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Dissemination of Mildew Spores in a Glasshouse [and Discussion]

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Dissemination of mildew spores in a glasshouse

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Epidemics of fungal plant diseases in a greenhouse sometimes show a rather rapid dispersal; in other cases, however, the epidemic stays isolated in a part of the greenhouse. The spore-dispersal system in glasshouses is very complex and as a consequence it will be extremely hard to design a model for it.

The system depends on a number of variables, relating to pathogen, environment and man. Much attention is given to the environment as the variable affecting the dispersal of fungal spores; man, however, appears to be the most underestimated and perhaps most important of the three.

In a series of experiments the dissemination of a typical airborne spore type, the conidiospore of *Erysiphe graminis* f. sp. *hordei* (barley powdery mildew), has been followed.

Introduction

In the literature on aerobiology the indoor spread of microorganisms is most frequently considered in relation to medical aspects, where it is of great importance. The literature concerning the spread of human pathogenic or allergenic microorganisms in closed and segregated environments is voluminous (Bourdillon *et al.* 1948; Silver 1970; Hers & Winkler 1973; Chatigny & Dimmick 1979), but references to the spread of plant pathogens in glasshouses are scarce, and solid papers even lacking. This paper will review the subject from the literature and will primarily be based on studies made in the glasshouses of the Centre of Entomology and Phytopathology at the Agricultural University in Wageningen.

Botanical versus medical epidemiology

This is not the place to discuss in detail the differences between medical and botanical epidemiology, but we need to compare the fundamental factors involved in both cases. In the disease tetrahedron for botanical epidemiology of Zadoks & Schein (1979) the base symbolizes the interaction of host, pathogen and environment, with man at the apex, the factor affecting each of them (see figure 1). For medical epidemiology we could use the same tetrahedron, but in this case man serves not only as the factor influencing the development and control of epidemics but also as host in the base triangle of the tetrahedron.

The most important difference between the two cases is the host, which in botanical epidemiology consists of a population of plants incapable of moving from one place to another, except when man serves as a carrier, which can happen in glasshouses if plants are grown in pots. In medical epidemiology, however, man can serve as a moving source or as a moving reservoir of the disease in question. It will be no surprise that in the special case discussed here, epidemics in glasshouses, man shows up as a disturbing factor, for here man cannot be neglected as a possible vector of plant diseases. The glasshouse situation is much more complicated than the situation of epidemics in the field.

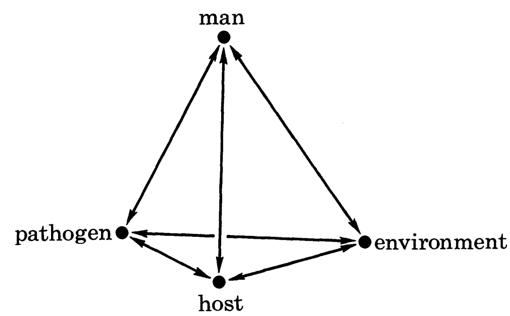

FIGURE 1. Disease tetrahedron for botanical epidemiology, showing the relations between host, pathogen, environment and man (Zadoks & Schein 1979).

GLASSHOUSE VERSUS FIELD EPIDEMICS

The influence of man in field epidemics is limited to the control of disease development. He is able to slow the development or even to stop it by taking special measures, but will function as a tool for the spread of the fungal or bacterial particles in few cases only. An exception might occur when sprayings are applied, but less labour is done in the field than in the glasshouses during the vegetative period. The spread of the fungal spores in the field is much more affected by environmental factors such as wind, air turbulence and rain, which cause mechanical shaking of the leaves and by this the liberation of the spores from their mycelium or sporechain (Bainbridge and Legg 1976). This does not imply that environmental factors should be ignored when studying epidemics in glasshouses; on the contrary, because, in contrast with the situation in the field, conditions in closed or segregated environments are relatively constant and more favourable for the development of fungal pathogens on the aerial parts of the plants and as such for the build-up of the inoculum, the number of spores likely to become airborne.

What are the variables responsible for the rate of development of an epidemic? We recognize: (1) the length of the latent period, (2) the relative rate of spore production, (3) the length of the infectious period, which also controls the rate of removals, and (4) the proportion of dispersal units that initiate new infections.

In glasshouses the first three variables are favourably influenced by the constant, mostly sub-optimal environmental conditions mentioned above. They can be predicted. In the field, however, these conditions are of a varying and unpredictable nature, at least in our temperate climate. The fourth variable – the proportion of dispersal units initiating new infection, which is a part of the proportion of spores enabled to leave their place of origin, to move along airstreams or to diffuse from one air unit to another, and to land on a part of the same or another susceptible plant – will be larger in the field than under conditions indoors. Air motion within a cereal crop will perhaps hardly reach the speed of 0.5 m s^{-1} (Bainbridge & Legg 1976), but in glasshouses this speed already means a 'storm'. In experiments done in glasshouses we have found that windspeeds normally did not exceed 0.25 m s^{-1}. Exceptionally when doors were opened against each other, the windspeeds could be increased to about 0.5 m s^{-1}.

FACTORS AFFECTING DISSEMINATION OF SPORES IN GLASSHOUSES

Which factors have to be taken into consideration when discussing the dissemination of spores in glasshouses? We shall of course consider only the plant pathological consequences of spore dissemination at this time, although allergic problems could also be discussed because the dissemination of spores in glasshouses can cause various physical complaints. Allergic problems are not only caused by plant pathogenic airborne spores, but can also show up if treated soils (heating, fungicides, etc.) are used. The treatment can disturb the balance between the different populations of fungi. This results in a rapid development of soil fungi, unimportant in the first place but finally causing concentrated spore clouds in glasshouses. In most experiments we were only interested in the epidemiological consequences, looking only for the viable spores able to arrive at a host and to produce a new infection.

The host

To classify the factors involved we can again make use of the disease tetrahedron. We shall start with the host.

In our experiments we used barley seedlings as host plants and barley powdery mildew (*Erysiphe graminis* f. sp. *hordei*) as the airborne disease. This was an unusual pathosystem, maybe, but suited to the present purpose: unusual because we have to do with a field crop never cultivated in glasshouses, but suitable because the barley seedlings can be bred in great numbers and in a short time, and, moreover, the pathogen has a sporetype that is typically airborne and unable to attack other plants in the glasshouse.

In some glasshouses market gardeners cultivate one crop only, in others different crops are grown together. This of course does affect the epidemiological reflections on the subject, but it does not affect the aerobiological reflections at all. Much more important are the crop density, the crop structure and crop height. It is obvious that glasshouses full of cucumber, tall plants with sometimes huge leaves, and with carnations, medium sized plants with fine leaves, and with lettuce, a very short crop with a very dense rosette of leaves, show different dispersal patterns.

Burdon & Chilvers (1976) in Australia did a series of experiments, examining the infection rate of barley powdery mildew in a barley stand with different plant densities. The experiments were carried out under controlled environmental conditions. Besides a constant rate of a so-called 'within-plant' spore transmission, they could discern a linear relation between infection rate and host density. An almost fourfold increase in plant density showed an almost doubling of the infection rate, of which the 'between-plant' spore transmission formed an important part. They interpreted their results 'to mean that the relationship was mainly controlled by the number of host targets available to collect inoculum, rather than the distances between them'. We do not intend to discuss this subject matter in more detail here. It has already been done by Legg (this symposium), and though he was concerned with the movement of spores in a crop canopy under field conditions, there is no reason to believe that the crop, as single factor regulating spore dissemination, will act in different ways in the field and glasshouse situation. Returning to our own experiments in the glasshouse we should mention that we never used barley as a 'crop', as was done by Burdon & Chilvers, but we simply used pots with ten seedlings 7 days old as spore traps to mark where, when and how many spores were able to arrive at these targets. The plants cultivated in the experimental glasshouse usually were ornamentals of different height and structure, used for all kinds of tests and experiments.

The pathogen

The second base angle of the tetrahedron is formed by the pathogen, the aerobiological particles considered here. Depending on, among other things, their morphology, fungal spores

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can be transported in different ways. The one studied here is transport by air: what is the behaviour of the spores once becoming airborne?

Velocities of air movement in glasshouses are lower than in the open air. This implies that sedimentation of the fungal spores, besides impaction by air currents, plays a more important role in glasshouses than under field conditions. The rate of sedimentation is defined by Stokes's law for smooth spheres, in which the radius of the sphere and its density are essential elements. However, spores seldom belong to the category of smooth spheres, so that their morphology influences the terminal velocity and so the time a spore is able to remain airborne and to be dispersed. The terminal fall velocity plays a role in all cases of aerial transport of spores. In general the surface:mass ratio of the spores, their external form and their surface texture are defining factors in their dissemination. For more information we refer the reader to Gregory (1961).

Besides these factors, attention must be given to the methods of spore liberation, which are described so excellently by Ingold (1965, 1971). It makes a great difference if a spore is liberated actively by some kind of special mechanism, like the 'squirt gun' mechanism of the ascomycetes, or if the spores are liberated passively by environmental factors. An example of this method of spore liberation can be found in our case study with powdery mildew, the spores of which have to be liberated by air movement and vibration of the leaf.

The sporulation of most fungi has a circadian rhythm. Some sporulate abundantly during the daytime, others during the night. Our fungus, *E. graminis* f. sp. *hordei*, is a day-flyer: experiments in our glasshouse showed that more spores were disseminated during the daytime than during the night. Again we refer to the work of Ingold (1965, 1971), only mentioning the fact that daytime in glasshouses is often prolonged by artificial illumination, which results in a longer daily sporulation period for those fungi using light for the formation and maturation of their spores (Frinking 1977). Some of our experiments show clearly that a prolonged illumination during the night causes a prolonged dissemination period (figure 2, table 1).

Last but not least the place of origin of the spores is of importance. The plant pathogen can be introduced in different ways. First, from an outside source, the spores enter the glasshouse by windows, doors, and often simply by chinks or seams in badly isolated glasshouses or by cracks in the glass of greenhouses not kept in good repair. We often see disease first appearing in the glasshouse crop in those places where the point of entrance is unambiguous. It is often more difficult to locate the outside source than to show that the spores have been introduced by air movement from the glasshouse surroundings. The introduction of a pathogen by seed, by soil, by plant material or even by man creates the second case, where a pathogen source can develop anywhere in the glasshouse, contrary to the first case, where the source must be located in certain specific areas. The place where the pathogen appears in relation to the basic air-movement pattern of the glasshouse will, among other things, determine the dispersal pattern of the spores and the rate of development of an epidemic.

We did some experiments with the source of the pathogen situated at different places in our glasshouse. The conclusions we drew from these experiments were: at places where least air movement could be measured, for example near the well isolated back wall of our glasshouse, far less dissemination of spores was observed than at the centre of the glasshouse, where most labour is done and where turbulence is considered to be 'normal'. Most spores, however, were disseminated when the source was placed near one of the doors, where of course strong air currents were regularly recurring phenomena (figure 2, table 1).

The environment

The environment, the third determining factor, is most complex, and the only course open to us is to give an impression of this complexity. Spores are liberated or disseminated, or both, by moving air, especially by the 'gustiness' of it. How can this movement be generated? There exists an important difference in air pressure between the relatively still glasshouse air and the air outside the glasshouse, which is in constant motion. This difference, which depends on the

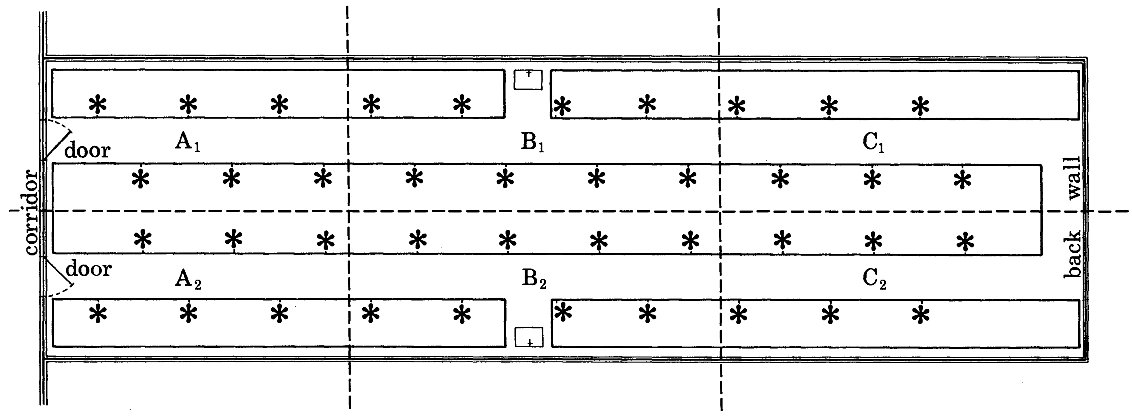

FIGURE 2. Ground plan of the glasshouse in which the experiments in table 1 were carried out. Solid lines represent fixed tables in the glasshouse, broken lines represent an imaginary division of the glasshouse into sections, as used for table 1. The asterisks show the location of the pots with spore-trapping barley seedlings.

windspeed of the outside air, will be able to generate an air current through windows, chinks and cracks. Besides the air movement generated by wind, there is an air movement generated by temperature differences between the outside and inside air. Together they show a linear relation between the quantity of exchanged air, the total surface and the windspeed. The relation is expressed by the formula

$$\phi_V = KAu,$$

where ϕ_V is the quantity of exchanged air, K is a constant factor, A is the total surface of opening, and u is the windspeed. The exchange between outside and inside air, expressed as the number of times the total glasshouse content is renewed per hour, is about 0.2 for well isolated glasshouses and 0.5 for normal glasshouses, without perfect isolation, in both cases when windows are closed. When windows are open, the exchange can increase to 100 times per hour (G. P. A. Bot, personal communication). An experiment in our glasshouse with open windows and windspeeds of about 2.50 m s^{-1} outside the glasshouse showed that all spores in the air disappeared to the outside world by the renewal of glasshouse air, which could be estimated at about 30–40 times per hour (figure 2, table 1).

From measurements and experiments with smoke puffs in glasshouses we observed that the air currents in window openings were different for each window, and that many fluctuations could be measured. This means that the air movement in a glasshouse is influenced to different degrees by different openings in walls and roofs, that the fluctuations of the inside air movement can be considered as being normal phenomena, and that the speeds of these movements are low.

Another air movement to be considered is the movement generated by the so called free convection above warm and cold surfaces. In glasshouses this takes place above heating systems (upwards) and along walls and roofs (downwards). One may expect this process to be rather stable for each individual glasshouse. Some glasshouses are provided with a cooling or an air-circulation system causing forced ventilation, and in these circumstances the whole theory of air-exchange has to be reconsidered.

TABLE 1. NUMBER OF NEW COLONIES OF POWDERY MILDEW (*ERYSIPHE GRAMINIS* f. sp. *HORDEI*) ON BARLEY SEEDLINGS

(The new colonies were initiated by the dissemination of spores from a source in a glasshouse. Pots with ten barley seedlings 7 days old acted as spore traps. Sections $A_{1,2}$, $B_{1,2}$, and $C_{1,3}$ correspond to the sections of the glasshouse as illustrated in figure 2. All experiments except experiment IV were carried out with closed windows.)

expt	location of the source	exposure time/h	day or night	remarks	section A_1	A_2	section B_1	B_2	section C_1	C_2	total
I *a*	centre (section B)	15	night	total darkness	1	0	2	1	0	0	4
b	centre (section B)	15	(night)	prolonged illumination for 8 h	0	0	18	7	1	3	29
II *a*	centre (section B)	9	day	—	5	12	88	34	6	6	151
b	centre (section B)	9	night	—	1	0	2	3	0	1	7
III *a*	door (section A)	3	day	—	> 1000	4	3	8	0	0	> 1000
b	back wall (section C)	3	day	—	0	0	0	0	3	3	6
IV	centre (section B)	3	day	open windows	0	0	0	0	0	0	0
V *a*	centre (section B)	3	day	—	0	0	2	1	0	0	3
b	centre (section B)	3	day	intensive labour around the source	2	0	31	10	4	0	47
VI	back wall (section C)	3	day	source transferred from section C to section A, settling time 3 h	6	2	10	1	17	6	42

In conclusion, each glasshouse has its own air-movement pattern, based on its construction, fittings and situation in the outside environment. The last factor also determines differences in air pressure between outside and inside air and between different areas in the glasshouse. The pattern will be subject to many fluctuations, the timescale of which is of the order of minutes.

Man

Glasshouses are segregated environments for agricultural purposes. The intensification of agricultural practices in glasshouses implies, among other things, that glasshouse crops require more frequent care than crops growing in the field. This care consists of regular harvesting, watering, spraying, pruning, weeding etc. Workers enter the glasshouse regularly, and all work done by them results in an unpredictable pattern of air movement and great disturbance of plants, which in its turn causes high concentrations of spores in the glasshouse air. Together this means a better chance for a rapid increase of epidemics.

In our glasshouse we demonstrated the results of work done during ¾ h around a source of inoculum without touching the diseased plants of the source. The work gave such a turbulence around the diseased plants that spread of the spores was inevitable. This resulted in 47 new colonies as opposed to 3 new colonies after exposure for the same period without people working in the glasshouse.

There is certainly no need to mention that movement of diseased plants is a mortal sin in glasshouse aerobiology: 42 new colonies were observed along the route of transportation from glasshouse back wall to the front door (figure 2, table 1).

Hirst (1959) reports an experiment executed in 1953 by Gregory & Hirst, in which they compare the concentrations of spores inside and outside a tomato glasshouse in Surrey, England. The number of spores trapped by Hirst's volumetric spore sampler inside was more than four times that outside the glasshouse. Sprinkling foliage and soil with water increased the total number of spores inside the glasshouse 30 times, and 1 h after finishing the action the spore concentration had been reduced to only about one third of the starting concentration. This illustrates once more the result of work done in glasshouses.

Conclusion

A glasshouse can be considered as a closed agricultural system with components, linkages between these components, and well defined boundaries, separating this system from the outside environment (Edens & Haynes 1982). The system itself could be compared with a kaleidoscope, showing a rapid changing colour pattern. The words 'colour pattern' can be translated, for this system, into 'air-movement pattern'.

The balance of the system is unstable by its nature, for a complete separation of the system from the environment will be a fiction. Each minor disturbance of the inner system by the environment or by the intervention of man, normal phenomena in a glasshouse cropping system, potentially results in an increase of the rate of development of epidemics. A good knowledge of the circumstances inside and outside the glasshouse together with a well considered management of the activities by glasshouse workers could help to protect the crops from undesirable disease levels.

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Discussion

P. K. C. Austwick (*Aerobiology Unit, Cardiothoracic Institute, University of London, Frimley, U.K.*). In view of the potential allergenicity of fungal insecticides to operatives in glasshouses, could Dr Frinking give any guidance as to the precautionary measures that might be taken as a result of his study of spore distribution and concentration of powdery mildew?

H. D. Frinking. The increasing interest in the use of fungi as 'insecticide' can pose important problems for men, especially when these practices are carried out in closed environments such as glasshouses. So far, the allergenicity of entomogenous and plant pathogenic fungi has not been studied very well. Precautionary measures that could be considered are of the same kind as those taken when chemical insecticides and fungicides are applied. Besides these measures medical tests will be important.

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