particles have, deliberately, involved the use of single cell particles (mono - disperse aerosols) to obviate the possibility of cells being protected by others surrounding them in the particle. Most natural aerosols - including those produced by biotechnology - will include packets of cells; see for example, May and Pomeroy (1973). Indeed, Andersson, Bergstrom and Bucht (1973) found that locally produced natural aerosols of bacteria, sampled 1.5 meters above ground level, consisted mainly of particles greater than 6 μ m. During 24 hour trials of a high volume sampler, Benbough, 1973, (pers. comm.) found that there was a particular time in each summer night when a large natural cloud of organisms was released into the atmosphere; the time of release became later as the year progressed until it ceased in early winter. This sudden release, which was not dependent upon rain, was marked by an increase from about 3 particles/litre of sampled air to more than 200 within 2½ hr, followed by a return to about 3 organisms/litre after a further 3 hr. Using a three stage sampler, set about 11m above ground level, he found that often the number of particles greater than 6 μ m equalled or exceeded the 3 - 6 μ m particles which in turn exceeded the particles of less than 3 μ m. Zhalko-Titarenko (1965) and May (1966b) have shown that, with some bacteria at least, survival is greater in aggregates than in single-cell particles. Subsequently, May (1972) showed that survival is inversely proportional to particle size, and that clumping protects organisms against more than the effects of radiation.

d) the eventual growth environment

Again there is much uncertainty; we have shown in the previous section how the viability of an organism is affected by the ways in which it is recovered from the aerosol - processes which equate with deposition of the organism from the "natural" aerosol. As shown in earlier chapters, plant pathogens are fairly specific for the type of host that they can infect, so the chances of an organism reaching an acceptable host - assuming that the organism is still infective - will depend, inter alia, on the frequency of occurrence of suitable hosts, the meteorological conditions on arrival, and the preferred point of entry into the host. Corn is grown in many large fields so the chances of a successful landing by a rust organism are relatively great; similarly with bacteria and viruses that infect potatoes. As we said in Chapter IV, intensive farming increases disease risks.

The climatic conditions will govern the method of landing which can either be wet or dry. In the case of a wet landing, larger particles can be washed out of the aerosol by rain, sleet, hail or snow (precipitation), and the type of precipitation can itself increase the chances of infection being established; rain, by creating splashes, can generate secondary aerosols to spread further the infection (see Quinn, Sells & Graham, 1980), and hail, by damaging plant structures, can facilitate entry of the organism. In the case of a dry landing, deposition of the organism will depend first upon the position of the aerosol in the moving air mass and the rate at which that mass is moving: if the aerosol particle is high above the ground it will travel on; if it is near the ground it may be in turbulent air which can help it to break through the boundary layer of air associated with the crop; if the rate of airflow is very low it is possible for deposition to occur through settlement, but only large particles will fall out since air is seldom truly still, if only because of thermal effects. For small particles it is more likely that they will be impacted onto a plant surface; then the nature of that surface becomes important. Chamberlain (1967) has shown that those surfaces having hairlike roughness are more efficient than smooth ones at causing particles in the 1 - 5µm range to stick, possibly due to the micro turbulence that the roughness creates. Subsequent entry of a bacterial pathogen into the plant may possibly depend upon its possession of flagella which may enable it to swim to a stoma and so obtain entry to a host plant and start an infection. There is no evidence as yet for bacteria swimming on leaf surfaces, but conditions do need to be suitably moist for infection to occur. If, at landing on a leaf the climatic conditions are too dry, the soft rot erwinias, at least, can remain quiescent for more than seven weeks and resume growth when the surface becomes wet again (Perombelon, 1978). If the landing is on soil they can survive, and Perombelon (1981) has also shown that a high proportion of samples from lochs, streams and rivers in Scotland - even those far removed from arable areas - are contaminated with erwinias.

Transport of airborne particles

The ability of the atmosphere to serve as a medium for the transport of quite large objects has been recognised by some and doubted by others for centuries. Aristotle recorded the occurrence of showers of frogs, and in 1840 a M. Pontier recorded a shower that deposited small frogs over an area that could be traversed by 15 minutes of brisk trotting on horseback (Allen, 1979). These had been sucked from the surface of the water in which they lay by a severe storm and carried, in the turbulence, many miles before their relatively high sedimentation velocity allowed them to be deposited on the land; showers of fish, slugs and snails have also been reported. In 1968 people in the south of England awoke to find that a severe rain storm in the night had deposited a layer of red dust; Stevenson (1969) was able to show that this had been transported from the southern Sahara - 4,000km - and estimated the weight of material at one million tonnes! By plotting the course of the dust laden air, she was further able to show that it had been carried at a height of between 16,000 and 20,000ft. In a similar way, Bovallius *et al.* (1978) were able to identify the provenance of a layer of red snow in Sweden as the Black Sea area of Russia; this parcel of air had taken two days to travel about 1600 km and had brought grains of pollen and spore-forming bacteria. Earlier, Bucht, Rydgren and Wallin (1971) (quoted by Andersson *et al.*, *loc. cit.*) had shown, by trajectory analysis, the transport of bacteria, almost exclusively Gm⁺ spore-forming rods, over a distance of 1,800km. It is very unlikely that a bacterial plant pathogen could survive airborne transport over anything like this distance or for such a time because fortunately, and perhaps surprisingly, no known bacterial

phytopathogen forms spores.

Mould and fungal pathogens, however, do form spores and figure frequently in accounts of long-range spread of plant diseases. One of the best known is the transmission of the rust fungus Puccinia graminis from the USA to Canada (see, for example, Stakman & Harrar, 1957), but it now seems that there are similar migrations in other parts of the world of, for example: oat crown rust from Tanzania to Israel, black rust of wheat from the Iberian peninsula to England (Ogilvie & Thorpe, 1966) and yellow rust of wheat from Turkey into India (Zadoks, 1973). Hirst, Stedman and Hurst (1967) found, at a height of between 500 and 1500 meters over the North Sea, spores of, inter alia, Cladosporium from the United Kingdom and uredospores of Puccinia graminis possibly from east of the Baltic. They concluded that distant spore transport is both frequent and extensive, and probably important in temperate latitudes; see also Chapter V.

Less work has been done on the airborne transmission of bacterial plant pathogens although such a mechanism for their spread has been suspected for many years (see, for instance, the paper by Baker, 1971); but it is only during the past 10 years or so that systematic air sampling and laboratory tests of aerostability have been carried out. Understandably, most of the work has been done on two of the most prevalent diseases; potato black-leg and fireblight. Graham et al., (1979) examined the aerostability of Erwinia carotovora sub sp. atroseptica using the microthread technique of May and Druett (1968) and were able to show that even in the open air some organisms could survive for up to two hours, i.e. equivalent to a distance of up to 20-40km, in conditions of high relative humidity. They went on to show (Quinn et al., 1980) that from summer to early winter erwinias could be detected near infected potato crops on some occasions when it rained (not during dry spells) and sometimes even at sites removed from any known potato growing areas; the latter bacteria had most probably travelled, at least, several km. The work of Southey and Harper (1971) has already been mentioned; they were able to show, using the microthread technique, that significant numbers of Erwinia amylovora were able to survive in the open air for at least two hours with survivals similar to those of a robust Escherichia coli. Unfortunately they were unable to follow this work by sampling in an infected area.

Apart from the possibility of their spread as discrete aerosol particles, bacterial and fungal plant pathogens can be spread in association with pollen grains and seeds. Phatak (1980) stated that 60 species of bacteria from five genera can be transmitted in the seeds of over 100 host species, and that a large number of fungal pathogens, belonging to about 90 genera, are seed transmitted in several hundred hosts. Survival in seeds is greater for fungi than for bacteria, but in either case can exceed 20 years. There are no data on the transport of fungi in association with pollen, but some bacteria can infect, and others merely contaminate, pollen grains thereby increasing, in many cases, the chances of the spread of disease (Phatak, 1980).

Two other important genera of bacterial plant pathogens are those of Pseudomonas and Xanthomonas. Ps. syringae glycinea is a pathogen of soya bean plants and it has been shown by Venette and Kennedy (1975) that aerosols of this organism formed when infected crops were irrigated by overhead sprinklers, and during rainstorms. Claflin, Stuteville and Armbrust (1973) showed that, although they are not soil pathogens, Xanthomonas campestris pv. alfalfae and X. campestris pv. phaseoli are carried on wind-blown soil particles to spread infections of, respectively, leaf spot of alfalfa and common blight of beans in Kansas, USA. X. campestris pv.

malvacearum, which produces black arm of cotton, has been reported by Zadoks (1973) to be transported over many km in the Sudan by driving rains. Both these genera contain many organisms that are known to be ready producers of polysaccharides, indeed X. c. pv. malvacearum has been mentioned in a number of patents (see for example McNeely, 1968; Patton & Lindblom, 1962, and Chapter VIII).

Although we have considered principally the airborne transport of organisms that attack the above-ground parts of plants, those that gain entry through the roots should also be mentioned. It is generally believed that these are chiefly spread by water flow, but Burke (1965), for instance, found that Fusarium solani f. phaseoli was unable to spread in the soil even 1.3cm from an infected to a non-infected plant. For such, some other method of dispersal must be at work, and as Wallace (1978) has stated: "it seems likely that dispersal of soil pathogens by wind and water occurs frequently, intensively, and sometimes over several miles".

To sum up: while it is well recognised that many fungal diseases can be spread by airborne transport, there is good evidence to support the hypothesis that at least some bacterial plant diseases can be similarly spread.

Meteorology of aerosols

We cannot here give a full account of the meteorology of aerosol generation, transport and deposition, only a brief outline of some of the mechanisms involved; for a fuller account see, for example, Tyldesley (1967), Pasquill (1974) or Mason (1979). We will assume that little transport of microorganisms will occur in winter since, with temperatures low and crops relatively sparse, there will be little growth and production of microorganisms to be transported: Benbough's unpublished results support this assumption.

Even the fresh air we breath in the country is microbiologically unclean. In warm weather organisms can be lifted from the ground by rain splashes and thence, by thermal uplift and frictional turbulence, into the lower atmosphere; but as Benbough has shown, rain is not a prerequisite: combinations of frictional turbulence, plant movement and thermal currents can be sufficient to lift microorganisms from an infected crop area, plant or soil. Once the aerosol has left the boundary layer of the crop or ground, sedimentation velocity of the particles will, except for very large particles, be small by comparison with the eddy velocities in turbulent air and with convection currents. At night there is the possibility of cold air flowing down an incline to carry away particles which can then be lifted further by convection currents and turbulence. Once in the lower atmosphere further up-lift can be arrested by a stable air layer, such as an inversion, or particles can be redeposited by frictional resistance, turbulence or down draughts. If this does not occur, the aerosol can rise into the atmosphere carried by convection (particularly when the sun returns), by a warm front slipping under the cold air carrying the aerosol, by convective bubbles of warm air which can be from 300m - 2km across and which can rise rapidly at up to 6m/sec. (Hirst & Hurst, 1967); (since bacterial survival decreases with increasing temperature it is not surprising that disease episodes resulting from long distance transport of relatively inert mould spores are much more frequently reported than those caused by bacteria). If sufficient height is thus achieved then further transport will depend upon the movement of large parcels of air in large pressure systems as was the case with the Sahara and Black Sea dust storms to which we have already referred.

It is evident that the higher a particle rises the further it is likely to travel; it is also clear that the further an aerosol particle travels the greater will be the stress to which it is subjected. However, it may not need to ascend more than a few meters above ground level in order to be transported many meters or more. Pasquill (1974) quotes results of Chamberlain (1966) which showed that if *Lycopodium* spores (30 μm diameter) were released, as tracers, 1 meter above ground level in suitable conditions, the cloud retained 76, 50 and 28% of the original spores, at 100, 1,000 and 10,000 meters distance respectively. From greater heights the descent can be occasioned by rain (for large particles), by subsidence (the sinking of air behind a fast-moving depression), by turbulence or by down-draughts of colder air, the particles then being filtered out by a suitable host crop, or by deposition onto soil in which the host crop is to be planted.

Methods for sampling airborne particles

Here we attempt to present a brief resume of the principle types of samplers for airborne particles; further details of principles, construction and methods of use can be found by recourse to the references. We shall not consider the non-specific particle counters, such as the Royco, since they cannot discriminate between different types of microorganisms, biological detritus and organic dusts, nor between viable and non-viable organisms.

Although, throughout this Chapter, we have mainly considered mono-disperse aerosols composed of particles in the 1 - 5 μm range, a natural biological aerosol may be, as we have mentioned, composed of particles varying in size from 1 μm (bacteria) to more than 100 μm (fungal spores, mycelial fragments and pollens). Within these limits there are also smaller individual units which can form aggregates with or without fibres, polysaccharides, soil, plant particles and exudates, etc. Although the terminal velocity of a 100 μm spherical particle of unit density in still air at 760mm Hg and 20 $^{\circ}\text{C}$ is 24.7cm/sec. (Green & Lane, 1964), this does not preclude it from airborne transport if the meteorological conditions are suitable; we have already referred to the airborne transport of froglets, etc. Moreover, shape can significantly affect the sedimentation velocity, a fact utilised by some seeds and pollens.

Because there is such a range of particle sizes distributed throughout the atmosphere and up to a height of several miles (Postgate, 1969), it is not possible to use a single type of sampler. Moreover, although there may be times when it is sufficient to count, and even identify, particles microscopically without consideration of whether they be alive or not, for most epidemiological purposes it is essential that the particles be caught in a manner that will ensure that they are not further stressed to an extent that will prevent their subsequent growth, if viable.

Apart from the settle plate, which can only be used to estimate fall out, there are two stages in entrapping an aerosol: first, a stream of air must be drawn into the apparatus in such a manner that it is a truly representative sample with no concomitant change in the concentration of particles; this so-called "isokinetic sampling" is an ideal that cannot wholly be achieved in the open air where wind speed and direction fluctuate: second, unless subsequent examination is only to be by microscope, the particles must be concentrated in a manner that will not further diminish their ability to grow or to infect a suitable host, nor, if collected in a liquid milieu, to allow them to grow and so produce a specious, high count. It has been said that the number of samplers that have been developed is roughly equal to the number of investigators; a public health monograph (Wolf *et al.*,

1959) lists and describes no less than 37, but since then many more have been described in the literature (see May, 1967 and 1972 for a general review of theory and methods, and May, Pomeroy & Hibbs, 1976 for a particularly valuable comparison of the efficiencies of 17 different sampling techniques for large windborne particles).

In the laboratory it is very often sufficient to use apparatus that samples air at only 28 litres/minute because the air being sampled is largely contained within the relatively small volume of a room and in which the concentration of suspended particles is often rather high. In the open air the situation can be very different: particles from a point source (plant, field or factory) are diluted by vast volumes of air, the dilution increasing with distance from the source. There is a further complication in that the direction of the wind is seldom steady; whereas a 10° wind shift will have, if any, very little effect upon samples taken at the middle of the downwind edge of a heavily infected field 100m square, it could cause the cloud of airborne particles to miss completely a sampler placed 300m away. A plume of smoke from a chimney or bonfire, observed from a variety of suitable vantage points in different meteorological conditions, gives a visible demonstration of wind variation and also of atmospheric dilution as the originally thick plume of smoke may quite quickly disappear into the atmosphere. There may thus be need for more than one sampler to cover wind shifts and, to overcome the effect of dilution, for samplers able to concentrate the aerosol content of large volumes of air.

Types of sampler. The Casella large model slit sampler samples 700 litres of air per minute directly onto a 14cm petri dish of agar. This, a direct descendant of the Bourdillon slit sampler, (Bourdillon, Lidwell & Thomas, 1941) can only be run for a few minutes before the agar dries out to an unacceptable extent unless OED (oxyethylene docosyl ether) (May, 1969) has been previously poured over the plates, in which case about 40,000 litres of air may be sampled in one hour. In a similar way the Andersen (1958) sampler, which samples 28 litres of air/minute, can be used to sample 13,000 litres during the course of a working day with only one set of plates. The Casella large model sampler does not discriminate different sizes of particles; the Andersen does, but is inefficient at collecting particles larger than 18µm. A limitation of this type of sampler is that if the concentration of organisms in the aerosol is too high, colonies of interest will be confluent, uncountable and may overgrow or be overgrown by other organisms. Selective media can be used to overcome this problem, but an aerosolised (stressed) organism may be less able to grow even on a medium designed to select it (Lidwell, 1966).

A different approach is to sample the air in such a way that particles are impacted onto a liquid film which is continuously collected and renewed. Three such samplers have been reviewed by Decker *et al.* (1969): the electrostatic precipitator, the multi slit, and the cyclone samplers. The electrostatic Litton sampler is 80-90% efficient for 1 µm particles at 1,000 litres/minute and relies upon the attraction of negatively charged particles to a liquid covered disc that is at ground potential; it is capable of operating at up to 10,000 litres of air/minute, concentrating the particles into 10ml. It needs to be worked by trained operators. The multislit sampler impacts particles through eight slits in a manner similar to the Bourdillon sampler, but, like the Litton sampler, onto a wetted disc. It is about 90% efficient for 1 µm at 1,000 litres/minute and is more compact and easier to use than the Litton, although still large and complicated. The Aerojet cyclone is simpler and much cheaper: a fine mist is generated and injected into the fast moving air stream where it entraps the aerosol particles. The mist with

the entrained particles is then removed by centrifugal action in the cyclone, and the liquid is collected. For an account of the theory of cyclones see Errington and Powell (1969) who also describe perspex or stainless steel models sampling at 75 and 350 litres/minute. Another development of the cyclone, sampling at 20,000 litres/minute, was described by Fontanges and Isoard (1973); however, the 1,000 litre/minute cyclone is virtually as useful as any of the higher volume samplers which merely concentrate the particles into a proportionately larger volume.

A still simpler and cheaper derivative was described by Buchanan *et al.* (1972). Made from plastics material, the "Simple Liquid Scrubber" samples air at up to 950 litres/minute with a concentration factor of 400,000:1; it has an efficiency of 90% for 1.8µm particles, and an efficiency greater than the All Glass Impinger (the standard against which all the samplers so far described were measured) for particles in outdoor air. Like the aerojet cyclone it is portable and easy to use.

Even simpler and more portable is the battery operated Rotorod sampler, developed by Perkins and described by Gregory (1961) which consists of two rods, 1/8" in section and six inches high that rotate about a four inch radius at 2,500 rev./min. sampling the air, independently of wind direction, at an effective rate of 120 litres/minute; this system was used by Asai (1960) to study the movement of the fungal spores of black stem rust of wheat in the upper Mississippi Valley. However, it is of no effective use for sampling particles smaller than 7 µm (May *et al.*, 1976). Fungal spores have also been sampled using "sentinel" plants or tissues, held in containers through which the air to be sampled is drawn (Schwarzbach, 1979); the presence of a specific plant pathogen can be detected in this way if the appropriate host plant has been used. Although this is a rather slow method of detection, it has the singular advantage that it can detect infectious, as opposed to viable, particles; these being not necessarily synonymous, as Hood (1961) and Henis and Ben-Yaphet (1970) showed. The sampler is particularly suited to sampling mildew spores which tend to stick to dry surfaces from which, being sensitive to water, they are difficult to remove.

The morphology of fungal spores is fairly characteristic of some genera and species which can therefore be identified microscopically by trained personnel. There are many spore samplers which deposit the sampled particles onto microscope slides: cascade impactor (May, 1945), Kramer-Collins spore sampler (Kramer & Pady, 1966) which gives discrete hourly samples on one microscope slide for 24 hours, the Hirst spore trap (Hirst, 1952) to mention but a few. In parenthesis, it should be mentioned that, although widely used for supplying pollen counts, the Hirst spore trap has a low efficiency for particles of even 20µm and that this diminishes as the particle size increases; although based on the second stage of the May cascade impactor, this is largely due to the extraordinary, large rain shield which imparts such inertia to the sampler that it may, momentarily, be 90° out of phase with changes of wind direction (May *et al.*, 1976). Impacted spores can be removed with a micropipette and transferred to a suitable growth medium to give some indication of the viability of a small portion of the total sample; for a review of methods of dealing with samples see Noble (1967).

Strange (1973) has reviewed various rapid methods for detecting and/or identifying airborne microorganisms which he classified into three groups:

- a) those that detect viable organisms; these rely upon the metabolic activity of the sampled organisms to cause a pH or

turbidity change, produce CO₂ or to take up phosphate from the medium;

- b) those that detect the presence of microorganisms; these detect various chemicals that are ubiquitous in microorganisms, such as DNA, RNA, ATP or, using chromatography, products of their pyrolysis;
- c) those that are specific for organisms of predetermined interest; these use specific antibodies that are either fluorescent or have been labelled with a radio isotope of, for example, iodine.

For bacteria the detection limit of the most sensitive methods is about 10⁵ organisms, so, if the concentration of the organism of interest is only 1/litre of air it will be necessary to sample at least 100,000 litres to obtain sufficient identifiable material. Such a concentration will increase proportionately the concentration of background particles i.e. detritus, pollutants and other organisms not of interest. If the particles of interest are formed of aggregates of the organism, as the work of May, Benbough and others would suggest, the volume sampled could be reduced, but this would only slightly reduce the high concentration of background particles.

In general, the specific methods will be of most use in detecting plant pathogens, but they require the prior preparation of antisera specific to the pathogen of interest. Such methods have the advantage that they are rapid, do not depend upon the ability of the sampled organisms to grow, are applicable to bacteria and fungi, and are amenable to automation (see also Harper, 1976). However, they give no indication of the ability of the sampled organisms to cause disease; this the Schwarzbach sampler does, but at the expense of considerable delay.

From this brief survey of methods it is clear that the choice of sampling method for the detection and possible identification of airborne particles must depend upon the purpose of the experiment and the resources available. In conclusion, we strongly advise anyone interested in sampling the large particles of "natural" aerosols to read the paper of May *et al.* (1976) wherein the difficulties, and some methods of surmounting them, are most clearly explained.

This Chapter has been written at some length, and in the style of a review, because only a very limited number of people have hitherto needed to take an interest in the subject. We hope it will be useful not only to the plant pathologists and biotechnologists interested in the growth of plant pathogens, but also to biotechnologists in general.

In the next Chapter we go on to consider the ways in which biotechnological processes can give rise to aerosols.

CHAPTER VII

AEROSOL GENERATION IN PROCESSES USING PLANT PATHOGENS

It follows from Chapters IV, V and VI that the release of plant pathogenic microorganisms as an aerosol, at times when atmospheric conditions favour survival and dissemination of the aerosol to a susceptible crop, constitutes a potential threat to plant health. Let us therefore examine the means, most often in liquids, by which microorganisms are propagated, and processed, on an industrial scale, identifying the quantities involved at the various stages. Let us then consider how aerosols of living microorganisms might be released into the atmosphere during the processes concerned.

Five stages are commonly involved in the industrial use of microorganisms and their products: culture preservation, inoculum preparation, large scale growth, separation of the microorganisms in the form of a damp solid from the liquid medium in which they have been grown, and their subsequent use. These stages, as they apply to aerobes, will be described and considered in turn. For anaerobes the processes are similar, except that steps are taken to exclude air from the cultures.

Culture preservation has as its objective the preservation of the microorganisms in such a way that a very high proportion of them remains viable for long periods, while retaining their desirable properties unaltered. Typically, the microorganisms to be preserved are cultivated in volumes of less than 100 ml, a preservative is added and the resultant mixture is transferred in quantities of up to 1 ml into small tubes. The tubes are either sealed and stored at -180° or -196° , above or in liquid nitrogen, or submitted to freeze drying to remove most of the water, sealed, and stored between room temperature and 0° . These tubes form the starting point for all future growth, and in the case of bacteria, each sealed tube will contain a few milligrams (dry weight) of the microorganisms - say 10^{10} cells.

Inoculum preparation has the aim of providing the required microorganism in a quantity, and of a quality, suitable to initiate the large scale cultivation. A tube of the preserved culture is opened and its contents transferred into a flask containing about 100 ml of a sterile nutrient solution or suspension. The flask is closed with a filter which permits the passage of gases, but not of microorganisms, and maintained at a suitable temperature (in the authors' experience, depending upon the organism involved, this may be between 4° and 75°) until satisfactory growth has occurred; perhaps 18 hours. This culture is further multiplied by transfer to a similar, but larger, flask containing up to a litre of sterile nutrient medium: this flask is maintained at the same temperature and is often automatically shaken to increase gaseous interchange across the liquid/air interface; again the process may take about 18 hours, after which the culture is transferred to a sterile container specially designed to facilitate the further transfer of its contents into a culture vessel.

Up to this point it may have been necessary to limit the amount of growth at each stage lest the organisms, in growing, should cause the medium to become too acid or too alkaline and so halt growth or even kill the organisms; besides, the rate at which oxygen can be transferred to the culture in a shake-flask is often too slow to permit rapid growth of dense cultures. Once the seed culture has been transferred to the culture vessel

these restrictions no longer apply. Typically, there may be only a few grammes (dry weight) of organisms at this stage.

Large scale growth in a fermenter allows the microorganisms to grow under conditions which are controlled so as to maximise the yield of desired product. It is usually carried out in a stainless steel tank which is fitted with a mechanical stirrer. Often the final process is preceded by growth in successively larger tanks; for example, successful growth on a 20,000 litre scale might require the intermediate propagation of 20, 200, and 2,000 litre stages. Most industrially important microorganisms require oxygen for growth in good yield, and for these compressed air, that has been sterilised by filtration, is allowed to enter the vessel, near the base, at flow rates as high as one culture volume per minute. The vessel's contents are stirred vigorously, with energy inputs of up to 10 watts per litre, and the spent air, which is inevitably charged with an aerosol of microorganisms, leaves the vessel via a tube situated near its top.

The final production stage of cultivation can be carried out either in the batch mode or the continuous mode; if in the batch mode, the whole culture is harvested as a batch after, usually, not more than a few days; if in the continuous mode, the culture is fed continuously with fresh culture medium and simultaneously harvested at the same rate. At present most production processes are carried out batchwise, but some more recently developed processes (for example ICI "pruteen") use the continuous mode. Indeed, if the production of bulk chemicals by fermentation is ever to become economically successful, very large continuous systems will be needed. (A. Spinks, The Philips Lecture at the Royal Society London, 8 June 1981).

A harvested culture will usually contain between one and 20 kilograms (dry weight) of microorganisms per 1000 litres, but it should be remarked that a manufacturer normally seeks to maximise the yield of cells in order to minimise production costs and that in some cases yields several times greater than this are obtained.

Separation of the microorganisms is usually the first step in the purification of the desired product which, if not the organism itself, is normally present either inside the cell, or dissolved in the medium; rarely, in both. Moulds are usually harvested by a system of continuous filtration; bacteria by use of a centrifuge. At this stage one might recover, very roughly, up to about 100 kg of wet cell material from 1,000 litres of culture. However, a centrifuge does not remove all the cells from the culture broth - in our experience an initial count of 10^{10} organisms per ml may be reduced to not less than 10^4 per ml. If a sterile culture fluid is required for further processing these residual organisms can be removed by some second process, such as filtration or perhaps killed by passage through a heat exchanger in which the liquid can be rapidly heated and as rapidly cooled.

Subsequent processing of the microorganisms varies widely, depending upon the purpose of the fermentation. In some cases the whole culture may be used without separation of the cells; some of the uses of the gums produced by Xanthomonas spp. exemplify this. In other cases the separated microorganisms may be used without further processing beyond drying; yeasts, moulds and bacteria used as "single cell protein" fall into this category. In yet other cases the cells must be subjected to some form of physical or chemical treatment to promote the release of a desired chemical product which may then be purified; the asparaginases from Escherichia coli and Erwinia chrysanthemi are examples of this. As we have seen, up to

about 100 kg of wet cell material might require to be processed from each 1,000 l of culture.

Release of aerosols during growth Aerosols of living microorganisms will inevitably be generated during all the stages described, and if precautions are not taken to contain them they may be released into the environment. However, it is clear that the quantities of microorganisms handled during all the stages from culture preservation to inoculation of the fermenter are so small that it is possible to do all the necessary operations in approved safety cabinets and so, virtually, to guarantee that microbial aerosols are not released. Within the fermenter dense aerosols are produced, due to the continuous formation and breaking of liquid films. Measurements carried out using small scale apparatus suggest that the effluent gas might contain as many as 10^9 viable particles per m^3 ; thus from a large fermenter of $1,000m^3$ working volume, operated under the conditions already described, $1,000m^3$ of effluent gas might be expected to emerge each minute carrying perhaps 10^{12} particles.

However, large industrial fermenters contain features, different from those of small-scale apparatus, which may affect the concentration of viable microorganisms in the effluent gas. Many factors will influence this, for example: type of cells, nature of the culture broth, rate of aeration, distance between liquid surface and air outlet, foaming, and design of the air outlet.

Some of these factors were investigated at Grangemouth by the BP Company using a $150m^3$ fermenter growing their "Toprina" yeast (not a plant pathogen). Viable cells in the exit air could be as low as 10^5 per m^3 (J. Shennan, pers. comm.). It was found that cyclone separators, similar in principle to the aerogel sampler described in Chapter VI, could reduce the organisms escaping, but at the cost of increased risk of contamination of the culture.

For fermenters up to a few thousand litres, filtration of the effluent air can be carried out at reasonable cost, but the effluent gas stream from large fermenters is neither filtered nor heat sterilised; to do so were both difficult and expensive with such large volumes of water saturated air involved. Whether or not it is safe to release an aerosol in this way will depend upon the organism and the way in which it is being grown. We have mentioned in Chapter VI the influence of growth conditions on aerostability and pathogenicity, at least for bacteria, and shall, in Chapter VIII, give examples of the loss of pathogenicity in process strains.

In comparison with the numbers of viable aerosol particles that might be released in the culture effluent gas, all other releases of aerosols associated with the culture process are negligible. For example, only very small amounts of aerosol (similar in quantity to those released during laboratory scale work) will be generated during sampling and these can readily be contained using an appropriately enclosed sample point (eg, see Harris-Smith & Evans, 1974).

Release of aerosols following growth Separation and subsequent processing always involve the breaking of liquid films which, as we have seen in Chapter VI, releases airborne particles. For infective plant pathogens therefore it is desirable that, prior to processing, the microorganisms should first be killed, by heat or by chemical treatment for example.

In many cases, however, this will not be possible because of the lability of the desired product and the live culture must then be processed. Such processing can be carried out in ventilated cabinets or rooms, which have been fitted with suitable air filtration equipment. It should be noted, however, that the aerosols released during processing will, in all probability, be much less than those released via the air outlet from the fermenter. For processing a few hundred kilograms of cells, this is a feasible, if somewhat costly solution.

We therefore conclude that, provided the scale of manufacture does not exceed a few thousand litres, containment of the aerosols generated at all stages is possible. As the scale increases so the cost and the difficulty of containment inevitably increase. We do not, therefore, believe that a plant pathogen, which is still fully virulent after growth in a fermenter, can, at present, be handled safely on a very large scale; at least, in the vicinity of crops of suitable host plants. We shall consider this further in Chapter IX.

In the next Chapter we consider the uses to which plant pathogens are, or might be put.

CHAPTER VIII

PRESENT AND POSSIBLE FUTURE INDUSTRIAL USES OF PLANT PATHOGENS

The past few years have seen an explosion of European interest in Biotechnology, the use of microorganisms or their products to obtain materials in return for the minimum expenditure of energy, which has led to the growth of organisms on a scale approaching that of the heavy chemical industry. Those who have watched Biotechnology develop elsewhere in the world feel this interest to be long overdue. For example, 3.5 billion litres of fermentation alcohol were produced in Brazil during 1979 to spare petrol, and the Government, which is determined that production shall increase, has forecast that production in 1985 shall be 10.5 billion litres (Bazin, 1979). In Russia, heroic efforts have been made in the last 15 - 20 years to ease the shortage of protein for animal feed by the production of single cell proteins (SCP); much of it from agricultural and other industrial wastes. Carteg (1981) has estimated that in 1979 production was already greater than 10^6 tonnes per year; but the Government seem determined that this shall ultimately exceed 10^7 tonnes per year, so making Russia self sufficient for protein.

By comparison, European efforts seem puny; for example, the present ICI "Pruteen" factory is capable of producing an estimated 60,000 tonnes of bacteria per year containing about 43,000 tonnes of crude protein (Sherwood, 1980); but this is merely one plant; in Russia there are probably at least 86, but even so the protein produced on 10 acres by ICI is roughly equivalent to that produced on 25,000 acres by soyabeans (P.J. Senior, pers. comm.). There may be a factory in the next few years, and even the possibility of the production of materials for the manufacture of plastics by microorganisms (King, 1981) who also underlined the fact that the economics of processes improve with increasing scale. Another source of SCP is that produced by Ranks Hovis McDougall Ltd. from the fungus Fusarium graminearum; after 10 years of development, this was given clearance for human consumption in 1980 (Dunnill, 1981).

The increased interest in Biotechnology is principally evident in the number of Government sponsored reports (Canada, France, Germany, USA, the Netherlands, Ireland, Japan, Britain), the founding of new scientific journals of Biotechnology, the inclusion of a Biotechnology section in the Forecasting and Assessment in Science and Technology (FAST) Programme of the EEC, this report, and a multitude of articles in the popular and semi-scientific press, some of which have announced the financing of biotechnological projects by organisations as far removed from Biotechnology as Banks and Insurance Companies.

At present "Pruteen", the high grade bacterial protein produced by ICI, is acceptable only as an animal feed supplement, because, despite very extensive, and expensive, toxicity testing, there are still doubts about hypothetical risks attendant upon its use as a human feed supplement. The use of plant pathogens to provide useful products is as unlikely to produce direct, adverse consequences for man; but what, it will be asked, of the consequences for the plants upon which man, ultimately, depends? In this chapter we consider the present and possible industrial uses of plant pathogens.

In a previous report, Sargeant and Evans (1979) drew attention to the

increasing industrial use of plant pathogens and cited the use of Erwinia chrysanthemi for the production of the proven anti-leukemia enzyme asparaginase on a 400 litre scale. It is germane to consider the philosophical thinking that led to the choice of this organism in 1967: plants contain high levels of the amino acid asparagine and it was thought that the ability to metabolise this amino acid, which plays an important part in the ammonia storage system of plants, could confer an advantage upon a would-be pathogen. Following an extensive screening of many bacteria, including plant pathogens, an isolate of E. chrysanthemi was selected that produced, on solid media, about 5 International Units (iu) of asparaginase/mg of protein (Wade, Robinson & Phillips, 1971), but using continuous culture Callow, Capel and Evans (1975) were able to increase this to 210 iu/ml, about 36 iu/mg protein. This demonstrated that initially low levels of a desired product can, by appropriate choice of growth conditions and by careful strain selection, be increased to levels adequate for economic production. At present, batch production of the organism selected in continuous culture is only about 200 kg dry weight per year.

There is an even smaller scale demand for restriction enzymes produced by plant pathogens such as: Xanthomonas campestris pv. badrii (Xbal), X.c. pv. malvacearum (Xmal) and X.c. pv. holicola (Xhol), but while use of these may have a profound effect upon biotechnological processes through the new manipulated organisms that their use may help to create, production of enzymes from these organisms will be satisfied by their growth on a small, readily contained scale.

These applications of plant pathogens are very small compared with the present and forecast scale of use for polysaccharide gums produced by bacteria and yeasts. Following the pioneering work of the Northern Regional Research Laboratory, Peoria (eg, see Jeans, Pittsley & Senti, 1961) where the first industrial-scale production of the polysaccharide from X.c. pv. campestris was performed (Rogovin, Anderson & Cadmus, 1961) the production of xanthan gum has become an important industrial process. In 1979 the world production of xanthan was about 5.5k tonnes/year (Sutherland & Ellwood, 1979), but it has been estimated (Wells, 1977) that, by 1990, use by the oil industry alone will reach 50k tonnes/year. This may now prove to have been an over-estimate because, increasingly, there are reservations about the suitability of xanthan for EOR (enhanced oil recovery); nevertheless, there is likely to be a continuing demand for use in drilling muds on account of its pseudoplastic properties (Gabriel, 1979). However, it is the only microbial polysaccharide approved for food use by FDA and EEC, indeed the largest current demand is from the food industry where the gum is used as an emulsifier, suspending agent and gel former, to control ice crystal formation in frozen foods, and to improve "mouth feel" or flow characteristics (Andrew, 1977).

Xanthan gum also has applications in printing inks, paints, abrasives, adhesives, ceramics, cleaning agents, explosives and agricultural products such as sprays and feeds (eg, see McNealy & Kang, 1973). X.c. pv. campestris (brassicas) is the organism currently used to produce xanthan gum, but a variety of other pathovars of X. campestris that has been used for experimental production of gums; for example: pvs. juglandis (walnut), vesicatoria (tomatoes and peppers), malvacearum (cotton), vasculorum (sugar cane), phaseoli (beans and lupins), begoniae (Begonia spp.) and oryzae (rice). Some of these pathovars, eg vesicatoria, have been reported to be less productive than others (see, Lilly, Wilson & Leach, 1958): but Evans, Yeo and Ellwood (1979 & 1980) found considerable differences between different isolates of the same pathovar so it should not be assumed, on the

basis of a single isolate, that a particular plant pathogen is not suitable for polysaccharide production. Moreover, there is evidence that different isolates of the same pathovar can produce polysaccharides with slightly different properties; other differences can be induced by the use of different growth conditions.

Although those from Xanthomonas c. pv. campestris are currently produced in the greatest quantity, polysaccharides of industrial use are produced by other genera of plant pathogens. Kang, Veeder and Richey (1977) have reported the production of a polysaccharide (trade name Zanflo) by a soil isolate of Erwinia sp. which, unlike xanthan, is produced in good yield when the organism is grown with lactose as the carbon source. Since whey residues, which contain lactose, are readily available (indeed they can be something of an embarrassment) it were not surprising if the output of this polymer increased: the viscosity of a 1.5% solution of Zanflo is double that of xanthan and, although it is more sensitive to pH and temperature changes, it is claimed to have good compatibility with most cations and to have good freeze-thaw stability. It is possible that it will be of use to the paint and other industries that use basic dyes. Although naturally occurring Xanthomonads have not been reported as able to produce polysaccharides from lactose, a mutant of X.c. pv. phaseoli (syn X. fuscans) has been obtained which produces low levels of a potentially interesting material having a greater mannose: glucose ratio than that produced by the parent strain from glucose (Konicek, Lasik & Wurst, 1977). Charles and Radjai (1977) have also reported the production of a gum from acid whey, apparently by a variant of X.c. pv. campestris.

In nature, Erwinia amylovora, the causative organism of fireblight disease, produces copious amounts of polysaccharide material, much of it as threads which probably help airborne dispersal of the disease to other plants (Eden-Green & Billing, 1972, 1974). While it is unlikely that this organism will ever be produced industrially; we mention it here only to draw attention to the part played, in the dissemination of disease, by the polysaccharides produced by so many bacterial plant pathogens.

Another type of polymer that has attracted some commercial interest (see for instance Halleck, 1967) is the scleroglucan gum produced by various phytopathogenic fungi of the genera Sclerotium, Sclerotinia and Stromatinia. These fungi attack the stems, including corms and tubers, of various important European crop plants, although not in the U.K. The gum has uses similar to those of xanthan gum, and was certainly being produced in 1976 by the French firm Ceca S.A. as "Polytran". The production method included heat sterilisation of the culture within the fermenter prior to further processing (Manufacturer's literature).

The line of thought that led Wade to look for an asparaginase enzyme in phyto-pathogens could well be extended to other enzymes likely to facilitate an invasion of the plant host, such as pectinases, cellulases and even ligninases. Indeed, mould and bacterial phytopathogens could be used to supply the pectinases used in the food industry for clarifying fruit juices and for macerating fruit pulp (see Rombouts & Pilnik, 1971 for a review of applications), but at present these, and other, enzymes are obtained from the large-scale growth of Aspergillus niger, usually by cultivation on moist bran (Barbesgaard, 1977). Fungal enzymes, which are attractive commercially because of their low pH and high temperature optima, could also be produced from the plant pathogens Fusarium oxysporum and Botrytis cinerea, but at present only the enzymes from A. niger are approved for food use (Rombouts & Pilnik, 1972, 1980). In 1980 these authors estimated

the world sale of pectinases at about US \$10 million.

Recently phytopathogenic Erwinia spp. have been shown by Kobayashi et al. (1979) to be effective in retting the bast fibres for paper making; the alkaline pH at which the polygalacturonase works best, causes the fibres to swell improving the accessibility of enzyme to substrate without the need for the usual preparatory mechanical separation of the fibres. Other plant pathogens that produce endopectate lyase, polygalacturonase, and pectinesterase are various Erwinia and Xanthomonas spp. and Pseudomonas marginalis. These could all be used to advantage since they will produce well in stirred liquid culture.

With the increasing pressures in favour of renewable energy sources, it is likely that cellulases more active than those of Trichoderma viride and A. niger will be sought. Recently, Gong, Maun and Tsao (1981) suggested that an unidentified species of Monilia, isolated from bagasse compost, could be a potential candidate for the production of ethanol from cellulose. This organism is able, anaerobically, to hydrolyse cellulosic materials to glucose and xylose which it simultaneously ferments to ethanol: such a single-stage process could have obvious advantages. Since cellulose provides the primary wall of defence for many plants, it may be that plant pathogens will, increasingly, be examined and exploited; indeed Sudo, Nagayama and Tamari (1973) have shown a correlation between cellulase activity and the pathogenicity of Pyricularia oryzae. Nevertheless, as pectinases and cellulases are produced by many saprophytes and by some strains of Bacillus and Clostridium, important as "spoilage" organisms rather than plant pathogens, it is likely that the search for more active enzymes will concentrate upon these organisms because they will not be subjected to controls that might, eventually, be applied to pathogens.

A different application of plant pathogens has been their use to produce substances with hormonal or pharmacological activity. Two examples of this stand out; the gibberellins that affect plants, and the ergots that affect man and other animals.

The gibberellins, which have a variety of hormonal effects upon plants, are named after the fungal pathogen of rice plants Gibberella fujikuroi from which the substances were first isolated. It was noticed that in the early stages of the disease the rice plants grew with unusual rapidity until root growth was inhibited and the plant wilted. The gibberellins were isolated from the fungus and found to be a mixture of related compounds (see for example, Turner, 1975); of these, gibberellic acid is used to improve the quality and yield of grapes, oranges and lemons, as a fruit set for some pear varieties, and to speed the malting of barley. In 1972 Turner estimated these uses to consume 4 - 5 tons/year. On a smaller scale, a mixture of gibberellins is used to reduce the "June drop" of apples and so increase the yield. Greater use of these substances will probably depend upon a reduction in price, i.e. an increased yield per culture; increasing demands for food and energy may encourage research in this direction. Other plant pathogens, such as Agrobacterium tumefaciens (Romanow, Chalvignac & Pochon, 1969) and Corynebacterium fascians (Helgeson & Leonard, 1966) are known that produce cytokinins - substances that, like the various gibberellins, can affect the growth and development of plants - but as far as we can discover, no attempt has been made to produce these in quantity.

As G. fujikuroi has a hormonal effect upon plants other than those for which it is pathogenic, so some plant pathogens have pharmacological effects upon man. The most notorious of these is the ergot fungus Claviceps

purpurea; Its effects upon European populations have been graphically described by Barger (1931). Even as late as 1954 the fungus was routinely produced by deliberately infecting rye crops which were later harvested and extracted; the infection of neighbouring crops and pasture was regarded as a minor consideration (Rochelmeyer, 1954)! However, in 1972, Szczyrbak reported the isolation of a strain of C. fusiformis from Senegal millet which produced ergot alkaloid. Banks, Mantle and Szczyrbak (1974) later reported the 400 litre scale production of this fungus to produce agroclavine which inhibited both implantation and lactation in mice. Previously C. paspali had been used for production in fermenters, but it had proved difficult to manage due to the tendency of the culture to produce glucan, and consequently, to foam. The organism selected by Szczyrbak produced a glucanase and a glucosidase that hydrolysed the glucan produced during the first few days of the fermentation. C. fusiformis produces only clavine alkaloids which have no direct pharmaceutical use, but can be used as precursors, by addition to C. purpurea fermentations, for the production of lysergic acid. After a long selection process it is extremely unlikely that the C. purpurea strain used in fermenters would be able to infect a plant; the greatest dangers are from its product, a danger, common to all pharmacologically active compounds, and the danger of losing the selected strain. Unlike C. purpurea which has a wide host range, C. fusiformis is parasitic only on Senegal millet. For further details of culture selection, manufacture, composition and use of ergot alkaloids see the excellent review of Mantle (1975). Sherwood and Peberdy (1972) have reported on the production of fusidic acid by Fusarium spp. that cause vascular wilts in tomato, cotton, pea and banana plants. This compound has oestrogenic and anabolic effects on animals, but we have seen no reports of its possible application.

Some plant pathogenic microorganisms are able to transform steroids: by mono- or di-hydroxylations of various positions depending on the steroid structure, by dehydrogenation and/or oxidation of various selected rings (for a review, see Kieslich, 1980). Such organisms include: Calonectria decora - a snow mould of cereals, Fusarium graminearum, F. lini, and F. solani (many of the Fusaria are plant pathogens), Glomerella cingulata - causes bitter apple rot, Helminthosporium buchloes (many of the Helminthosporia are phytopathogens), various species of Alternaria (a genus containing many pathogens) and Verticillium spp., a genus that contains very many pathogens, some of which can produce disastrous infections. Some of these, eg Calonectria decora, are organisms of a type that cause problems on the mainland of Europe; others cause only occasional problems. For example, F. graminearum, which causes scab or earblight of cereals, was a problem in Wales in 1942; the disease is, however, distributed world wide, affecting rice and maize in the tropics, and may cause economically important losses in the USA especially when modern "no tilling" techniques are used. It is possible that the disease may be controlled in the future by the development of resistant cultivars, but such control might be only temporary. Yet others cause problems outside Europe, eg: Curvularia lunata which is common in the Tropics on several crops. Nevertheless, the potential pathogenicity of these organisms will depend upon whence they came, what effect they were having on the plant when they were isolated and, probably, how they were subsequently handled during research that led to the industrial process.

Plant pathogens have already contributed to the armoury of antibiotics with no effect upon the environment other than the beneficial results of their use. Cephalosporin C is produced by members of the genus Cephalosporium some species of which are wheat pathogens. Also produced is cephalosporin P, an agent inducing chlorosis in plants. Strains of

Cephalosporium can also produce fusidanes, including the antibiotic fusidic acid, active orally, against penicillin resistant staphylococci. The fusidanes are tetracyclic triterpenes which, like the steroids lanosterol, zymosterol, bile acids and steroid hormones are formed from squalene oxide (Turner, 1975). It is, therefore, not inconceivable that organisms naturally producing fusidanes might be used to produce intermediates for steroid synthesis.

A different type of transformation is that described by Vandamme (1980) who has reviewed the bioconversion of penicillin (usually penicillin G), by removal of the side chain, to the penicillin nucleus 6-aminopenicillanic acid. This conversion, an important step in the production of semi-synthetic penicillins, can be accomplished by many different microorganisms including the plant pathogens Erwinia and Xanthomonas spp. An anaerogenic strain of E. carotovora (syn. E. aroidea) produces an acylase that will similarly convert penicillin V. However, it is unlikely that these will be used commercially because the temperature optima and the substrate affinities of the enzymes are too low. In any case, the scale of their production is likely to be relatively small since Kulhanek and Tadra (1963) have shown that Escherichia coli cells containing the enzyme can be re-used at least four times. The recent report by Mayer, Collins and Wagner (1979) of a ten-fold increase in activity following genetic transfer of the acylase gene to a hybrid E. coli 5K (pHM12) makes even more remote the likelihood of a plant pathogen being used for penicillin acylase production. It is probable that immobilised cells are now used for the transformation of penicillin.

It has been known for many years that microorganisms, especially yeasts and fungi, are able to produce and accumulate oils, but, apart from the period of World War II, it is only in the past few years that unstable prices, the uncertain supply of traditional plant oils, and the increasing awareness of biotechnology, have combined to re-awaken interest in their commercial possibilities. Ratledge (1981) lists 62 fungi that have been reported to produce, variously, from 23 - 66% of their biomass as oils, and about 20 yeasts that produce from 15 - 71%. In many instances, especially with the fungi, little attempt seems to have been made to optimise oil production since much of the quoted work had been done in unstirred vessels or in shake flasks; Ratledge emphasises the improvements that could follow the use of proper fermenters, and how much work has yet to be done on the choice of strain and growth conditions. Choice of an organism for production is not merely a matter of selecting the highest producer of oil, because the proportions of different fatty acids vary with the organism used; for example, some can produce oils very similar to plant oils, others oils which, although very different, could have interesting properties or serve as feedstocks for other processes including, perhaps, steroid production. The carbon source used also affects the oils' composition. Among the strains listed by Ratledge are strains of plant pathogens such as some species of Fusarium that can infect banana, grass and flax, and Gibberella fujikuroi that can infect rice. Other species, such as Rhizopus, Mucor and Absidia are associated with stored grain, and other foods, but are also used for the preparation of traditional oriental fermented foods such as Tempeh, Sufu and Lao Chao (Wood & Yong, 1975).

Commercial production of microbial oils will probably depend upon the price of available plant oils, the availability of suitable cheap substrates, the discovery of some singular oil with especially valuable properties, and a lot more dedicated work.

Our final example of the use of plant pathogens is the production of an amino acid. Yamada et al. (1973) reported the production of D-alanine by

Corynebacterium fascians; this had the particular advantage that the amino acid was the optically pure D isomer and therefore dispensed with the need for subsequent resolution. This is an example of the sort of special ability that might encourage the use of a plant pathogen.

The names of plant pathogens that are known to be used now, for industrial purposes, are listed in the Table. However it must be remembered that, although bearing the name of a pathogen, the strain of organism used may have been derived from a non-pathogenic isolate, or, as a result of strain development and mutation, have lost its ability to penetrate or cause disease in a plant. For instance, the strain of Fusarium graminearum used for SCP production has, after extensive tests upon different cultivars of wheat, barley and maize, proved to be not pathogenic, as indeed was the original isolate (G.L. Solomons, pers. comm.).

Table Showing the present uses of plant pathogens, with some indication of their scale of use

Organism	Product	Scale
Xanthomonas campestris	Polysaccharide gum	industrial
X. c. pvs.	polysaccharide gum	laboratory
Sclerotium spp.	polysaccharide gum	industrial
various organisms	restriction enzymes	laboratory
Erwinia chrysanthemi	asparaginase	small industrial
E. spp.	alkaline pectinases	? laboratory
Gibberella fujikuroi	gibberellins	industrial
Claviceps spp.	ergot derivatives	industrial
Endothia parasitica	microbial rennin	industrial
Fusarium graminearum	SCP	industrial
various fungi	fermented foods	local and industrial

In complete contrast to all the above uses, we would mention two examples of plant pathogens being used deliberately to infect and control plants regarded by man as weeds (Templeton & Smith, 1977). The bacterial plant pathogen Pseudomonas solanacearum, normally pathogenic for potatoes, has been mutated and selected for maximum virulence on bitter nightshade, a weed competing with tomato plants. The second example is of a fungus that is endemic in the Mediterranean region: in 1971 the rust Puccinia chondrillina was introduced into Australia to control rush skeleton-

weed which competes with wheat plants. Following a single introduction, the pathogen spread widely and has caused spectacular damage to the weed.

It is unwise to attempt to predict the future, especially the future activities, interests and discoveries of a discipline that grows as rapidly as Biotechnology is likely to. As Pelissolo (1980) has said: thirty years ago there was no true Electronics Industry, merely Industries, such as radar, television, telecommunication etc that used valves and, later, transistors; only during the sixties, by regroupings and associations of different interests and technologies was this New Industry created. We would go even further back, to the invention of the typewriter, the cathode ray tube and the valve powered computer, and ask: who then would have predicted the desk top word processor of today? Even so, our suggested developments would surely seem to have been naive in 10 years time. Developments depend upon various factors, many of which are not predictable and some that may not now appear to be relevant. For example, the development of microbial oil production to a commercial scale will depend, inter alia, upon the availability and cost of plant oils, which will depend upon level of demand, upon climatic, economic and political factors, and upon the discovery of microbial oils with especial industrial or medical properties. Industry and Medicine may also need to develop new interests before those special properties are seen to be relevant. Nor, at this time when so many people equate Biotechnology with Genetic Engineering, should we forget the possibility that desirable genes may be moved from undesirable organisms eg: from plant pathogens to innocuous organisms such as E. coli. However, some care may be needed in this since the transfer of a cellulase gene to a saprophytic fungus enabled the fungus to behave as a plant pathogen (C-G. Heden, pers. comm.). In contrast, although attempts to implant the genes for rennin production into E. coli have been successful, the resultant yields are, at present, so low that microbial rennin must still be produced industrially (Sardinas, 1968), in the USA, from the phytopathogenic fungus Endothia parasitica that destroyed the chestnut trees of the eastern USA (Butler & Jones, 1949), or from Mucor spp. (Aunstrup, 1980).

The terms of reference and the title of this report have caused us to concentrate this chapter upon the uses, actual and potential, of plant pathogenic microbes; but there is little doubt that the potentials of saprophytic organisms remain relatively unexplored. This may be because pathogens are more obvious in culture collections than saprophytes; but it is likely that many desirable products, such as pectinases and cellulases are produced in nature by the saprophytes at least as readily as by the pathogens. The use of non-pathogenic organisms would have obvious advantages for an intending manufacturer, and should be encouraged.

CHAPTER IX

A DISCUSSION OF THE POSSIBILITY OF HAZARDS FROM THE INDUSTRIAL PRODUCTION AND USE OF PLANT PATHOGENS

We have seen, in Chapters IV and V, that naturally occurring microbial plant pathogens can spread and infect crops, and that the consequent economic loss can be very large. We have also seen that such microbes have evolved, and continue to evolve, in response to selection pressures exerted by man's cultivation of crop plants and the methods used. Further, we have pointed out that the selection pressures that apply within a fermenter are different, and unlikely to encourage pathogenicity. Indeed, it is a common experience that plant pathogens tend to lose pathogenicity when grown in media other than their normal host: often an infuriating problem for plant pathologists trying to study them. Animal pathogens behave similarly; a fact that has been exploited in the manufacture of live, avirulent vaccines.

The vulnerability of crops has been further increased by practices which have greatly enhanced productivity, such as:

- higher cropping densities
- larger, contiguous areas devoted to one crop
- narrower genetic base of many crop plants
- protected cropping, especially in glasshouses, reduces the range of environmental conditions employed in efforts to optimise yields and to produce crops at specific harvest times.

Other changes which have increased the vulnerability of crops include:

- development, in the field of plant pathogens resistant to pesticides
- unacceptability, on environmental grounds, of some previously effective pesticides such as: DDT, streptomycin and mercury seed dressings
- changes in cultivation methods such as: no tilling and reduced crop rotation (see Chapter V).

This vulnerability is exacerbated by the extreme dependence, highlighted in earlier chapters, of man and his farm animals upon the grasses; any major disaster affecting this family would have far-reaching consequences.

As far as we know, plant diseases have not hitherto been spread by the limited industrial use of any phytopathogen listed in Chapter VIII; however, the possibilities may increase for the following reasons:

- we are about to deploy many more biotechnological processes which will entail the industrial use of microorganisms not previously grown on an industrial scale
- some of these processes may be operated on a very large scale indeed

- some of the organisms used may be, or be derived from, known plant pathogens
- others, specifically constructed to degrade plant materials such as lignocellulose, might turn out to be pathogenic for some plants.

We have already, in Chapter VII, stated our belief that a plant pathogen that is still virulent after growth in a fermenter, cannot be handled safely on a very large scale. Nevertheless, if the kinds of precautions described in Chapter VII are employed, there is no reason to expect that the industrial processing of plant pathogens on a scale not exceeding a few thousand litres will lead to their release, either as bulk liquid or, much more importantly, as an aerosol. Therefore, at this scale, we need only consider the likelihood and the possible consequences of a systems failure.

The likelihood of such a failure can be reduced to a very low level, but never eliminated; a fact that applies to all industrial operations (Kletz, 1980). Accidental release might, for example, follow mechanical failure, filter failure or "foam-out" of the culture. The consequences for crops of such a failure would depend upon a number of factors:

- quantity of microorganisms released: establishment of disease can be related to dose
- strain of microorganism used: establishment of disease is related to strain
- physiological state of the microorganism: pathogenicity, infectivity, spore production and aerostability depend upon growth conditions
- meteorological and environmental conditions: dispersal, dilution, survival and ability to enter the host plant depend upon the weather
- position of the factory in relation to susceptible crops
- physiological state of the crop: susceptibility to infection depends upon condition and development of the host plant.

These factors are interrelated and, since all are subject to variation, in the present state of knowledge it is quite impossible to predict their relative importance. It is, however, possible to influence three of these factors, namely, the strain of microorganism used, its physiological state, and the siting of the factory. Thus:

- it may be possible to operate an industrial process based on a saprophyte or a specially developed non-pathogenic strain
- if a pathogenic strain must be used, it may still be possible, by using appropriate growth conditions in the fermenter, to reduce the risk to crops
- by appropriately siting the factory the risk may be further reduced.

Nevertheless, by far the best way to affect the hypothetical hazard of growing plant pathogens is to eliminate it altogether by using a saprophyte.

If a plant pathogen must be used, a less hazardous organism might be discovered, selected, or created, in one of 3 ways:

- screening for non-pathogenic strains
- selection, possibly preceded by mutation
- removing from the pathogen the genetic apparatus responsible for its useful properties and incorporating this in a different, and non-pathogenic organism (genetic engineering).

These methods might very simply reduce the risk. Some pathogenic fungi for example differ little from non-pathogens, or from saprophytes, and a search among these may well furnish equally useful organisms.

Again, as we have already mentioned, spontaneous selection of a non-pathogenic strain can occur during in vitro culture, and this can be encouraged by repeated serial transfers or by prolonged continuous culture. Recourse may also be had to the mutation/selection techniques which have been so successful in transforming wild strains, particularly of penicillia and streptomycetes, into very exceptional organisms, capable of producing enormous antibiotic yields, but unable even to survive outside the artificial environments of the laboratory or factory.

Genetic engineering techniques have not yet been widely adopted, but promise to be a particularly elegant long-term approach, when a more complete understanding of processes which affect the stability and expression of acquired genetic information has been obtained.

If, in spite of everything, a pathogenic strain of plant pathogen must be used, the organism should on no account be one of those prohibited in Annex 1 of the EEC Directive and the EPPO A1 list (see Chapter X). Neither should any other non-indigenous pathogen able to cause disease of any economically or environmentally important European plant be used. To use such organisms might not only contravene the law, it could also expose European plants to the sort of catastrophe that followed the introduction of Peronospora tabacina referred to in Chapter V.

If an indigenous pathogen were to be used the risk could be much reduced by killing the organism in the fermenter before further processing. If this is not possible the risk may still be reduced by using a suitable growth method, which, as mentioned in Chapter VI, can reduce aerostability and the ability to cause disease. Some suitable testing should then follow, under the aegis of the appropriate government plant protection service, and any consequently apparent risks weighed and considered against the possible benefits of the proposed process.

By siting the factory away from susceptible crops, or in areas where the environment opposes the establishment of disease, the risk may be further reduced. Let us take a purely hypothetical example: Puccinia graminis, the cause of a wheat rust, requires a warm, moist spring; in northern Europe the spring is moist enough, but too cool, whereas in southern Europe it is warm enough, but too dry for epidemics to occur. In central Europe conditions are virtually optimal; consequently little wheat is grown there. As the prevailing climatic conditions in both north and south are so unfavourable for the establishment of an epidemic (Horsfall & Cowling, 1978) if P. graminis could be grown industrially it might, paradoxically, be grown safely in either wheat-growing area. However, as

little wheat is grown in central Europe, it might be deemed safer to site the factory there.

There could also be another reason for care in siting the factory: the fact that plant pathogens were being produced could raise suspicion in lay minds that any local crop disease was caused by the factory; if the disease were of a kind that could be induced by organisms of the same species as the production organism, disproof of this suspicion could be difficult.

Thus the industrial exploitation of a plant pathogen might well be best carried out in a country where the organism is unknown, but where no susceptible host plant exists. Paradoxically, some present legislation, which forbids the import of non-indigenous plant pathogens, may appear to prevent this, although usually a licence from the appropriate plant health authority will allow import in such cases.

This brings us to a most important point: as we shall see in Chapter X plant health regulations were designed long before the large-scale industrial use of plant pathogens was envisaged, and so do not control the growth of all indigenous plant pathogens. While there is no need to prevent the controlled laboratory growth of a few grams of such organisms, since many kilograms of the same organisms might naturally be present in the environment, there might well be a need to regulate the growth of many tonnes.

CHAPTER X

A REVIEW OF SOME CURRENT INTERNATIONAL POLICIES OF MEMBER STATES TOWARDS PLANT PATHOGENS

The development of plant pathology as a scientific discipline (see Chapter IV) has historical roots going back to the plant diseases which first troubled man when he became a crop producer, as distinct from a gatherer from the wild; Bunt, or Stinking Smut, a fungal disease of wheat caused by Tilletia caries, is one of these ancient diseases. Pasteur's discoveries and Koch's innovations in laboratory technique made modern plant pathology possible and led to the development of rational policies for the control of plant disease in the old world and the new.

A most significant event was the setting up of Agricultural Experiment Stations in the USA during the 1880s, concerned, amongst other things, with the causes and control of plant diseases. Plant pathology then began to develop an international as well as a national aspect. For example, a Board of Agriculture was set up to consider these matters in Great Britain at this time; in 1881 there was an International Convention on measures to be taken against the vine root aphid, Phylloxera vastatrix - a pest rather than a plant pathogen as defined in Chapter IV, but destroying a crop nevertheless. It is not possible in this review to outline the historical background fully, but probably the first national legislative acts designed to control a plant pest were undertaken by France, Germany, Belgium, the Netherlands, Spain and Russia in the 1860s. These prohibited the import of potatoes from North America, where the depredations of the Colorado Beetle, Leptinotarsa decemlineata, were so bad that potatoes were becoming a luxury. Britain took similar legislative action in 1877, invoking wide powers to inspect crops and undertake other actions necessary to restrict or prevent the arrival of L. decemlineata. In 1903, Denmark passed a law prohibiting the cultivation of Berberis vulgaris, the alternate host of the fungal plant pathogen Puccinia graminis. This was followed by legislation in the USA fifteen years later. By 1907, the British Government had in operation a much more widely ranging "Destructive Insects and Pests Act" arming its Board of Agriculture with sweeping powers to regulate the import of material likely to introduce pests and plant pathogens into Britain, to appoint crop inspectors, control movement of infected plant material within Britain, and so on.

As Prentice (1971) puts it so clearly: "Legislation is effective in two areas: there are regulations to reduce the risk of introducing plant diseases from overseas (known as international quarantine), and regulations about diseases already present in a country (domestic quarantine)". Two fungal plant pathogens were involved in the first British legislation: Sphaerotheca mors-uvae (causing American Gooseberry mildew) and, more importantly Synchytrium endobioticum (the cause of Potato Wart Disease). In 1912, legislation was passed in the USA to check the spread of Cronartium ribicola (the cause of White Pine Blister Rust). About fifty "statutory instruments" are at present in force in Great Britain alone, concerned with various aspects of plant health (Ebbels & King, 1979).

Plant pathologists were becoming established as professional scientists during the first two decades of the 20th century. The first professional society, The American Phytopathological Society, was founded in 1907, and

is still by far the largest of the learned societies of plant pathology in the world. In May 1914, the first International Phytopathological Conference took place in Rome. Again briefly summarizing the situation: during the First World War (1914-18) and after it, both in Europe and elsewhere, there was a great expansion in research, advisory (or extension) work, teaching, in the commercial development of crop protection chemicals, and in legislative efforts to know, understand and control plant pathogens.

The International Phylloxera convention of 1881 was followed by the International Convention for the Protection of Plants, in Rome in 1929. Forty six countries attended the Convention, but only 12 signed it and it did not become an effective instrument.

The Hague Conference, convened in 1950 by the FAO and the Kingdom of the Netherlands, led to the International Plant Protection Convention (IPPC) which was approved by the FAO in 1951. The convention's agreed 15 articles have important provisions, designed to protect the crops of the world from plant pests, therein defined as "any form of plant or animal life, or any pathogenic agent, injurious to plants or plant products". It strengthens international efforts to combat plant pathogens, and to restrict their movement across international boundaries. Articles IV and VII of IPPC (Chock, 1979) require each nation:

- to have an official plant protection organisation
- to survey and inspect growing plants
- to inspect consignments of plants and plant products moving in international trade
- to issue phytosanitary certificates
- to strengthen advisory services and research in plant protection
- to report on the outbreak and spread of plant diseases
- to publish its plant quarantine regulations and requirements, and distribute these immediately to other governments and to FAO.

These principles, and the detailed recommendations and activities associated with them, form the basis of existing EEC policies.

Eight regional plant protection organisations were set up to comply with the IPPC agreements. EPPO (the European and Mediterranean Plant Protection Organisation) is one of these. It has 35 member countries including Iran, and its work, with particular reference to its regulatory aspects, has been reviewed in detail by Smith (1979). All EEC member countries together with Spain and Portugal belong. Its headquarters secretariat operates from Paris through various Working Parties and Conferences.

The EPPO Working Party on Phytosanitary Regulations is the one most concerned with plant health; the EEC has been regularly represented to ensure awareness of activities paralleling its own. This Working Party has been active for 20 years, and its main purpose (Smith, 1979) has been to apply agreed principles to certain immediately important quarantine pests not already present in Europe, and to those which, although already present in one or more, but not all EPPO countries, seem very likely to spread to

new areas. These harmful organisms were selected, after consultations with member countries, only if they:

- were known to cause major damage to plant hosts that are economically important in some or all EPPO countries
- are unlikely to spread in EPPO countries by natural means, so that plant health action could not be expected to be effective
- appear capable of surviving and reproducing in some or all EPPO countries, either in the field or in protected crops (eg, under glass).

List A1 refers to pests and diseases not yet introduced into the area covered by EPPO. For these quarantine organisms a zero tolerance is required for all countries. As well as parasitic plants, insects and mites, viruses and mycoplasmas, it covers three bacterial species (all Xanthomonas spp.) and more than 20 fungi, of which only some can be grown away from the host plant and so might be of use to Biotechnology.

List A2 refers similarly to pests and diseases already introduced into some EPPO countries. For these organisms a zero tolerance is normally required. Countries are invited to include into their national regulations only those pests and diseases which are of importance according to the prevailing ecological conditions. The list contains 21 species of bacteria, and 24 fungi of which many can be grown away from the host plants. It includes only two of the organisms already mentioned in Chapter VIII, Erwinia chrysanthemi and Endothia parasitica, that are used industrially.

This listing is still at the recommendation, not regulation, stage in most EPPO countries (Smith, 1979), but the EEC has a Directive governing the import and export of plant materials by member states. This is the Council Directive (77/93/EEC) of 21 December 1976, on protective measures against the introduction into the member states of harmful organisms of plants or plant products. It was published in the Official Journal of the European Communities, 20, L26, 20-54. (Southey, 1979). Since 1980, plant health legislation has been based on this Directive in all EEC countries except the Federal Republic of Germany, and Greece, which have been granted periods of derogation.

Here we can only briefly summarise this long and complicated document which contains 21 Articles and four Annexes. The Articles prescribe protective measures to be taken by member states to prevent the introduction of organisms, (insect, plants, bacteria, fungi, viruses, mycoplasmas and other pathogenic agents) harmful to plants and to plant products; the Annexes enumerate those organisms and the plant materials on which they are likely to be introduced. Annex I specifies organisms whose introduction must be forbidden into all member states; it includes three bacterial and 17 fungal species. Of some relevance to this report are: the bacterium Erwinia amylovora and the fungus Endothia parasitica, and it is this Annex that is directly relevant to the importation of organisms. In addition, under section 7 of Article 3, the member states can declare that the introduction of organisms in the isolated state, other than those enumerated in Annex I and II, and which could be considered as harmful, is prohibited in their territory or subject to special authorization.

Any potential importer of such plant pathogens should, therefore, check the regulations of the country concerned which may, in some cases, allow import under special conditions. All EEC countries prohibit import of

organisms listed in Annex I. Most, indeed, go further: for instance, the Netherlands prohibits import of all harmful organisms in an isolated state from all countries, whereas the United Kingdom prohibits any non-indigenous "plant pest". However, both countries provide, as do many others, for licenced import.

Thus individual localities, nations and groups of nations, have policies directed towards the control of plant pathogens. Restricting the entry of non-indigenous plant pathogens is an important part of these policies, as is internal restriction of spread within each state by "domestic" policies for nationally indigenous plant pathogens.

The common feature of all the policies discussed in this chapter is that they seek to prevent the spread of plant disease by controlling the movement of contaminated plant materials and organisms in an isolated state. As already mentioned in Chapter IX these policies were designed before the large-scale industrial use of plant pathogens was envisaged. In the next chapter we consider whether the EEC now needs new regulations to guide this unexpected development.

CHAPTER XI

THE POSSIBILITY AND POTENTIAL BENEFITS OF SETTING UNIFIED COMMUNITY REGULATIONS FOR THE INDUSTRIAL USE OF PLANT PATHOGENS

In Chapter IX we have said that existing plant health regulations were drawn up before industrial use of plant pathogens was envisaged. Although, in principle, they do provide protection against the importation of non-indigenous plant pathogens for use in Biotechnology, they do not control the use of indigenous pathogens. Nevertheless, we do not believe that there is a need, at this time, for new regulations.

We conclude that it will not be possible to unify the plant health regulations of member states more closely than they have been by the recent EEC Directive. The environmental conditions, and the crop plants grown in the various member states, are too diverse; a fact recognised by the inclusion or exclusion of some pathogens from the lists for some countries.

The proposals that we make in Chapter XII would allow the collection of information sufficient for a re-appraisal to be made in a few years. It may then be seen that the indiscriminate use of some indigenous organisms, which remain pathogenic after growth in fermenters, should be controlled, at least in some areas, much as non-indigenous pathogens are now controlled.

CHAPTER XII

PROPOSALS FOR ACTIONS TO BE CARRIED OUT BY THE COMMUNITY

1. **List of plant pathogenic bacteria and fungi to be commissioned.**

Because of the increasing industrial use of plant pathogens it is important that European biotechnologists be aware of which organisms might be pathogenic for European plants. It might be expected that this should already be the case, but our experience in gathering information for this report has convinced us that it is not so. Indeed two of us found great difficulty in discovering whether or not several organisms that are used or might be used were plant pathogens; further, we discovered two instances of other experienced biotechnologists being unaware that particular organisms with which they were concerned belonged to pathogenic species.

For these reasons we recommend that lists of indigenous and non-indigenous bacteria and fungi capable of growing away from a host plant and potentially able to cause major damage to crop plants important to the economy or the environment of member states should be commissioned.

Annex I of the EEC Directive includes the most dangerous non-indigenous organisms (absent from some or all EEC countries) potentially able to cause major damage to economically or environmentally important crop plants within the EEC. Our recommendation would extend this list to cover a wider range of non-indigenous plant pathogens and, also, important indigenous pathogens that can be grown in culture away from their hosts and are thus of possible interest to biotechnologists. These lists should be prepared by a plant pathologist(s), commissioned by the EEC, in consultation with the Plant Protection Services of the EEC countries and with EPPO.

It is most important to recognise that these lists will be no more than a valuable guide. As we have explained throughout this report, pathogenicity is a complex character which is not conferred by a name. Saprophytes and many strains and races of pathogenic species do not cause disease and could safely be used by industry. It is to these that industry should be guided by the list.

If in spite of everything a pathogenic strain or race must be used, the Plant Protection Services of the country concerned should first be consulted to verify whether any plant health regulations apply, and the conditions under which the organism may be used. The local plant protection authorities should also be informed of the use of indigenous pathogens. This leads us to the second proposal.

2. **List of pathogenic strains or races in current industrial use to be prepared.**

Just as we discovered biotechnologists unaware that some of the organisms with which they were concerned belonged to pathogenic groups, so we found experienced plant pathologists unaware of current industrial uses of such organisms.

We recommend that a list of pathogenic strains or races of bacteria and fungi in current industrial use within the EEC should be made. This should include details of where, and on what scale, they are used.

The purpose of this list is to inform the plant protection authorities within the EEC of possible biotechnological sources of plant disease. It should be prepared by the Plant Protection Services of each country from information voluntarily supplied by manufacturers, and collated with the lists of proposal 1. This could provide a convenient central assemblage of information about the use of non-indigenous pathogens within the EEC and, for the first time, similar information about indigenous pathogens.

CHAPTER XIII

APPENDIX 1: PEOPLE WHO HAVE BEEN CONSULTED

In preparing this report we received help in the form of discussion and criticism from many people. We include here a list of most of the people that we have approached, apologising to any whose names we have omitted. Especially must we thank Valerie M Bowden, a Secretary sans pareil et sans reproche, for her skill, patience and dedication beyond the call of duty.

While we thank all who have helped us, we must accept full and sole responsibility for the views expressed in the report.

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CHAPTER XIII

APPENDIX 2:

REFERENCES

- Ainsworth, G.C. (1971). Ainsworth and Bisby's Dictionary of the Fungi, 6th. Edn. Commonwealth Mycological Institute, Kew, Surrey.
- Allen, A. (1979). "Frogs that fall in the rain". *The Field*, 12 December pp. 1556-7.
- Andersen, A.A. (1958). "New sampler for the collection, sizing and enumeration of viable airborne particles". *J. Bacteriol.* 76, 471-484.
- Anderson, J.D. & Cox, C.S. (1967). "Microbial survival". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press, pp. 203-226.
- Anderson, J.D., Dark, F.A. & Peto, S. (1968). "The effect of aerosolization upon survival and potassium retention by various bacteria". *J. gen. Microbiol.* 52, 99-105.
- Andersson, R., Bergstrom, B. & Bucht, B. (1973). "Outdoor sampling of airborne bacteria; results and experiences". in *Airborne Transmission and Airborne Infection*, eds. J.F. Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 58-62.
- Andrew, T.R. (1977). "Applications of xanthan gum in foods and related products". in *Extracellular Microbial Polysaccharides*, eds. P.A. Sandford and A. Laskin. Amer. Chem. Soc. Wash. D.C. pp. 231-241.
- Anon. (1981). "Forestry; scotch mist aids growth". *Chemistry in Britain*, 17, July, p. 319.
- Asai, G.N. (1960). "Intra- and inter-regional movement of uredospores of black stem rust in the upper Mississippi valley". *Phytopathol.* 50, 535-541.
- Aunstrup, K. (1980). "Proteinases". in *Microbial Enzymes and Bioconversions*, ed. A.H. Rose. Academic Press, Lond., pp. 49-114.
- Baker, K.F. (1971). "Fireblight of some pome fruits: the genesis of the concept that bacteria can be pathogenic to plants". *Hilgardia* 40, 603-633.
- Banks, G.T., Mantle, P.G. & Szczyrbak, C.A. (1974). "Large-scale production of clavicine alkaloids by Claviceps fusiformis". *J. gen. Microbiol.* 82, 345-361.
- Barbesgaard, P. (1977). "Industrial enzymes produced by members of the genus Aspergillus". in *Genetics and Physiology of Aspergillus*, eds. J.E. Smith and J.A. Pateman. Academic Press, Lond. pp. 391-404.
- Barger, G. (1931). *Ergot and Ergotism*. Gurney and Jackson, Lond. 279 pp.

- Bateman, J.B., Stevens, C.L., Mercer, W.B. & Carstensen, E.L. (1962). "Relative humidity and the killing of bacteria: the variation of cellular water content with external relative humidity or osmolarity". *J. gen. Microbiol.* 29, 207-219.
- Bazin, M. (1979). "Brazil: running on alcohol". *Nature (Lond.)* 282, 550-551.
- Benbough, J.E. & Hambleton, P. (1973). "Structural, organisational and functional changes associated with envelopes of bacteria sampled from aerosols". in *Airborne Transmission and Airborne Infection*, eds. J.F. Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 135-137.
- Blanchard, D.C. & Woodcock, A.H. (1957). "Bubble formation and modification in the sea and its meteorological significance". *Tellus* 9, 145-158.
- Bourdillon, R.B., Lidwell, O.M. & Thomas, J.C. (1941). "A slit sampler for collecting and counting airborne bacteria". *J. Hyg. Camb.* 41, 197-224.
- Bovallius, A., Bucht, B., Roffey, R. & Anas, P. (1978). "Long-range air transmission of bacteria". *Appl. env. Microbiol.* 35, 1231-1232.
- Brooks, D.H. & Dawson, M.G. (1968). "Influence of direct-drilling of winter wheat on incidence of take-all and eyespot". *Ann. appl. Biol.* 61, 57-64.
- Brown, A.D. (1953). "The survival of airborne microorganisms: I, experiments with *Escherichia coli* near 0°". *Aust. J. biol. Sci.* 6, 470.
- Brown, A.D. (1954). "The survival of airborne microorganisms: III, effects of temperature". *Aust. J. biol. Sci.* 7, 444.
- Buchanan, L.M., Harstad, J.B., Phillips, J.C., Lafferty, E., Dahlgren, C.M. & Decker, H.M. (1972). "Simple liquid scrubber for large-volume air sampling". *Appl. Microbiol.* 23, 1140-1144.
- Burke, D.W. (1965). "The near immobility of *Fusarium solani* f. *phaseoli* in natural soils". *Phytopathol.* 55, 1188-1190.
- Butler, S.G. & Jones, E.J. (1949). *Plant Pathol.* Macmillan, Lond.
- Callow, D.S., Capel, B.J. & Evans, C.G.T. (1975). "Improvements relating to the production of L-asparaginase". *Brit. Pat.* 1, 379, 328.
- Carefoot, G.L. & Sprott, E.R. (1967). *Famine on the Wind.* Rand McNally & Co., 231 pp.
- Carter, G.B. (1981). "Is biotechnology feeding the Russians?" *New Scientist*, 23 April, 216-218.
- Chamberlain, A.C. (1967). "Deposition of particles to natural surfaces". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press, pp. 138-164.
- Charles, M. & Radjai, M.K. (1977). "Xanthan gum from acid whey". in *Extracellular Microbial Polysaccharides*, eds. P.A. Sandford and A. Laskin. Amer. Chem. Soc., Wash. pp. 27-39.

- Chock, A.K. (1979). "The international plant protection convention". in *Plant Health*, eds. D.L. Ebbels, & J.E. King. Blackwell Scientific Publications, Oxford pp. 1-11.
- Clafin, L.E., Stuteville, D.L. & Armbrust, D.V. (1973). "Wind-blown soil in the epidemiology of bacterial leaf spot of alfalfa and common blight of bean". *Phytopathol.* 63, 1417-1419.
- Collison, W.E. (1935). *Inhalation Therapy Technique*. Heinemann, Lond.
- Cox, C.S. (1966). "The survival of Escherichia coli atomised into air and into nitrogen from distilled water and from solutions of protecting agents as a function of relative humidity". *J. gen. Microbiol.* 43, 383-399.
- Cox, C.S. (1968). "The aerosol survival of Escherichia coli B in nitrogen, argon and helium atmospheres and the influence of Relative Humidity". *J. gen. Microbiol.* 50, 139-147.
- Cramer, H.H. (1967). *Plant Protection and World Crop Production*. Pflanzenschutz-Nachrichten, Bayer, No. 20.
- Dark, F.A. & Callow, D.S. (1973). "The effect of growth conditions on the survival of airborne E. coli". in *Airborne Transmission and Airborne Infection*, eds. J.F. Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 97-99.
- Darlow, H.M. (1972). "Safety in the microbiological laboratory: an introduction". in *Safety in Microbiology*, D.A. Shapton & R.G. Board. Soc. appl. Bacteriol. Tech. Ser. 6, eds. Academic Press, Lond. & N.Y. pp. 1-20.
- Darlow, H.M. & Bale, W.R. (1959). "Infective hazards of water-closets". *Lancet*, June 6, 1196-1200.
- Decker, H.M., Buchanan, L.M., Frisque, D.E., Filler, M.E. & Dahlgren, C.M. (1969). "Advances in large-volume air sampling". *Contamination Control Magazine*, 8, August, 13-17.
- Dimmick, R.L. (1960). "Delayed recovery of airborne Serratia marcescens after short time exposure to ultra violet irradiation". *Nature (Lond.)* 187, 251.
- Druett, H.A. & May, K.R. (1969). "The open air factor". *New Scientist*, 13 March, 579-581.
- Dunnill, P. (1981). "Biotechnology & Industry". *Chemistry and Industry*, 4 April, 204-217.
- Dyrssen, D. (1972). "The changing chemistry of the oceans". *Ambio*, 1, 22-25.
- Ebbels, D.L. & King, J.E. (1979). *Plant Health*. Blackwells, Oxford.
- Eden-Green, S.J. & Billing, E. (1972). "Fireblight: occurrence of bacterial strands on various hosts under glasshouse conditions". *Plant Pathol.* 21, 121-123.

- Eden-Green, S.J. & Billing, E. (1974). "Fireblight". *Rev. Plant Pathol.* 53, 353-365.
- Errington, F.P. & Powell, E.O. (1969). "A cyclone separator for aerosol sampling in the field". *J. Hyg. Camb.* 67, 387-399.
- Evans, C.G.T., Harris-Smith, R., Stratton, J.E.D. & Melling, J. (1974). "Design and Construction of a ventilated cabinet for a continuous flow centrifuge". *Biotechnol. Bioengng* XVI, 1681-1687.
- Evans, C.G.T., Yeo, R.G. & Ellwood, D.C. (1979). "Continuous culture studies on the production of extracellular polysaccharides: Xanthomonas juglandis". in *Microbial Polysaccharides and Polysaccharases*, eds. R.C.W. Berkeley, G.W. Gooday and D.C. Ellwood. Academic Press, Lond. N.Y. & San Francisco. pp. 51-68.
- Evans, C.G.T., Yeo, R.G. & Ellwood, D.C. (1980). "Continuous culture studies on the production of extracellular polysaccharides: Xanthomonas juglandis and X. campestris". in *Continuous Cultivation of Microorganisms, Proc. 7th Symp. Prague, 1978*, eds. B. Sikyta, Z. Fencel and V. Polacek. Inst. Microbiol., Czech. Acad. Sci. Prague. pp. 689-698.
- Fontanges, R. & Isoard, P. (1973). "A new apparatus with large air intake for the sampling of atmospheric microconstituents". in *Airborne Transmission and Airborne Infection*, eds. J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 62-66.
- Gabriel, A. (1979). "Economic value of biopolymers and their use in enhanced oil recovery". in *Polysaccharides and Polysaccharases*, eds. R.C.W. Berkeley, G.W. Gooday and D.C. Ellwood. Academic Press, Lond. N.Y. and San Francisco. pp. 191-204.
- George, M. (1981). President, UK Agricultural Suppliers Trade Association (UKASTA) Press Release, 14 Sep.
- Goldberg, L.J. & Ford, I. (1973). "The function of chemical additives in enhancing microbial survival in aerosols". in *Airborne Transmission and Airborne Infection*, eds., J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 86-89.
- Gong, C.S., Maun, C.M. & Tsao, G.T. (1981). "Direct fermentation of cellulose to ethanol by a cellulolytic filamentous fungus, Monilia sp.". *Biotechnol. Letters* 3, 77-82.
- Graham, D.C., Quinn, C.E., Sells, I.A. & Harrison, M.D. (1979). "Survival of strains of soft rot coliform bacteria on microthreads exposed in the laboratory and in the open air". *J. appl. Bacteriol.* 46, 367-376.
- Green, H.L. & Lane, W.R. (1964). *Particulate clouds, dusts, smokes and mists*. 2nd edn. Spon, Lond. Ch. 3.
- Gregory, P.H. (1961). *Microbiology of the Atmosphere*. Hill, Lond. 251 pp.

- Halleck, F.E. (1967). "Polysaccharides and methods for production thereof". U.S. Pat. No. 3, 301, 848.
- Hambleton, P. & Benbough, J.E. (1973). "Damage to the envelopes of gram-negative bacteria recovered from aerosols". in *Airborne Transmission and Airborne Infection*, eds. J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 131-134.
- Harper, G.J. (1976). "The possible role of automation and rapid methods in air microbiology". in *Proc. 2nd. Int. Symp. Rapid Methods and Automation in Microbiology*, Cambridge, England, Sept, 19-25. Learned Information Ltd. (Europe) Oxford.
- Harrison, M.D. (1980). "Aerosol dissemination of bacterial plant pathogens". in *Airborne Contagion*, ed. R.B. Kundsinn. Ann. N.Y. Acad. Sci. Vol. 353, 94-104.
- Harris-Smith, R. & Evans, C.G.T. (1974). "Bioengineering and protection during hazardous microbiological processes". in *Biotechnol. & Bioengng Symp. No. 4*, ed. E.L. Gaden. Wiley & Sons, N.Y. Lond. Sydney & Toronto. pp. 837-855.
- Hedges, F. (1926). "Bacterial wilt of beans (Bacterium flaccumfaciens, Hedges) including comparisons with Bacterium phaseoli". *Phytopathol.* 15, 1-22.
- Helgeson, J.P. & Leonard, N.J. (1966). "Cytokinins: identification of compounds isolated from Corynebacterium fascians". *Proc. natn. Acad. Sci. U.S.A.* 56, 60-63.
- Henis, Y. & Ben-Yaphet, Y. (1970). "Effect of propagule size of Rhizoctonia solani on saprophytic growth, infectivity, and virulence on bean seedlings". *Phytopathol.* 60, 1351-1356.
- Hirst, J.M. (1952). "An automatic volumetric spore trap". *Ann. appl. Biol.* 39, 257-265.
- Hirst, J.M. & Hurst, G.W. (1967). "Long distance spore transport". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press, pp. 307-344.
- Hirst, J.M., Stedman, O.J. & Hurst, G.W. (1967). "Long-distance spore transport: vertical sections of spore clouds over the sea". *J. gen. Microbiol.* 48, 357-377.
- Hood, A.M. (1961). "The infectivity of Pasteurella tularensis". *J. Hyg. Camb.* 59, 497-504.
- Horsfall, J.G. (1972). *Genetic Vulnerability of Major Crops. Report by a Committee of the Agricultural Board, Division of Biology and Agriculture, National Research Council, National Academy of Sciences, Washington, D.C.*
- Horsfall, J.G. & Cowling, E.B. (1978). "Some epidemics man has known". in *Plant Disease*, 2. eds. J.G. Horsfall and E.B. Cowling. Academic Press, N.Y. & Lond. pp. 17-32.
- Huddleson, I.F. & Munger, M. (1940). "A study of an epidemic of Brucellosis due to Brucella melitensis". *Amer. J. Public Health* 30, 944-954.

- Hugh-Jones, M.E. (1973). "The epidemiology of airborne animal diseases". in Airborne Transmission and Airborne Infection eds. J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 399-404.
- Hurst, G.W. (1968). "Foot and mouth disease". Vet. Record 82, 610-614.
- Jeans, A., Pittsley, J.E. & Senti, F.R. (1961). "Polysaccharide B-1459: a new hydrocolloid polyelectrolyte produced from glucose by bacterial fermentation". J. appl. Polymer. Sci. V, 519-526.
- Johansson, K.R. & Ferris, D.H. (1946). "Photography of airborne particles during bacteriological plating operations". J. infect. Dis., 78, 238-252.
- Kang, K.S., Veeder, G.T. & Richey, D.D. (1977). "Zanflow - a novel bacterial heteropolysaccharide". in Extracellular Microbial Polysaccharides, eds. P.A. Sandford and A. Laskin. Amer. Chem. Soc., Wash. pp. 211-219.
- Kennedy, B.W. & Alcorn, S.M. (1980). "Estimates of US crop losses to procaryotic plant pathogens". Plant Diseases 64, 674-677.
- Kenny, M.T. & Sabel, F.L. (1968). "Particle size distribution of Serratia marcescens aerosols created during common laboratory procedures and simulated laboratory accidents". Appl. Microbiol. 16, 1146-1150.
- Kieslich, K. (1980). "Steroid conversions". in Economic Microbiology 5, ed. A.H. Rose. Academic Press, N.Y. and Lond. pp. 369-465.
- King, P.P. (1981). "Biotechnology - an industrial view". Lecture at 2nd Congress of European Federation of Biotechnology, Eastbourne, England, April 6-10.
- Kletz, T.A. (1980). "Benefits and risks: their assessment in relation to human needs". Endeavour, New. Ser. 4, (2), 46-51.
- Kobayashi, Y., Matsuo, R. & Yoshihara, K. (1979). "Pulping of bast fibres by alkaline pectolytic enzymes". ACS/CSJ Chemical Congress, Honolulu, Hawaii, Cell, 184. Quoted by Yoshihara, K. & Kobayashi, Y. (1981). Biotechnol. Letters, 3, 118-123.
- Konicek, J., Lasik, J. & Wurst (1977). "Production and characteristics of exocellular polysaccharide in mutant strains of Xanthomonas fuscans". Folia Microbiol. 22, 12-18.
- Kramer, C.L. & Pady, S.M. (1966). "A new 24 hr. spore sampler". Phytopathol. 56, 517-520.
- Kramer, C.L. & Pady, S.M. (1968). "Viability of airborne spores". Mycologia 60, 448-9.
- Kulagin, S.M., Fedorova, N.I. & Ketiladze, E.S. (1962). "Laboratory outbreak of haemorrhagic fever with a renal syndrome: clinico-epidemiological characteristics". Zh. Mikrobiol. Epidemiol. i. Immunobiol. 33 (10), 121-126.

- Kulhanek, M. & Tadra, M. (1963). "Notes on the preparation of 6-amino-penicillanic acid by bacterial hydrolysis of Benzyl penicillin". *Folia Microbiol.* 8, 301-303.
- Large, E.C. (1940). *The Advance of the Fungi*. Jonathan Cape, Lond.
- Large, E.C., (1952). "The interpretation of progress curves for potato blight and other plant diseases". *Plant Pathol.* 1, 109-117.
- Large, E.C. (1966). "Measuring Plant Disease". *Ann. Rev. Phytopathol.* 4, 9-28.
- Larson, E.W. (1973). "Environmental variables and microbial survival". in *Airborne Transmission and Airborne Infection*, eds. J.F.Ph. Hers & K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 81-86.
- Leach, J.G., Lilly, V.G., Wilson, H.A. & Purvis, M.R. (1957). "Bacterial polysaccharides: the nature and function of the exudate produced by Xanthomonas phaseoli". *Phytopathol.* 47, 113-120.
- LeClerc, E.L. (1964). "Crop losses due to plant diseases in the United States". *Phytopathol.* 54, 1309-1313.
- Lidwell, O.M. (1966). "Discussion". *Bacteriol. Rev.* 30, 573-575.
- Lilly, V.G., Wilson, H.A. & Leach, J.G. (1958). "Bacterial polysaccharides: II, laboratory scale production of polysaccharides by species of Xanthomonas". *Appl. Microbiol.* 6, 105-108.
- Mantle, P.G. (1975). "Industrial exploitation of ergot fungi". in *The Filamentous Fungi: 1 Industrial Mycology*, eds. J.E. Smith and D.R. Berry. Edward Arnold, Lond. pp. 281-300.
- Mason, C.J. (1979). "Atmospheric Transport". in *Aerobiology: the Ecological Systems Approach*, ed. R.L. Edmonds. Dowden, Hutchinson & Ross Inc., Stroudsburg, Pennsylvania. pp. 85-95 & 106-109.
- May, K.R. (1945). "The Cascade Impactor: an instrument for sampling coarse aerosols". *J. sci. Inst.* 2, 187-195.
- May, K.R. (1966). "Spinning-top homogeneous aerosol generator with shockproof mounting". *J. sci. Inst.* 43, 841-842.
- May, K.R. (1966). "Multistage liquid impinger". *Bacteriol. Rev.* 30, 559-570.
- May, K.R. (1967). "Physical aspects of sampling airborne microbes". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press, pp. 60-80.
- May, K.R. (1969). "Prolongation of microbiological air sampling by a monolayer on agar gel". *Appl. Microbiol.* 18, 513-514.
- May, K.R. (1973). "The Collison nebulizer: description, performance and application". *Aerosol Sci.* 4, 235-243.
- May, K.R. (1972). "Assessment of viable airborne particles". in *Assessment of Airborne Particles*, eds. T.T. Mercer and W. Stober. Charles C. Thomas, Publisher, Springfield, Illinois. pp. 480-494.

- May, K.R. & Druett, H.A. (1968). "A microthread technique for studying the viability of microbes in a simulated airborne state". *J. gen. Microbiol.* 51, 353-366.
- May, K.R. & Pomeroy, N.P. (1973). "Bacterial dispersion from body surfaces". in *Airborne Transmission and Airborne Infection*, eds. J.F.Ph. Hers & K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 426-432.
- May, K.R., Pomeroy, N.P. & Hibbs, S. (1976). "Sampling techniques for large windborne particles". *Aerosol Sci.* 7, 53-62.
- Mayer, H., Collins, J. & Wagner, F. (1979). "Cloning of the penicillin G acylase gene of *Escherichia coli* ATCC 11105 on multicopy plasmids". in *Plasmids of Medical, Environmental and Commercial Importance*, eds. K.N. Timmis and A. Puhler. Elsevier, N. Holland Biomedical Press, pp. 459-470.
- McCubbin, T.K. (1953). "The particle size distribution in fog produced by ultrasonic radiation". *J. acoust. Soc. Amer.* 25, 1013-1014.
- McNeely, W.H. (1968). "Process for producing a polysaccharide". U.S. Patent 3 391 060.
- McNeely, W.H. & Kang, K.S. (1973). "Xanthan and other biogums". in *Industrial Gums*, 2nd edn. eds. R.L. Whistler and J.N. BeMiller. Academic Press, N.Y. & Lond. see pp. 492-495.
- Muromtsev, S.N. & Nenashev, V.P. (1960). "The study of aerosols: III, an ultrasonic aerosol atomizer". *Zh. Microbiol. Immunobiol.* 31, 50. see *Biol. Abstr.* (1962) 37, 6505.
- Noble, W.C. (1967). "Sampling airborne microbes - handling the catch". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press, pp. 81-101.
- Odum, E.P., (1959). *Fundamentals of Ecology*, 2nd edn. W.B. Saunders Company, Philadelphia, U.S.A.
- Ogilvie, L. & Thorpe, I.G. (1966). "Black stem rust of wheat in Great Britain". *Proc. Cereal Rust Conf.*, Cambridge, 1964, pp. 172-176.
- Pasquill, F. (1974). *Atmospheric Diffusion*, 2nd edn. Ellis Horwood Ltd., Chichester, 429 pp.
- Patton, J.T. & Lindblom, G.P. (1962). "Process for synthesizing polysaccharide". U.S. Patent 3 020 206.
- Pelissolo, J.-C. (1980). "La biotechnologie demain?" Rapport au Premier Ministre DGRST, Dec. 1980.
- Perombelon, M.C.M. (1978). "Contamination of potato crops by airborne *Erwinia carotovora*". in *Proc. 4th Inst. Conf. Plant Pathogenic Bacteria*, ed. Station de Pathologie Vegetale et Phytobacteriologie, INRA. pp. 63-65.
- Perombelon, M.C.M. (1981). "Towards an integrated control of potato blackleg". in *Proc. Crop Protection in Northern Britain*, pp. 101-109.

- Phatak, H.C. (1980). "The role of seed and pollen in the spread of plant pathogens particularly viruses". *Tropical Pest Management* 26, 278-285.
- Phillips, D.J. (1965). "Ecology of plant pathogens in soil. IV. Pathogenicity of macroconidia of *Fusarium roseum* f. sp. *cerealis* produced on media of high and low nutrient content". *Phytopathol.* 55, 328-329.
- Phillips, G.B. (1965). "Microbiological hazards in the Laboratory - part one - control". *J. chem. Education* 42, (1), A43-A47.
- Populer, C. (1978). "Changes in host susceptibility with time". in *Plant Disease* 2, eds. J.G. Horsfall and E.B. Cowling. Academic Press, N.Y. and Lond. pp. 239-262.
- Postgate, J. (1969). *Microbes and Man*. Penguin Books Ltd., England, 237 pages.
- Preece, T.F. (1971). "Disease assessment". in *Diseases of Crop Plants*, ed. J.H. Western. Macmillan, Lond.
- Preece, T.F. (1976). "Some observations on leaf surfaces during the early stages of infection by fungi". in *Biochemical Aspects of Plant - Parasite Relationships*, eds. J. Friend and D.R. Threlfall. Academic Press, Lond., pp. 1-10.
- Preece, T.F. & Dickinson, C.H., (1971). *Ecology of Leaf Surface Micro-organisms*. Academic Press, Lond.
- Preece, T.F. & Smith, L.P. (1961). "Apple scab infection weather in England and Wales, 1956-1960". *Plant Pathol.* 10, 43-51.
- Prentice, I.W. (1971). "Legislation and crop health". in *Diseases of Crop Plants*, ed. J.H. Western. Macmillan, Lond.
- Quinn, C.E., Sells, I.A. & Graham, D.C. (1980). "Soft rot *Erwinia* bacteria in the atmospheric bacterial aerosol". *J. appl. Bacteriol.* 49, 175-181.
- Ratledge, C. (1981). "Microbial oils and fats: an assessment of their commercial potential". in *Progress in Industrial Microbiology*, Elsevier, Holland, (in the press).
- Raven, P.H. (1981). "Natural heritage". at 5th Parliamentary and Scientific Conference, Council of Europe, Helsinki, 3-5 June, 1981.
- Rochelmeyer, H. (1954). *Deutsch. Apoth. Ztg.* 94, 1. Quoted by Vining & Taber in *Biochemistry of Industrial Microorganisms* eds. C. Rainbow and A.H. Rose. Academic Press, Lond. & N.Y. 1963, pp. 341-378.
- Rogovin, S.P., Anderson, R.F. & Cadmus, M.C. (1961). "Production of polysaccharide with *Xanthomonas campestris*". *J. Biochem. and Microbiol. Technol. & Engng* III, 51-63.

- Romanow, I., Chalvignac, M.A. & Pochon, J. (1969). "Recherches sur la production d'une substance cytokinique pour Agrobacterium tumefaciens (Smith et Town) Conn". Ann. Inst. Pasteur 117, 58-63.
- Rombouts, F.M. & Pilnik, W. (1971). "Pektolysches enzyme in der Fruchtsaft-industrie". Flussiges Obst. 38, 93-98.
- Rombouts, F.M. & Pilnik, W. (1972). "Research on Pectin depolymerases in the sixties - a literature review". CRC critical Rev. Food Technol. 3, (1), 1-26.
- Rombouts, F.M. & Pilnik, W. (1980). "Pectic enzymes". in Microbial Enzymes and Bioconversions, ed. A.H. Rose. Academic Press, Lond., pp. 227-282.
- Rutter, D.A. & Evans, C.G.T. (1972). "Aerosol hazards from some clinical laboratory apparatus". B.M.J. 1, No. 5800, 594-7.
- Sardinas, J.L. (1968). "Rennin enzyme of Endothia parasitica". Appl. Microbiol. 16, 248-255.
- Sargeant, K. & Evans, C.G.T. (1979). Hazards Involved in the Industrial use of Micro-organisms. European Communities - Commission, EUR 6349, Biological sciences series. ISBN 92-825-1088-3.
- Sargeant, K., Sheridan, A., O'Kelly, J. & Carnaghan, R.B.A. (1961). "Toxicity associated with certain samples of ground nuts". Nature (Lond.) 192, 1096-1097.
- Schwarzbach, E. (1979). "A high throughput jet trap for collecting mildew spores on living leaves". Phytopathol. Z. 94, 165-171.
- Sherwood, M. (1980). "ICI debugs Pruteen at last". Chemistry and Industry 20 December, p. 921.
- Sherwood, R.F. & Peberdy, J.F. (1972). "Zearalenone production by Fusarium species in grain". J. gen. Microbiol. 73, xxiii.
- Slepushkin, A.N. (1959). "An epidemiological study of laboratory infections with Venezueline equine encephalomyelitis". Probl. virol. USSR. English Translation 4, 54-58.
- Smith, I.M. (1979). "EPPO; the work of a regional plant protection organisation, with particular reference to phytosanitary regulations". in Plant Health, eds. D.L. Ebbels and J.E. King. Blackwells Scientific Publications Ltd., Oxford. pp. 13-22.
- Southey, J.F. (1979). "Preventing the entry of alien diseases and pests into Great Britain". in Plant Health, eds. D.L. Ebbels and J.E. King. Blackwells Scientific Publications Ltd., Oxford. pp. 63-70.
- Southey, R.W. & Harper, G.J. (1971). "The survival of Erwinia amylovora in airborne particles: tests in the laboratory and in the open air". J. appl. Bacteriol. 34, 547-556.
- Stakman, E.C. & Harrar, J.G. (1957). "Stem rust epidemics of 1925 and 1935". in Principles of Plant Pathology. Ronald Press, N.Y. 581 pp.

- Stevenson, C.M. (1969). "The dust fall and severe storms of 1 July 1968". *Weather* 24, 126-132.
- Strange, R.E. (1973). "Rapid detection of airborne microbes". in *Airborne Transmission and Airborne Infection*, eds. J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht. pp. 15-23.
- Sudo, T., Nagayama, H. & Tamari, K. (1973). "Occurrence and some properties of cellulase in the filtrates of conidiospores and mycelia of *Pyricularia oryzae* Cavara". *Agric. biol. Chem.* 37, 1651-1659.
- Sulkin, S.E. (1961). "Laboratory acquired infections". *Bact. Rev.* 25, 203-209.
- Sutherland, I.W. & Ellwood, D.C. (1979). "Microbial exopolysaccharides - industrial polymers of current and future potential". in *Microbial Technology*; S.G.M. Symp 29. eds. A. Bull, D.C. Ellwood & C. Ratledge, pp. 107-150.
- Szczyrbak, C.A. (1972). "Production of clavine alkaloids, in vitro, by *Claviceps fusiformis*". Ph.D Thesis, University London.
- Teltsch, B. & Katzenelson, E. (1978). "Airborne enteric bacteria and viruses from spray irrigation with wastewater". *Appl. env. Microbiol.* 35, 290-296.
- Templeton, G.E. & Smith, R.J. (1977). "Managing weeds with pathogens". in *Plant Disease* 1, eds. J.G. Horsfall and E.B. Cowling. Academic Press, N.Y. & Lond. pp. 167-176.
- Tomlinson, A.J.H. (1957). "Infected air-borne particles liberated on opening screw-capped bottles". *Brit. med. J.* July 6th, 15-17.
- Turner, J.N. (1972). "Practical uses of gibberellins in agriculture and horticulture". *Outlook on Agriculture* 7, 14-20.
- Turner, W.B. (1975). "Commercially important secondary metabolites". in *The Filamentous Fungi: 1, Industrial Mycology*, eds. J.E. Smith and D.R. Berry. Edward Arnold, Lond., pp. 122-139.
- Tyldesley, J.B. (1967). "Movement of particles in the lower atmosphere". in *Airborne Microbes*, eds. P.H. Gregory and J.L. Monteith. Cambridge University Press. pp. 18-30.
- Vandamme, E.J. (1980). "Penicillin acylases and Beta-lactamases". in *Economic Microbiology* 5, ed. A.H. Rose. Academic Press, N.Y. and Lond. pp. 467-522.
- Venette, J.R. & Kennedy, B.W. (1975). "Naturally produced aerosols of *Pseudomonas glycinea*". *Phytopathol.* 65, 737-738.
- Voinov, S.I. & Khor'kov, I.A. (1968). "On the survival of the Hoof-and-Mouth Disease Virus in the air". *Veterinariya* 11, 96-99. USSR. from USA/TT10. TSTC. HT-23-38-69.
- Wade, H.E., Robinson, H.K. & Phillips, B.W. (1971). "Asparaginase and glutaminase activities of bacteria". *J. gen. Microbiol.* 69, 299-312.

- Wallace, H.R. (1978). "Dispersal in time and space: soil pathogens". in Plant Disease, 2, eds. J.G. Horsfall and E.B. Cowling. Academic Press N.Y., San Francisco and Lond. pp. 181-202.
- Waller, J.M. (1979). "The recent spread of coffee rust (Hemileia vestatrix) and attempts to control it". in Plant Health, eds. D.L. Ebbels and J.E. King. Blackwells Scientific Publications Ltd., Oxford. pp. 275-283.
- Walton, W.H. & Prewett, W.C. (1949). "The production of sprays and mists of uniform drop size by means of spinning disc type sprayers". Proc. Phys. Soc. B. 62, 341-350.
- Webber, J. (1981). "A natural biological control of Dutch elm disease". Nature (Lond.) 292, 449-451.
- Wells, J. (1977). "Extracellular microbial polysaccharides - a critical overview". in Amer. Chem. Soc. Symp. Ser. 45, Extracellular Microbial Polysaccharides, eds. P.A. Sandford and A. Laskin. p. 299-313.
- Wilson, A.T. (1959). "The surface of the ocean as a source of air-borne nitrogenous material and other plant nutrients". Nature (Lond.) 184, 99-101.
- Winkler, W.G., Fashinell, T.R., Leffingwell, L., Howard, P. & Conomy, J.P. (1973). "Airborne rabies transmission in a laboratory worker". JAMA. 226, 1219-1221.
- Wittwer, S.H. (1980). "Future challenges and opportunities for agricultural and forestry research". CHEMRAWN, 1, 401-412.
- Wolf, H.W., Skalig, P., Hall, L.B., Harris, M.M., Decker, H.M., Buchanan, L.M. & Dahlgren, C.M. (1959). Sampling Microbiological Aerosols. U.S. Publ. Health Monograph, 60. U.S. Govt. Printing Office, Wash. D.C.
- Wood, B.J.B. & Yong, F.M. (1975). "Oriental food fermentations". in The Filamentous Fungi: 1, Industrial Mycology, eds. J.E. Smith and D.R. Berry. Edward Arnold, Lond., pp. 265-280.
- Worthington, A.M. (1963). A Study of Splashes. The Macmillan Co., N.Y., 169 pp.
- Yamada, S., Maeshima, H., Wada, M. & Chibata, I. (1973). "Production of D-alanine by Corynebacterium fascians". Appl. Microbiol. 25, 636-640.
- Zadoks, J.C. (1967). "International dispersal of fungi". Netherland J. Plant Pathol. 73, Suppl. No. 1, 61-80.
- Zadoks, J.C. (1973). "Long-range transmission of phytopathogens". in Airborne Transmission and Airborne Infection, eds. J.F.Ph. Hers and K.C. Winkler. Oosthoek Publ. Co., Utrecht, pp. 392-396.

Zadoks, J.C. & Schein, R.D. (1979). *Epidemiology and Plant Disease Management*. N.Y. Oxford University Press.

Zhalko-Titarenko, V.P. (1965). "A study of diphtheria bacillus aerosols". *Zh. Mikrobiol. epidem. Immunobiol.* 2, 68.