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SAMPLING AND MONITORING DISEASE IN WINTER WHEAT

SAMPLING AND MONITORING DISEASE IN WINTER WHEAT

by

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The work formed the basis of a doctoral thesis (1991) by S.R. Parker and supervised by D.J. Royle and M. W. Shaw. A copy of this document, entitled "Studies on some factors influencing the reliability of disease measurements in winter wheat crops" may be borrowed from the libraries of the University of Bristol, Long Ashton Research Station or through the Inter-library Loans system.

ABSTRACT

In a three year study some of the factors likely to affect the reliability of disease monitoring in winter wheat crops were investigated.

A novel large scale sampling procedure using randomly positioned transects and based on the theory of autocorrelation analysis is described. The great attraction of the technique is that it allows valid tests of significance to be made on the autocorrelation coefficients calculated. The sample data obtained are also suitable for use in mapping analysis and the production of semivariograms.

Over a period of three crop seasons the spatial pattern of some common diseases of winter wheat were investigated at growth stages 31/33 and 59/61 using the techniques outlined above.

The most complete data obtained were for *Septoria tritici* which was found to have an essentially random pattern at the growth stages investigated. Spatial pattern of the disease was detected on a small scale in some fields which were patchy as a probable consequence of low nutrient status.

With the exception of powdery mildew at GS31/33 and yellow rust at GS59/61 the other diseases also generally exhibited a random disease pattern. For this reason random (not haphazard) sampling paths can be recommended to be an adequate method of obtaining samples for monitoring purposes. A survey of observers employed in various areas of the agricultural industry indicated that such sampling patterns are already commonly used in disease monitoring despite the advice of ADAS to sample along two diagonal transects of the field.

The reliability of visual disease severity estimates was investigated. Observers were shown to be inaccurate, inconsistent and often imprecise in their estimates of disease severity. In a comparison of the efficacy of three seed treatments on the disease severity of powdery mildew the errors described above were shown to cause the wrong conclusions to be drawn about the efficacy of the treatments relative to one another.

The use of model leaves highlighted the possibility that the precision and consistency with which assessments were made was reduced with increasing speed of assessment. It is therefore suggested that the proficiency of observers may be adversely affected by having to make large scale disease assessments. There was also evidence that the design of disease assessment keys, being reliant on high contrast between the 'diseased' and 'healthy' areas, is fundamentally flawed.

A new key is proposed which aims to avoid some of the drawbacks of conventional disease assessment keys, but as yet the efficacy of the key is untested.

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CONTENTS

ABBREVIATIONS

Standard abbreviations for the SI units of measurement are used throughout the present work. In addition the following abbreviations are used:

AFRC	Agricultural & Food Research Council
cv	cultivar
df	degrees of freedom
F	calculated F value of the F distribution
GS	growth stage
IACR	Institute of Arable Crops Research
ICI	Imperial Chemical Industries
IHR	Institute of Horticultural Research
LARS	Long Ashton Research Station
ns	not significant at the 5% level
p	the level of probability
SE	standard error of the mean
%VAF	The coefficient of determination from a regression of least squares expressed as a percentage

1. INTRODUCTION

Since the second world war agriculture in the UK has undergone considerable change. A series of economic inducements and pressures has made the adoption of new technical and management practices and the cultivation of inherently less productive land more profitable (Raymond, 1984). Intensification and output has increased and, as a consequence, arable agriculture has become increasingly dependent upon chemical inputs.

Winter wheat has always tended to receive more pesticide applications than other cereal crops and during the late 1970's and early 1980's the use of routine prophylactic applications of fungicides was widely adopted by farmers. However, recent years have seen increasing economic restrictions. As a result, farmers now require more precise information so that agrochemical inputs can be reduced whilst maintaining profitability. Also, there has been a growing public awareness and concern over the environmental impact and possible health hazards associated with pesticide use. These factors act to create a greater demand and need for pest and disease management schemes which rationalize the use of pesticides generally.

1.1 Disease management schemes

A good disease management scheme invariably relies on an element of being able to predict or forecast disease. Its aim is to ensure that treatments are applied to crops only when economically sensible *i.e.*, when the cost of treatment is less than the economic loss in crop from taking no control action. Therefore, any effective forecast model must be applicable on a local level, ideally that of an individual field.

The farmer or his consultant will usually be responsible for the collecting the information needed to drive the model. Inevitably this will include disease monitoring in which estimates of disease are made in a crop at one or more times during the crop season. These should be as simple as possible, requiring little time or effort.

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1.2 Definition of basic concepts

To avoid any confusion in the discussion of disease monitoring it is necessary to introduce some commonly used terms, that have been well defined by Seem (1984):

Incidence is the proportion or percentage of diseased entities within a sampling unit, *e.g.* percentage of leaves diseased on a plant.

Severity is the quantity of disease affecting entities within a sampling unit, *e.g.* percentage of a leaf area as disease lesions.

Intensity is a general characterization of disease within a specific area. Thus, two of the attributes of intensity are incidence and severity.

There is a potential for errors to arise where the amount of disease affecting a crop is estimated visually. Both overestimation and underestimation of disease intensity will lead to pesticides being applied at the wrong times. Therefore errors in disease measurement can lead to a reduction in the economic success of a disease management scheme. When evaluating the errors made by observers in visual estimates of disease two considerations can be identified:

Accuracy refers to the closeness of an estimate to the true value of the quantity of the disease assessed.

Precision refers to the repeatability or variation associated with an estimate.

The nature of accuracy and precision are clearly illustrated in the analogy used by Campbell and Madden (1990) (Fig. 1).
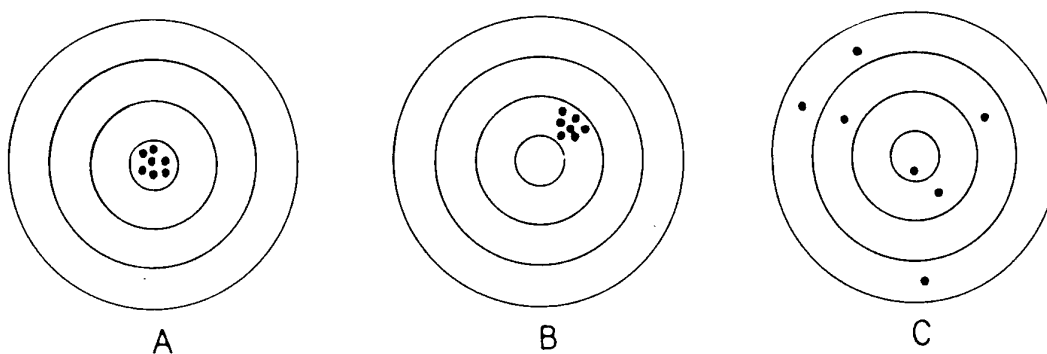
Fig 1. Accuracy & precision of an archer when the objective is to place all arrows in the central circle. A, accurate and precise; B, not accurate but precise; and C, not accurate and not precise.

1.3 Aims of the study

The assessment of disease stands as a cornerstone of plant pathology. Studies in epidemiology, assessment of crop losses and plant disease surveys and their applications are not possible without quantification of disease (Kranz, 1988). In addition, the implementation of disease forecast schemes will inevitably incorporate disease measurement, either by visual estimation or immunoassays, as an influential parameter of the underlying forecast model.

The first step in the quantification of disease is to acquire a representative sample of a crop from which an estimate of disease intensity can be determined. A pragmatic basis for obtaining field samples suitable for disease monitoring can only be achieved after consideration of the spatial pattern of disease and how it changes as the season progresses. Thus, **the first aim** of the work reported here was to obtain good information on the spatial pattern of some common diseases of winter wheat, so that sampling methods suitable for use in field monitoring could be devised.

The second aim of the work was to investigate the accuracy and precision of visual

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disease severity assessments. The broad definition of this aim can sensibly be subdivided in to the following problems:

1.   The accuracy and precision of individual observers, and their consistency over different sampling dates.
2.   The comparability of assessments made by different observers
3.   The type and extent of training needed to ensure similar results from different observers.

2. DISEASE MONITORING: A PERSPECTIVE ON CURRENT APPLICATION IN WINTER WHEAT

In autumn 1990' ca. 630 questionnaires (see Appendix I) were distributed to members of the Arable Research Institute Association (ARIA) and to advisors of the Scottish Agricultural Colleges (SAC) and ADAS. The aim was to gain information of the present use of sampling and monitoring in cereal crops. A response of around 16% was achieved. Unfortunately no returns were obtained from ADAS advisors.

Returns were categorized into 5 groups:

1. Farmers/Farm Managers   (38 = 36%)
2. Independent Advisors   (20 = 19%)
3. SAC Advisors   (20 = 19%)
4. Sales Advisors   (12 = 11%)
5. Researchers & Field Trials Officers   (15 = 14%)

Disease monitoring was clearly practised to some extent by all groups defined by the investigation. All Independent and Sales Advisors, 95% of Farmers and approximately 75% of SAC Advisors and Researchers, often made disease assessments. The remainder of all groups indicated that they occasionally made disease assessments.

Although individuals were generally confident of their ability to assess disease fairly accurately, few considered that disease thresholds were important when making disease management decisions; disease presence was the primary management concern. However, it might reasonably be argued that this is wasteful of the self proclaimed ability to quantify disease, and could lead to the treatment of disease at levels for which no economic return is gained. Moreover, the introduction of disease forecast schemes would almost certainly involve some form of quantifying disease more accurately than the crude measure of presence or absence in the crop. Certainly, if such schemes are to be used in the near future, visual assessments provide one of the few realistic options for disease measurement.

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For foliar cereal diseases, ADAS (Anon., 1976) recommends that 25 fertile tillers are taken on each of two diagonal traverses of the crop. However, random sampling paths were the most widely adopted means of obtaining disease assessments by all groups and the size of samples taken varied considerably.

Approximately 37% of Farmers assessed 20 or less plants from a field. Fewer than 8% indicated that they assessed more than 50 although a further 10%, who replied "many" on the questionnaire, may have done. Only one respondent from the group made any reference to altering the number of plants sampled depending on the disease in question (*i.e.*, "50+ for eyespot, otherwise fewer"). Similarly, only one respondent from the Independent Advisors and two from the Sales Advisors made any reference to the influence of this factor on the number of plants required to provide an accurate sample. However, the majority of respondents from the Independent Advisors indicated a range of sample sizes. Whether this was to account for variability in field sizes or differences in the biology of different pathogens, or a combination of the two cannot be determined. To some extent this was also true of the Sales Advisors, but the specified ranges of sample size were smaller and the upper limits of sample size were lower. It was concluded that Sales Advisors generally assess about 20 plants from a field. The majority of SAC Advisors did not specify a range of sample sizes. However, the number of plants sampled by individuals varied considerably between 10-100 plants. It was impossible to extract a typical sample size for the group.

The methods adopted by the various groups, for sampling winter wheat, are largely untested for their suitability. The protocol described by ADAS is either not widely known or else is ignored. Given the range of different strategies used, it seems likely that some monitoring is too cursory to provide reliable information. In the same vein, some monitoring may be excessively time consuming when considering the usefulness of the additional sample reliability achieved.

The questionnaire provided a list of three training methods: diagrammatic keys, by a colleague, or by a formal training course. Experience of other types of training not

included in the list were requested to be specified.

All Researchers and most SAC Advisors had been trained in making disease assessments. Three-quarters of Independent Advisors, two-thirds of Sales Advisors and a little over half of Farmers had experienced some form of training.

The training experience within groups was varied (Table 1). However, the predominant method of training was through the use of assessment keys. An exception to this was for Researchers of whom most had been trained by a colleague.

Table 1. Type of training for visual disease assessments experienced by five groups employed in the Agricultural industry.

Training experienced	Proportion in group with this experience (%)				
	Farmer	Independent advisor	Sales Advisor	SAC Advisor	Researcher
keys	21.1	35	25	25	23.3
colleague	2.6	0	8.3	15	30.8
course	0	5	0	10	0
keys & colleague	15.8	15	8.3	20	23.1
keys & course	0	5	0	15	15.3
colleague & course	2.6	0	0	0	0
keys, course & colleague	2.6	10	16.7	15	7.7
EPIPRE	2.6	0	0	0	0
none	47.6	25	33	10	0

Considering that the use of keys was the most widely experienced form of training, the proportion of individuals within each group who indicated that they always used a key was low. The most conscientious users of keys were Researchers, nearly third a of whom always used an assessment key. No Independent and Sales Advisors, and only a small proportion of Farmers and SAC Advisors were such frequent users of keys. The number of respondents in each group replying they never used keys was greater than the proportions from each group having experienced no form of

assessment training.

The absence of any coherent training strategy and the reluctance to make use of disease assessment aids is indicative of either a general lack of awareness or, more disturbingly, widespread complacency towards the importance of disease measurement.

3. SPATIAL & TEMPORAL DEVELOPMENT OF DISEASE

3.1 Introduction

The importance of knowledge of the spatial pattern of disease to the plant pathology discipline is now well recognized (*e.g.*, Jeger, 1989).

At the simplest level, an understanding of the spatial development of disease is needed for designing sampling programs to use when monitoring fields for disease management purposes. It is from this requirement, referring particularly to the future needs of disease forecast systems, that the proposal for this project arose.

In the wider context, spatial pattern analysis can provide a cornerstone of epidemiological studies. For example, enabling the development of biological and environmental hypotheses that account for the relationship between pathogen propagules and diseased plants.

3.2 Spatial pattern and its importance to sampling strategies

There are three basic spatial patterns ranging from random, through uniform to aggregated (Fig. 2). These patterns represent a continuum, so statistical evaluation is needed to classify the patterns exposed in experiments. The nature of the continuum is shown by the relationship between population variance and mean. For a uniform pattern population variance is less than the mean; for a random pattern (which only arises when every plant in the field has an equal probability of being infected) population variance equals the mean; and for an aggregated pattern population variance is greater than the mean.

Computer simulation has shown that for aggregated disease patterns, sampling paths with a large number of arms, for example a W pattern, are necessary for obtaining a reliable estimate of disease intensity. Whereas, for a random pattern it is the number of sampling units rather than arms that is important (Lin *et al.*, 1979).

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Uniform ($S^2 < \bar{x}$)   ←   Random ($S^2 = \bar{x}$)   →   Aggregated ($S^2 > \bar{x}$)
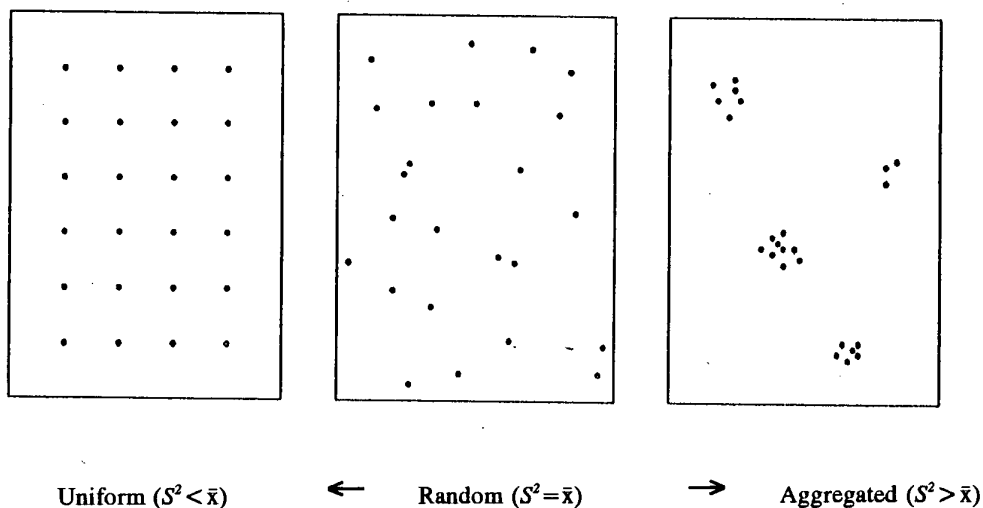
Fig. 2. Three basic forms of spatial pattern and the dispersion continuum.

Information on the spatial pattern of common diseases at critical periods of crop growth is required before recommendations about sampling strategies appropriate for use in disease management schemes can be provided. In this study growth stages 31 (the end of the vegetative overwintering stage and the start of stem elongation), (Zadoks *et. al*, 1974), and 59 (the end of ear emergence) were chosen for investigation.

3.3 Method

3.3.1 Field sites

Five fields of winter wheat were studied in southwest England over 3 years (Table 2). Two of them were managed according to the constraints of an organic system; the principal reason for this was to obtain substantial observations of disease. The other fields were conventionally managed.

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Table 2. *Sites investigated for spatial pattern of common disease pathogens, with year of study, winter wheat cultivar (cv) and growth stages sampled.*

Site	Year	cv	Sampled at
Field A Long Ashton Research Stn. Plot 30	1988	Slejpner	GS31, 59/60, 70
Field B 'Harnhill' RAC, Cirencester, Glos.	1989	Mercia	GS31/32 *(60% GS32)*
Field C 'Driffield' RAC, Cirencester, Glos.	1989	Mercia	GS31/32 *(80% GS32)*
Field D 'Saunders' Eastleach, Glos.	1989	Slejpner	GS31/32, 59 *(90% GS32)*
	1990	Mercia	GS31/32, 59/60 *(70% GS32)*
Field E 'Rough Ground' Eastleach, Glos.	1990	Mercia	GS32/33 *(80% GS33)*

3.3.2 Sampling method

The sampling technique was evolved over five sampling dates in 1988. The description which follows is for the method used in 1989 and 1990. Differences between this method and the one used in 1988 are indicated in the text.

Originating from a point equidistant between the two wheelings of a tramline, approximately situated at the centre of the field, cartesian coordinates x and y were marked out by 50m tape measures (Fig. 3).

Random values of x, generated using a Genstat 5 program, were marked with white canes along the x axis. For each cane on the x axis a second cane was placed at a predetermined interval along the y axis (Table 3). Each pair of canes provided a coordinate for a transect origin.

Numbered red marker canes were used to mark the transect origins. The accurate location of the marker canes at 90° to their respective coordinate canes was achieved

using an optical square (Hall Ltd) which is a hand held surveying device in which 2 reflecting surfaces are arranged to provide lines of sight at a fixed angle 90° apart.

Plants were sampled from along crop rows, away from the x origin, for even transect numbers; and at 90° to crop rows, away from the y origin, for odd transect numbers (Fig. 3). Five plants were taken from each of 5 distances along a transect, these were; cane, 31cm, 100cm, 310cm and 1000cm. Each sample of 5 plants was placed in a labelled polythene bag (39cm x 30cm).

3.3.3 Storage & assessment of samples

Storage of field samples

Prior to assessment the stem base and roots of the plants were carefully washed. The individual five plant sample was wrapped at the roots in moist absorbent paper and returned to the labelled bag. The samples were maintained at 2°C in a cold store, or on occasion, frozen at -18°C. Frozen samples were allowed to defrost at room temperature for approximately thirty minutes prior to assessment.

The samples for 14 transects from field D at GS59 in 1989 were lost due to a failure of the freezer, so foliar assessments could not be made; fortunately stem base diseases had already been scored.

Growth stage key

Plant growth stages were assessed as decimal codes using the key described by Zadoks et al. (1974); and also in terms of apical development as defined by Kirby and Appleyard (1984).

Assessment of foliar diseases

Disease severity on leaves was estimated as the proportion of the laminar area covered by disease pustules. In the case of Septoria spp., only the area containing pycnidia was assessed as diseased.

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Table 3. Transect number and y coordinate relative to the random x coordinate used in field sampling protocol. Predetermined repeated values of y were paired with random values of x to provide coordinates for transect origins. The number of transects sampled varied both within and between years. Thus y coordinates are shown for each possible combination of year and number of transects sampled. Refer to Fig. 3 for an explanation of the directional orientation of the transects.

Transect Number	y coordinate (m)			
	50 Canes	40 Canes (1989 onward)	40 Canes (prior to 1989)	20-40 Canes (April & June 1988)
1-5	8.5	8.5	2	random
6-10	-8.5	-8.5	2	
11-15	25.5	25.5	-2	
16-20	-25.5	-25.5	-2	
21-25	42.5	42.5	6	
26-30	-42.5	-42.5	-6	
31-32	8.5	8.5	18	
33	"	-8.5	"	
34	"	"	"	
35	-8.5	25.5	"	
36	"	"	-18	
37	"	-25.5	"	
38	"	"	"	
39	25.5	42.5	"	
40	"	-42.5	"	
41	"			
42	-25.5			
43	"			
44	"			
45	42.5			
46	"			
47	"			
48	-42.5			
49	"			
50	"			

KEY

→ direction of sampling along crop rows
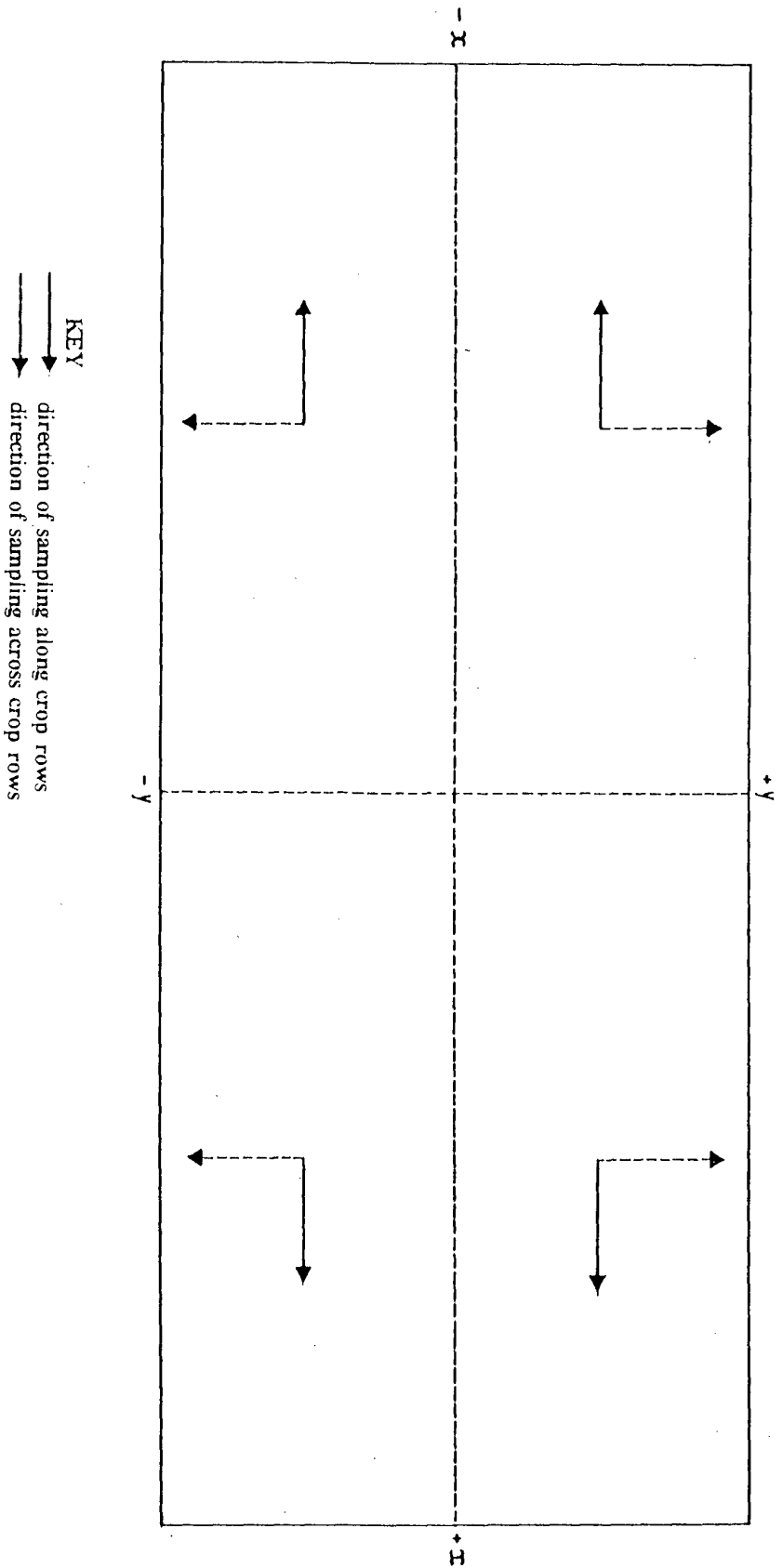
→ direction of sampling across crop rows

Fig. 3. Direction of sampling with respect to the origin of the catesian axes and crop rows. Five plants were taken from each of the distances 0cm, 31cm, 100cm, 310cm and for some fields 1000cm along a transect. In 1988, transects were sampled at random directions θ from North, and an additional sample was taken at 3100cm.

In 1988 stem base diseases were scored for incidence. In subsequent years the severity of eyespot and sharp eyespot was scored using the method described in section 5.2.

3.3.4 Statistical evaluation of spatial pattern

Most of the reported analytical sampling techniques are subject to inherent limitations. A discussion of these limitations is beyond the scope of this report (Parker, 1991). But, for example, where probability distributions are used data may fit several contradictory distributions.

Autocorrelation analysis

One technique of spatial pattern analysis that has great intuitive appeal is autocorrelation analysis. This statistic is closely related to the commonly used correlation coefficient. Where the occurrence of disease in one location makes the occurrence of the same disease in adjacent locations more (or less) likely, the disease is spatially autocorrelated. Since plant diseases often result from a process of biological contagion they would be expected to exhibit some degree of spatial autocorrelation. As an illustration, for a highly focal disease it would be expected that the autocorrelated distance would decline with increasing distance of separation and that the rate of this decline would be greater at the start of the epidemic than at the mid-point of the epidemic (Fig. 4).
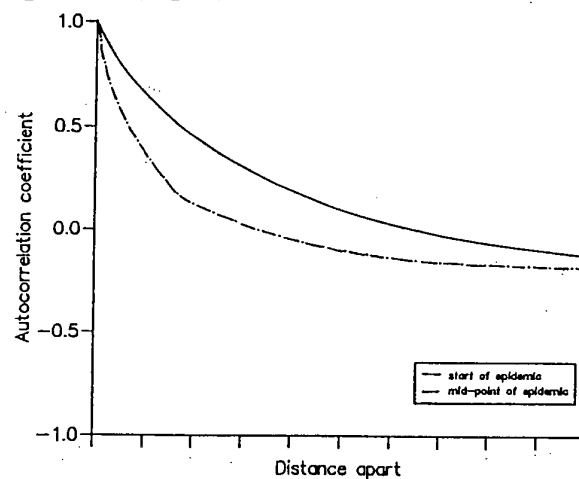

Fig. 4. Hypothetical plot of autocorrelation coefficient against distance of separation for a highly focal disease.

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The use of quadrat grids in autocorrelation analysis has been used with some success by a number of workers (*e.g.* Shew *et al.,* 1984; Madden *et al.,* 1987; Reynolds *et al.,* 1988). However, in common with other methods employing the use of quadrats the technique has a number of drawbacks:

1.   It constrains the investigation to intensive samples of relatively small areas due to considerations of time and effort.

2.   There is a problem of scale. The quadrats need to be small enough to detect the rate of decline in autocorrelation with increasing distance apart. Yet, simultaneously the quadrat grid must be larger than the spatial pattern that

is   to be detected and quantified. To some extent these requirements of scale are in conflict, particularly in the early stages of a study when researchers might only have a vague idea of the scale of the disease pattern.

3.   It is extremely difficult to conserve with any accuracy the position of individual sample units.

4.   The statistical significance of the calculated autocorrelation coefficients is not possible because the observations made for the grid are not independent.

To redress these problems the present investigation was based on transect samples. There are several advantages associated with the use of this method:

1.   The samples can be stratified by taking them at specific distances along a transect, such that the position of each sampling location is known and can be incorporated in subsequent analyses where necessary.

2.   The transects do not have to be divided in equal distances. Therefore, by ensuring the first distance is small and the final distance large it should be possible to detect any spatial pattern smaller than the field scale.

3.   The data collected are also suitable for several methods of analysis.

4.   The observations made for each transect are independent, thus enabling valid tests of significance to be made.

Autocorrelation coefficients (r) were determined as:

$$r_j = \frac{\sum (x_i - \bar{x})(x_{ij} - \bar{x})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (x_{ij} - \bar{x})^2}}$$

where : $\bar{x}$ = mean disease severity for all the field
x_i = mean disease severity at position i
x_{ij} = mean disease severity at distance j from position i

Semivariograms

Geostatistics is a method of spatial analysis, based upon the theory of regionalized variables, that accounts for location. A regionalized variable is a random variable that takes different values according to its location within some region. The method differs from spatial autocorrelation in that the only required assumption is that the variance of the difference between samples is a function of their separation (Clark, 1979). Hence, geostatistics detects spatial dependence by measuring the variation among samples separated by the same distance. Semivariance is a measure of the expected difference between all values separated by the same distance in a selected direction

Semivariance (γ) was calculated for all possible distances of separation along the transects using the following equation:

$$\gamma_j = \frac{1}{2N_j} \sum_{i=1}^{N} [x_i - x_{ij}]^2$$

where: N = the total number of plants in the sample

A semivariogram may be constructed by plotting semivariance against distance, and this can be used to provide a measure of the spatial variability within the field. The shape of the semivariogram will differ according to the spatial variable. For a purely random spatial pattern the semivariance is at its maximum for all distances, giving

a flat semivariogram. For other situations in which semivariance increases with distance, theoretical models, for example spherical or linear, may be fitted to the experimental plot. Chellemi *et al.* (1988), demonstrated the use of semivariograms for describing spatial pattern in three situations: simulated fields with random and aggregated disease patterns; glasshouse-grown capsicum plants suffering experimentally induced copper toxicity; and finally, in a field of pineapple plants to investigate the spatial variability of initial inoculum density and its relation to incidence of heart rot. Semivariograms have also been used by Todd & Tisserat (1990), to investigate the anistropic (directional) nature of phytoparasitic nematodes in soil collected from putting greens of creeping bentgrass (*Agrostis palustris cv.* Pencross).

Mapping

Mapping is a simple form of analysis which can provide a useful picture of the disease present in a field. Mapping has been done manually using dots (Jarvis & Hawthorne, 1972; Jeger *et al.*, 1987), or with varying shades and patterns (Punja *et al.*, 1985; Shew *et al.*, 1984; Smith and Rowe, 1984). Computers have been used in entomological and phytopathological studies, to assist the generation of maps in which shading or 3-dimensional peaks defines the intensity of aggregation (Goodell and Ferris, 1980a-b, Hau *et al.*, 1982). A further extension has been the use of contour maps showing isopleths (Noe & Campbell, 1985).

Mapping has usually relied on data collected from quadrats set out in systematic grids. At the simplest level mapping provides a visual display of density values for each individual quadrat and as such the analysis is inherently subjective. Although the use of computers allows the generation of contour maps, which smooth the data into discernible patterns by the use of algorithms, this inevitably causes the loss of significant details in the sample data.

Maps generated in this study were computed using the Uniras 6 graphics package running in the VAX VMS environment.

3.4 Results

The evaluation of spatial pattern from the information provided by autocorrelation analysis, semivariograms and mapping is a convoluted and time consuming procedure. Comprehensive details are not necessary for the purposes of the current discussion and are reported elsewhere (see Parker, 1991). However, it is useful to present some data that provides an insight to the mechanism of the analytical process.

3.4.1 Analysis of pattern of foliar diseases

(a) *Septoria tritici* Rob ex Desm.

A cube root transform of percentage severity of *S. tritici* was used to stabilize the variance (Shaw & Royle, 1987).

<u>Growth stage 31/33</u>
Disease incidence on the 1st fully expanded leaf, ranging between 0-0.7%, was too low at any of the study sites for sensible autocorrelation coefficients to be determined.

Tables 4 and 5 show the autocorrelation coefficients determined for the 2nd and 3rd fully expanded leaves respectively. Only one site, Field E sampled in 1990, still had a 4th leaf not completely senescent, the autocorrelation coefficients for which are presented in table 6.

In 1988, strong correlations were detected for the second leaf out to 100cm (Table 4), but to only 31cm on the 3rd leaf (Table 5), at field A.

The transects for the 1988 sample at GS32 were set to run at random directions $\theta°$ from north, so it was not possible to construct semivariograms to test for any directional dependent pattern.

In 1989 autocorrelation analysis detected no spatial structure to the disease for the

Table 4. Percentage disease incidence and autocorrelation coefficients for *S. tritici* severity on the 2nd fully expanded leaf at GS 31/33. Severity scores were cube root transformed prior to autocorrelation analysis.

Distance (cm)	Site, Year, Disease Incidence and Autocorrelation Coefficients					
	Field A 1988 *18.46%*	Field B 1989 *17.62%*	Field C 1989 *0.6%*	Field D 1989 *19.05%*	Field D 1990 *19.2%*	Field E 1990 *1.1%*
31	0.69****	0.12	-0.06	0.15	0.08	-
100	0.59*****	0.27	0.02	0.26	-0.18	-
310	0.04	-0.25	-0.02	0.19	0.36**	-
1000	-0.13				0.05	-
3100	-0.05					

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Asterisks denote significant correlation with $p \leq 0.025(**)$ and $0.005(****)$ respectively

Table 5. Percentage disease incidence and autocorrelation coefficients for *S. tritici* severity on the 3rd fully expanded leaf at GS 31/33. Severity scores were cube root transformed prior to autocorrelation analysis.

Distance (cm)	Site, Year, Disease Incidence and Autocorrelation Coefficients					
	Field A 1988 88.62%	Field B 1989 91.24%	Field C 1989 23.62%	Field D 1989 91.29%	Field D 1990 93.48%	Field E 1990 36.1%
31	0.39*	0.59******	0.45****	0.34**	0.06	0.04
100	0.32	0.13	0.16	0.34**	0.26	-0.03
310	0.15	-0.12	0.37***	0.20	0.11	0.09
1000	0.30				-0.21	-0.19
3100	-0.01					

Asterisks denote significant correlation with $p \le 0.05(*)$, $0.025(**)$, $0.01(***)$, $0.005(****)$ and $0.0005(******)$ respectively

2nd leaf layer at any site (Table 4). The semivariograms were in agreement with this for Field B and Field D. But for the organic Field C there was evidence of spatial structure along crop rows out to around 200cm (Fig. 5). However restricting the calculation of the autocorrelation analysis to the transect samples taken along rows provided no significant autocorrelation coefficients. The recorded disease incidence was extremely low at field C, at only 0.6% on the 2nd leaf, which represents less than 10 leaves showing symptoms. Most of the infected plants were found on one transect sampled along crop rows. The semivariogram must be treated with some scepticism, but it may be demonstrating the biological process by which *S. tritici* was spread to the 2nd leaf. In any crop, particularly a patchy one, the emergence of leaves is not perfectly synchronized. Thus a major splash event infecting the 3rd leaf with inoculum from the base of the crop could also have infected the tip of the emerging second leaf of a patch of slightly advanced plants. An alternative though less likely interpretation is possible. Ascospores of the perfect stage (*Mycosphaerella graminicola* (Fückel) Schroeter) of *S. tritici* are known to be released well into the spring (Hunter pers. comm.). Thus a single ascospore randomly deposited in late February or early March could have caused infection of the 2nd leaf when splash events are not intense enough to move pycnidiospores vertically in the crop. Later rain showers, or leaf rubbing, could have transported pycnidiospores small distances along crop rows for the 2nd leaf layer.

On the 3rd leaf in 1989 significant correlations were detected in disease for all three fields sampled (Table 5). The furthest correlation was 310cm detected for field C ($p < 0.025$). The correlation may have been significant at greater distances than this, but transects were not long enough to detect them. At Field D the autocorrelated distance was 100cm ($p < 0.025$), and at Field B only 31cm ($p < 0.0005$).

The semivariogram for field B was in agreement with the autocorrelation data indicating aggregation out to between 30 and 100cm. There is no evidence of any directional dependence to the aggregation. For field D the semivariogram suggested that aggregation was possibly up to 200cm which is further than

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indicated by the autocorrelation analysis (Fig. 6). However, this is because the autocorrelation analysis tested for spatial dependence at only five specific distances, the 200cm indicated by the semivariograms lies between the distances 100 and 310cm tested for autocorrelations. The semivariogram for field D along crop rows provided the most convincing model for spatial pattern, showing less fluctuation than evident across crop rows. Autocorrelation coefficients calculated for field D along crop rows were significant to 100cm ($p < 0.05$), but across crop rows were not significant at any distance. An aggregated spatial pattern could develop more easily along crop rows because neighbouring plants in the same row will tend to form a barrier intercepting rain splash. Hence, there is less chance of dispersal of pycnidiospores between crop rows.

In contrast to field D, the semivariogram for field C indicates that the component of spatial aggregation acted most strongly across crop rows. Along crop rows the autocorrelation coefficient was significant at 31cm ($p < 0.005$), close to significance (5% level) at 100cm ($r = 0.30$), and significant at 310cm ($p < 0.05$). Across crop rows significance was detected only at 310cm ($p < 0.05$). Field C was a very patchy crop. An open crop canopy will favour rain splash dispersal of the disease because droplets are less likely to be intercepted by neighbouring plants. Whilst disease spread along the rows will still, to a large extent, be constrained by the barrier of neighbouring plants, across the rows this will be far less effective in a patchy crop.

No significant correlations were detected on any leaf for the fields sampled in 1990 at this growth stage. Even at 31cm correlations were close to zero for leaves 2 and 3 at sites D and E (Table 4 & 5) suggesting a random disease pattern. Semivariograms constructed for the 1990 data confirmed the pattern suggested by the autocorrelation analysis. The semivariograms for both sites, and 2nd and 3rd leaves, show semivariance fluctuating with no relationship to distance. There was probably a different but constant underlying value of semivariance for each leaf; fluctuations around which could be caused by a combination of experimental errors.

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Table 6. Percentage disease incidence and autocorrelation coefficients for *S. tritici* severity on the 4th fully expanded leaf at GS 32/33. Severity scores were cube root transformed prior to autocorrelation analysis.

Distance (cm)	Field E, Disease Incidence and Autocorrelation Coefficients 92.25%
31	0.39***
100	0.05
310	0.07
1000	-0.07

Asterisks denote significant correlation with $p < 0.01$

Growth stage 59/60

Table 7 shows the autocorrelation coefficients determined for field D sampled in 1989 and 1990 at GS59.

No significant correlations were detected in 1989 though for leaf 4 autocorrelation was close to significance at the 5% level at 31cm. The semivariograms for the 2nd-4th leaves in 1989 did not show evidence of any spatial structure.

In 1990 there was no evidence of spatial structure to disease on the first three leaves in either the autocorrelation analysis or semivariograms (Table 7). Autocorrelation analysis detected significant spatial dependence out to 100cm on leaf 4 ($p < 0.05$; Table 7).

(b) Powdery mildew (*Erysiphe graminis* f.sp. *tritici* Marchal)

Use of the method of Box & Cox (1964) indicated that square root or cube root transformations were appropriate for stabilizing the variance of mildew severity scores. A square root transform was used in all the analyses reported for mildew in this study.
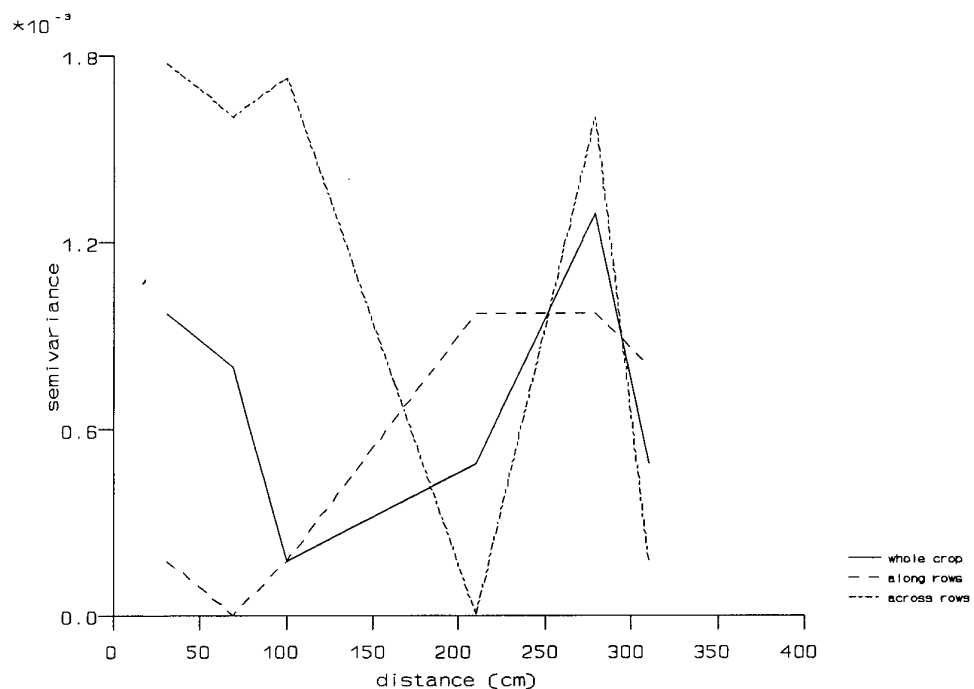
27

Fig. 5. Semivariance against distance for (*Septoria tritici* severity)$^{1/3}$ on the second fully expanded leaf for Field C.
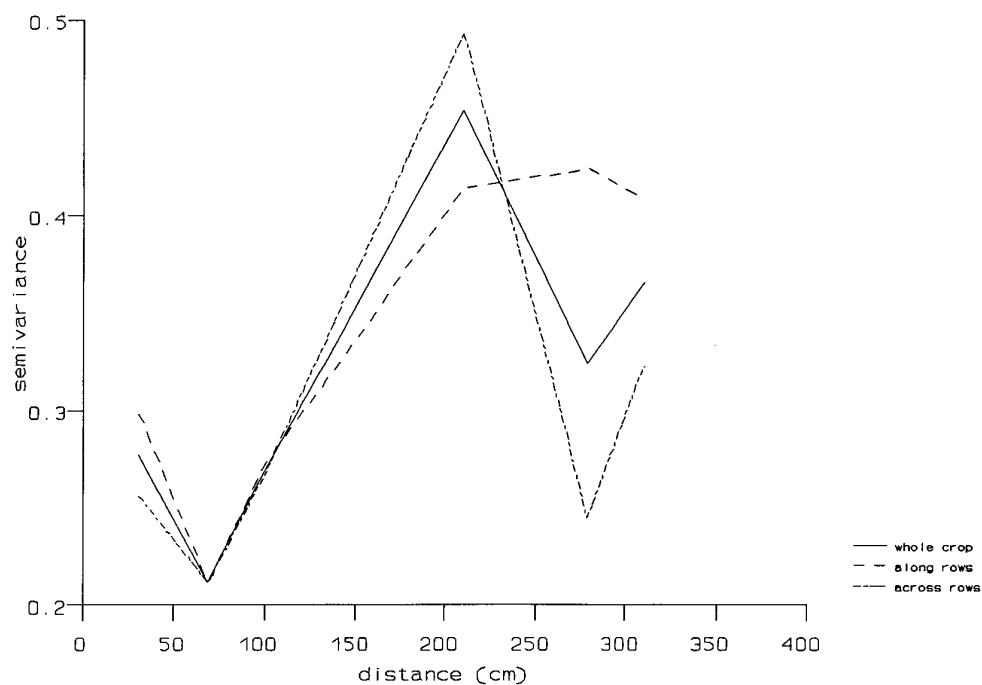

Fig. 6. Semivariance against distance for (*Septoria tritici* severity)$^{1/3}$ on the third fully expanded leaf for Field D (1989).

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Table 7. Percentage disease incidence and autocorrelation coefficients for *S. tritici* severity on leaves flag–4th at GS59/61. Severity scores were cube root transformed prior to autocorrelation analysis.

Distance (cm)	1989				1990			
	4 *47.82%*	**3** *8.46%*	**2** *1.23%*	**Flag** *0*	**4** *69.14%*	**3** *3.6%*	**2** *0.1%*	**Flag** *0*
31	0.30	0.27	-0.02	-	0.26*	-0.15	-	-
100	0.07	-0.20	-0.06	-	0.35**	0.23	-	-
310	-0.54	-0.18	-0.02	-	0.12	-0.15	-	-
1000	-0.14	-0.15	-0.08	-	0.24	-0.11	-	-

Asterisks denote significant correlation with $p \leq 0.05$(*) and 0.025(**) respectively

Growth stage 31/33

In 1988 active mildew was present at only very low levels (1% incidence on leaf 2). However, there was a substantial incidence of hypersensitive response (3.7% on leaf 1, 12.3% on leaf 2 and 7.7% on leaf 3), but this was at low severities, mainly less than 0.3%.

A large number of races of the mildew pathogen exist and these tend to have a restricted range of host varieties. It is impossible to determine with complete certainty whether the hypersensitive response recorded was due to unsuccessful infection by spores of incompatible races, active mildew wiped from the leaf by heavy rain, or the inhibition of conidial development by anaerobic conditions (caused by submersion in rainwater on the leaf surface soon after infection). Slejpner first appeared on NIAB's recommended varieties list in 1986 and has a good rating for mildew resistance, so a substantial number of unsuccessful infection sites would be predicted. However, in March and April at Long Ashton there were a number of intense rain showers with rates in excess of 40mm/hour. Although a recent study has shown that the ablution of mildew by short periods of rain is not significant 8 hours after infection has occurred (Holthius *et al.*, 1990), the same study indicated that fungal development was impaired by submersion in water. Whether the hypersensitive response was caused by one of these factors alone, or some combination of the three, the analysis of pattern for the active and hypersensitive data combined might provide useful information of the spatial pattern of mildew in the early stages of an epidemic.

Autocorrelation coefficients were not significant for leaf 1 or 3, but significant spatial dependence was detected at 31cm, 100cm and 1000cm for leaf 2 (Table 8). Semivariograms detected no spatial dependence on any leaf in 1988 which was in agreement for leaves 1 and 3, but not leaf 2. Because the 1988 sample was obtained using transects running at random directions $\theta°$ from north the semivariograms could only be constructed for the whole crop. Hence, significant directional spatial dependence may have been masked. The significant correlation coefficients detected could therefore be artefacts of any direction dependent correlations present in the crop. Because dispersal of mildew occurs through the

wind dispersal of conidia, spatial dependence would be expected to be anistropic in the direction of the prevailing wind.

Table 8. Autocorrelation coefficients for mildew severity (measured as combined hypersensitive response & active mildew) on the three fully expanded leaves at field A at GS31 in 1988. Severity scores were square root transformed prior to autocorrelation analysis.

Distance (cm)	leaf 1	leaf 2	leaf 3
31	-0.10	0.44**	0.13
100	0.22	0.53***	-0.03
310	-0.21	0.27	0.26
1000	0.13	0.43*	-0.20
3100	0.23	0.09	-0.01

Asterisks denote significant correlation with $p < 0.05(*)$, $0.025(**)$ and $0.01(***)$ respectively

In 1989 fields C and D were affected by active mildew at above trace levels of incidence (Field C: leaf 2 = 4%, leaf 3 = 16%; Field D: leaf 2 = 48%, leaf 3 = 34%)

Autocorrelation analysis detected no significant correlations for the 2nd leaf at field C. However the semivariogram for this leaf suggested spatial dependence was present along crop rows to around 200cm. The autocorrelation analysis was repeated separately for along crop rows and no significant autocorrelation coefficients were detected; at 31cm r was close to zero, but at 100cm ($r=0.32$) and 310cm ($r=0.31$) it was near to significance at the 5% level.

Field C had the greater levels of infection on leaf 3 with incidence at 15.7%. But severity was low at levels below 0.5% for most infected leaves. Autocorrelation indicated significant correlations out to the full length of the transect which was 310cm ($p<0.025$). The semivariogram for leaf 3 indicated that spatial structure was strongest along crop rows. Autocorrelation coefficients calculated separately for along crop rows and across crop rows were all significant to 310cm and the probability of the spatial dependence detected was the same for the two orientations at 31cm ($p<0.0005$) and 100cm ($p<0.025$), but at 310cm significance was greater along rows ($p<0.01$) than across rows ($p<0.025$).

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Crop C was most vigourous along a strip running parallel to the crop rows through the middle of the field. The map of mildew severity for leaf 3 suggests that most of the mildew was detected in the vigorous strip (Fig. 7). This is not surprising as mildew is known to be more severe on plants with high nitrogen levels (Darwinkel, 1980; Last 1953; Smith & Blair 1950).

Field D was heavily infected by mildew in 1989 at GS32. The autocorrelation analysis indicated significant spatial dependence to 31cm on leaf 2 ($p < 0.005$), and 310cm on leaf 3 ($p < 0.025$). On leaf 3 autocorrelation may have been significant at further distances, but transects were not long enough to test this.

The semivariograms constructed for field D on leaf 2 indicated spatial dependence was present to a greater extent than detected by the autocorrelation analysis. Furthest spatial dependence was detected along crop rows to around 200-250cm; but across crop rows the dependence was less than 100cm. Autocorrelation coefficients were calculated separately for along crop rows and across crop rows; spatial dependence was found to be highly significant to 100cm along crop rows ($p < 0.005$), but to only 31cm ($p < 0.005$) across crop rows. This suggests the disease was present in small ellipsoidal patches, with the longer axis orientated in the direction of the crop rows.

For the 3rd leaf the semivariogram detected no spatial dependence either across or along crop rows. The apparently random variation in semivariance in the 2 directions tested were cancelled out when data were pooled for the whole crop. This suggests that the significant autocorrelations are unreliable and that no legitimate spatial pattern was detected. Because *E. graminis* is an obligate biotroph pustules present on senescent tissue of the samples were not scored. Senescence was as high as 60% on the 3rd leaf at GS31 at this field. Thus the intensity of mildew present in the recent past of the crop was underestimated, and this may
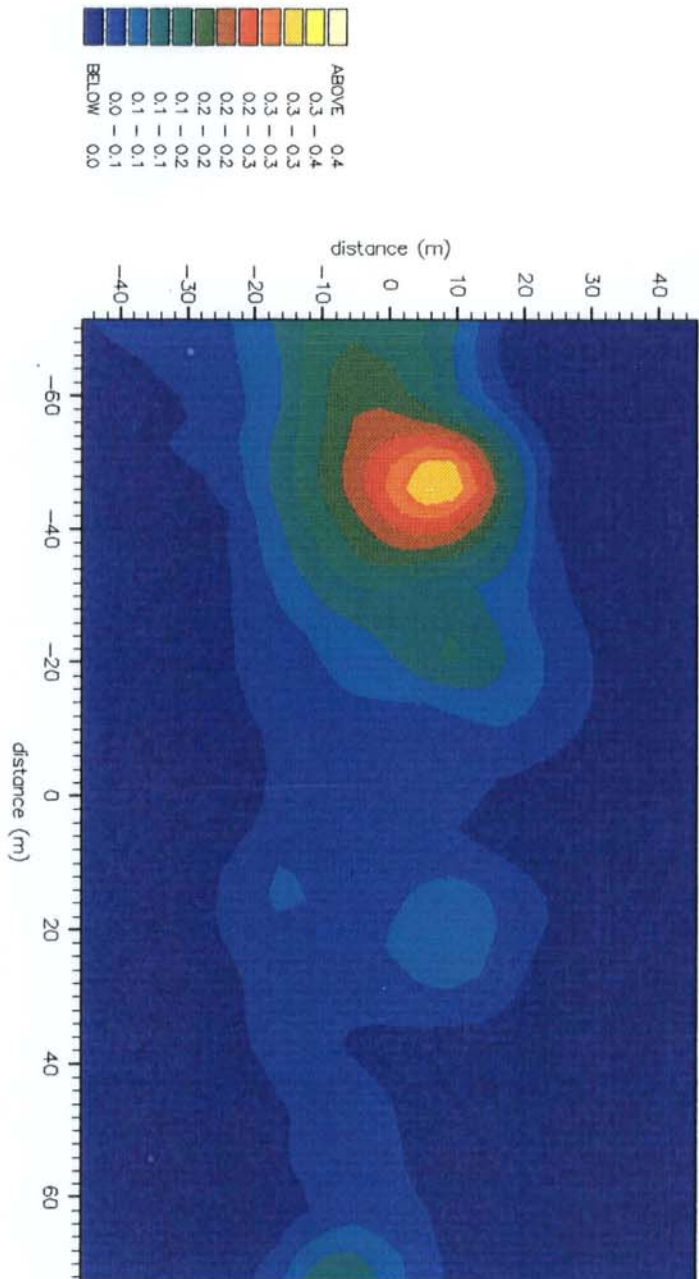
Fig. 7. Interpolated map (plotted using the Uniras V6 graphics package) of mildew severity on leaf 3 at GS31 in 1989 at Field C. Disease intensity is greatest along a strip running through the middle of the field which corresponded with a strip of plants that were more lush and vigorous than the rest of the crop.

have contributed to the violent fluctuations in semivariance.

Mildew was recorded at insignificant levels at GS31/33 in 1990.

Growth stage 59

Mildew was absent or at trace levels on the crops sampled at this growth stage.

(c) Yellow rust (*Puccinia striformis* Westend)

Yellow rust was detected at only one site during the course of the investigation. This was the conventionally managed field D in the season 1988/89. Several transforms were tried to stabilize the variance of which a *ln* transformed gave the best improvement for the data collected at both GS32 and GS59.

Growth stage 32

The disease was present at only trace levels on the 2nd fully expanded leaf and no significant autocorrelation coefficients were detected. This interpretation was supported by the semivariogram which also showed no evidence of spatial pattern.

Incidence was also low on the 3rd fully expanded leaf (leaf 7), being less than 2%. Significant correlations were detected to 100cm. The semivariogram for leaf 3 suggested spatial dependence was not present, and that semivariance fluctuated about an underlying constant value. However the map of severity for this leaf appeared to show the disease occurring in small clusters. Because severity was at low levels assessment errors may have confounded significant correlations at greater distances than 100cm and contributed an unknown variation masking trends in semivariance with distance.

Growth stage 59

At growth stage 59 the disease was present on the four leaf layers not completely senescent. On leaves 2 and 3 incidence approached 100% and was also high on leaf 4 at nearly 60%. Infection on the flag leaf was less acute with incidence below 2%.

Autocorrelation detected significant spatial dependence on leaves 2, 3 and 4. But the autocorrelation coefficient was close to zero for the flag leaf (Table 9). The longest distance over which correlations were significant was 1000cm on leaves 2 and 3. On leaf 4 spatial dependence was detected to only 310cm.

The significant correlation to 310cm on the 4th leaf was confirmed by the semivariogram plotted for this leaf. Along crop rows spatial dependence is evident to around 200cm, and across crop rows in the region of 500-600cm. In pooling the data for autocorrelation analysis the contribution of the directional dependence of spatial pattern is evened out to provide a general model for the whole field.

On leaves 2 and 3 where the disease approached 100% incidence the foci will have coalesced. In this situation the boundaries of an individual focus are not well defined and the spatial dependence of disease severity with distance becomes obscured. Whilst the autocorrelation analysis was unable to detect the underlying foci, semivariograms plotted for these leaves provided evidence of the presence of underlying foci. Semivariance increased to about 300cm where a constant value was reached; this value was maintained until between 700-800cm after which semivariance declined. This suggests that the typical size of the underlying foci was around 300cm and that the spacing between focal structures was around 500cm.

For the 2nd leaf the semivariance for data collected from across crop rows declined to the semivariance determined for separation distances of only 31cm, but this level was not quite reached for data collected from along crop rows. Semivariance began to increase again between 900-1000cm on the 2nd leaf.

In the case of the 3rd leaf the fall in semivariance did not reach the level detected for separation distances of 31cm, presumably because transects were not long enough to measure the complete decline. Any increase in semivariance at distances of separation in excess of 1000cm could not be detected.

Table 9. Percentage disease incidence and autocorrelation coefficients for yellow rust severity on 1st-3rd fully expanded leaf at GS32 and leaves flag–4th at GS59. Severity scores were *ln* transformed [*ln*(yellow rust severity + 0.0001)] prior to autocorrelation analysis.

Distance (cm)	GS32			GS59			
	7* 1.88%	6 trace	5 0	4 58.72%	3 90.77%	2 92.92%	Flag 1.85%
31	0.48****	0.04	-	0.44**	0.80******	0.87******	-0.07
100	0.28*	0.06	-	0.57*****	0.50****	0.70*****	-0.06
310	0.11	0.08	-	0.49***	0.67******	0.78******	-0.17
1000				0.31	0.64*******	0.69******	0.08

* 3rd fully expanded leaf at GS32

Asterisks denote significant correlation with $p \leq 0.025$(**) 0.01(***), 0.005(****) and 0.0005(*****) respectively

The spatial dependence defined to about 300cm by the semivariograms for leaves 2 and 3 are possibly the centres of the primary foci. This is plausible since 300cm is the same distance of spatial dependence detected for the 4th leaf on which disease incidence was lower and the spatial pattern presumably more clearly defined.

3.4.2 Analysis of pattern of stem based diseases

(a) Eyespot (*Psuedocercosporella herpotrichoides* (Fron.) Deighton)

Growth stage 31/33

In 1988 the disease was present at an incidence of 16.5%. The disease was scored as presence (1), or absence (0) in this year (*i.e.* incidence). These data were converted to percentage incidence of disease within each 5 plant sample and incorporated into the autocorrelation analysis. This analysis suggested spatial dependence was over only the shortest distance tested which was 31cm (Table 10). In the two subsequent seasons the disease was scored for severity.

At the fields tested in 1989 eyespot incidence was lower than in 1988. The organic fields B and C had disease levels below 5% (Table 10). This was not unexpected because of the way in which organic crops are managed: two year breaks with non-susceptible crops can reduce eyespot damage to negligible levels in the first wheat crop. Furthermore, the disease is favoured in crops which are lush due to high nitrogen input (Glynne & Slope, 1959). Incidence was slightly higher in the conventional Field D, at 8.5%. Several transformations were tried for normalizing the non-zero data of which a logit transform gave the best improvement. However, it should be noted that this was not ideal. Spatial dependence was not detected by autocorrelation analysis at any of the fields apart from at field C for 310cm. The correlation at this distance alone is not sensible and almost certainly arises as a consequence of the low incidence. Semivariograms plotted for the three fields sampled in 1989 also indicate that no spatial dependence was present.

Eyespot intensity was recorded at the highest levels in 1990. At field E incidence was nearly 20% and at field D in excess of 30% (Table 10). A logit was the

Table 10. Percentage disease incidence and autocorrelation coefficients for eyespot severity (or incidence where stated) at GS31/33. Severity scores were *logit* transformed prior to autocorrelation analysis.

Distance (cm)	Field A[a] 1988 *16.46%*	Field B 1989 *4.7%*	Field C 1989 *1.7%*	Field D 1989 *8.5%*	Field D 1990 *31.5%*	Field E 1990 *19.9%*
			Site, Year, Disease Incidence and Autocorrelation Coefficients			
31	0.29*	0.04	-0.07	0.13	0.56*****	0.56*****
100	0.06	0.07	-0.08	0.07	0.26*	0.36**
310	0.08	-0.02	0.40****	-0.09	0.29*	-0.20
1000	0.05				0.09	-0.16
3100	-0.25					

[a] Autocorrelation coefficients calculated for incidence data

Asterisks denote significant correlation with $p \leq 0.05$(*), 0.025(**), 0.01(***) and 0.0005(*****) respectively

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most successful of the transformations tried for normalizing the data.

Autocorrelation analysis indicated spatial dependence out to 100cm for field E and 310 cm for field D (Table 10).

The semivariogram for field E is in partial agreement with the autocorrelation coefficients, semivariance peaking at around 300cm (Fig. 8). There is no evidence of any difference in directional dependence along or across crop rows, suggesting the patches of disease were probably circular. In contrast the semivariogram for field D shows no evidence of any spatial dependence with distance. Semivariance fluctuates, apparently randomly, for both the directions plotted.

Growth stage 59/61

In 1989 eyespot was scored only for lesions penetrated to the stem, but in 1990 lesions present on the leaf sheaths were also included. However, only 0.8% of the disease incidence in 1990 was attributable to lesions not penetrated to the stem, so this is unlikely to have had a significant influence on subsequent analyses.

A logit transform again provided the best approximation of a normal distribution for both data sets.

In 1989 significant correlations were detected to 310cm ($p < 0.025$) though at 100cm the correlation was not significant. The semivariogram plotted for 1989 suggests that spatial dependence was not present along crop rows, but was weakly active across crop rows. Because this directional pattern was only weakly active any significant underlying autocorrelation at 100cm may have been confounded.

Autocorrelation coefficients calculated for the whole crop for 1990 indicated that disease levels were related to only 31cm, the shortest distance of separation ($p < 0.05$). The semivariogram constructed for these data indicates that spatial dependence was present along crop rows and across crop rows to the full length of the transects (Fig. 9). However autocorrelation coefficients calculated separately for along and across rows detected no significant correlations with the
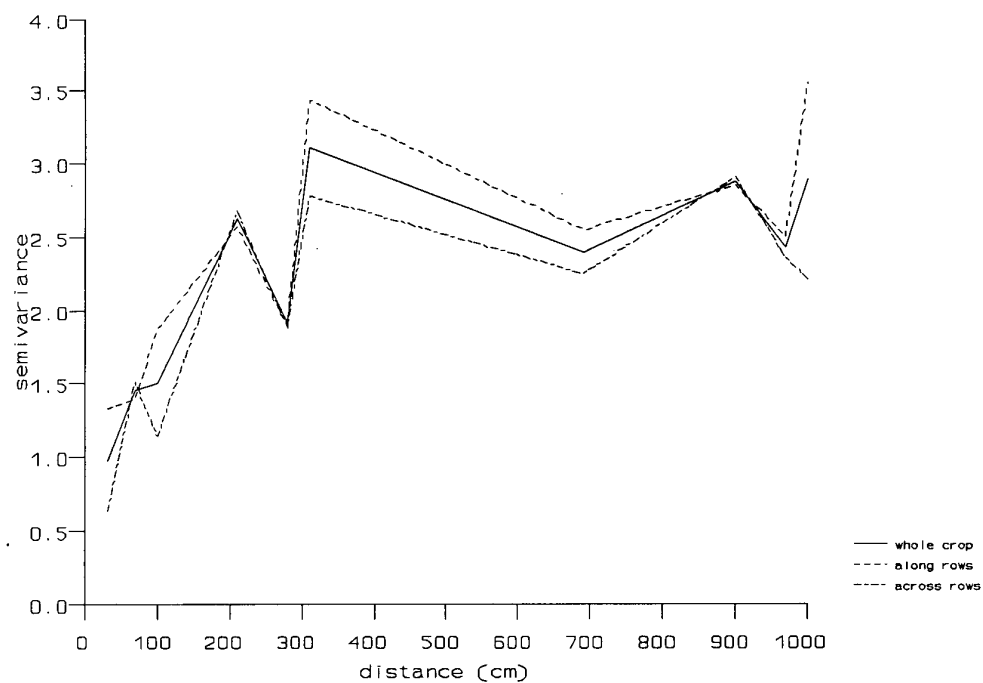
39

Fig. 8. Semivariance against distance for eyespot severity (logit transformed) for Field E in 1990 at GS33.
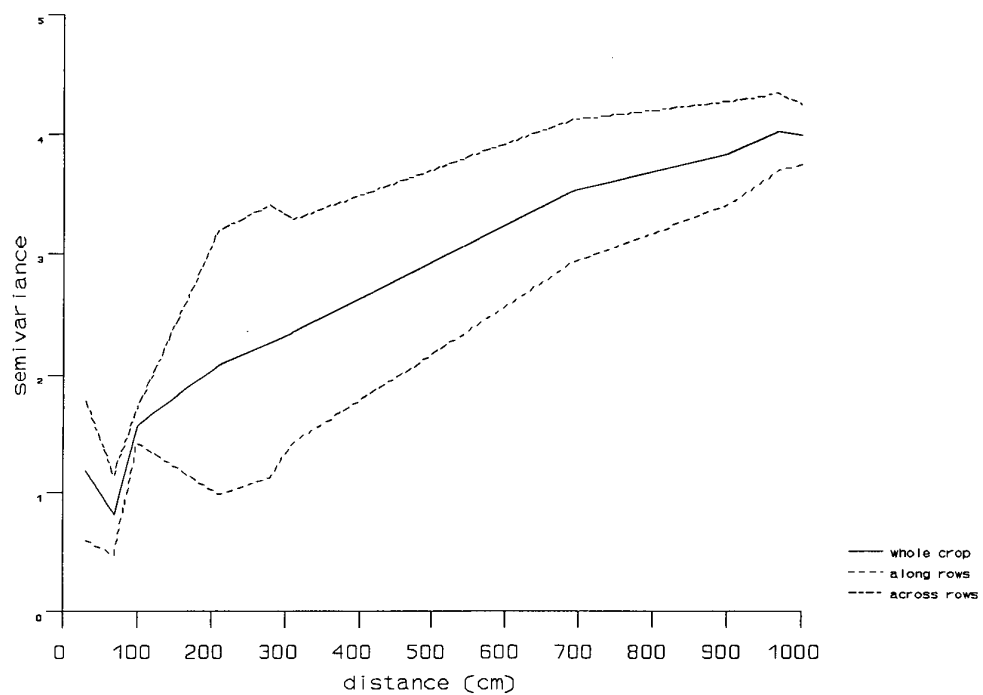

Fig. 9. Semivariance against distance for Eyespot severity (logit transformed) for field D in 1990.

exception of 31cm along crop rows ($p < 0.0005$).

Hoare (1987) found that infection of plants by eyespot decreased with increasing distance from an inoculum source experimentally introduced at GS11, for plants (cv Kador) grown in productivity beds. By GS60/63 only 16% of tillers were infected at a distance of 90cm from the inoculum source, compared with 30% at 45cm and 64% at 0-15cm. This provides evidence that spatial dependence acts over short distances.

(b) Sharp Eyespot (*Rhizoctonia cerealis* Van der Hoeven)

Growth stage 31/33

For seasons prior to 1990 sharp eyespot was measured only as incidence and these data were used in the autocorrelation analysis.

At field A in 1988 the disease was present at an incidence of approximately 10%. Autocorrelation analysis indicated significant spatial correlation only at 310cm which is biologically meaningless.

In 1989 sharp eyespot was insignificant at the organic fields with only trace levels at field B (0.8%) and absent at field C. Low incidence of the disease in organic crops is not unexpected since long rotations will inhibit the concentration of the inoculum. Incidence was approximately 10% at field D, but autocorrelation detected no significant spatial dependence to the epidemic.

In the season 1990 sharp eyespot was measured as severity, quantified by the amended assessment protocol described in section 5.2.

Significant spatial dependence was detected by the autocorrelation analysis at the two fields sampled out to 310cm ($p < 0.05$). However, the semivariograms constructed for the two fields did not show evidence of any biologically sensible spatial dependence.

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At field D the spurious correlations may have arisen as a consequence of the very low incidence level (2.4%). The same explanation may also account for the correlations detected at field E. but at this field incidence was relatively high at 12.4% and the correlation coefficients were very significant, declining in size as would be predicted for a strongly spatial dependent pattern.

Growth stage 59/61

In 1989 the incidence of sharp eyespot was high at field D at GS59: 68% of plants sampled having symptoms. Autocorrelation coefficients calculated for the sample were based on percentage incidence scores. No significant correlations were detected.

The same field sampled in 1990 at this growth stage was scored for disease severity. The overall incidence was much lower, at only 4%. Significant autocorrelation coefficients were detected out to 100cm ($p<0.05$). Semivariograms constructed for this field suggest that any pattern present was largely across rows. This was confirmed by the autocorrelation analysis, calculated separately for along and across crop rows. No significant correlations were detected along the rows, but across the rows significant correlations were detected at 31cm ($p<0.025$), 100cm ($p<0.01$) and 1000cm ($p<0.0005$).

The spread of the sharp eyespot pathogen is, to a large extent, limited by the ability of the mycelium to spread through the soil. Consequently, although over a number of years patches of concentrated inoculum might develop within a continuous wheat field ploughed at the end of each season, it is unlikely that individually these would be of significant area. However, when a crop is direct drilled the machinery will tend to pick up and move debris from the previous crop in the direction of the crop rows, eventually depositing it straddling across the new crop rows. This debris may therefore be in close proximity to developing seedlings, providing a potential substrate via which inoculum can reach the crop. Such a mechanism of transporting inoculum might provide an underlying disease pattern orientated across crop rows.

3.5 Conclusions

The novel application of spatial autocorrelation analysis based on transect samples proved to be an excellent research tool capable of providing fundamental information on the pattern of disease in winter wheat crops. Although alternative methods of analysis were able to provide strong suggestions about spatial pattern, the great advantage of the autocorrelation analysis was that it allowed valid significance tests to be used. Thus autocorrelation coefficients provided a rigorous means of testing any hypotheses suggested by the other analyses.

The spatial patterns of *S. tritici*, eyespot and sharp eyespot within crops of winter wheat were essentially random at GS31 and 59. Where spatial pattern did exist it was normally in patches of less than 3m.

The development of spatial pattern for *S. tritici* on a small scale was suspected to be due to patchy open crop structures, and during the three years of this investigation such crops were associated with low nutrient status; or in other words the organic crops.

There was no obvious reason for the spatial dependence detected over short distances for eyespot and sharp eyespot. It was suspected that, for sharp eyespot, pattern on this small scale might develop in direct drilled continuous winter wheat crops as a result of the concentration of inoculum. However, spatial aggregation on such a small scale ($<3m$) is of consequence only from a fundamental interest in the biology of the pathogen, rather than as a serious influence on the way that winter wheat should be monitored. In any case, further work is necessary to confirm the observation.

The absence of any large scale spatial pattern for these diseases has implications which have a convenient benefit for sampling strategies. Accurate and representative samples can be obtained by sampling the field at one or two locations chosen at random. In a patchy crop it is probably sensible to use several sampling locations, separated by around 5m, to ensure that small patches of disease are not sampled

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giving biased information.

Yellow rust was detected in only one of the crops sampled during the period of study. At GS31 the spatial dependence of the disease was only over very short distances. Thus a sampling pattern as described for *S. tritici* would probably be adequate. By GS59 the disease was spatially dependent over long distances. At this stage in a yellow rust epidemic there would be nothing to be gained for management purposes in making a sample. The disease would be evident from the side of the field and significant yield penalties would already be incurred.

Unfortunately, the development of mildew epidemics only occurred in 1989 at GS31 when short transects of only 310cm were used. The disease developed in patches of greater breadth than this so it was not possible to determine the scale of the disease pattern. For the two fields in which the disease was detected the autocorrelation coefficients were highly significant to the full length of the transects which suggests that the spatial scale was probably much greater than only 310cm. Previous work has shown that the incidence of powdery mildew on leaves of wheat progressed from distinct foci to a distribution that was more random as a result of dispersion of inoculum (Rouse *et al.*, 1981). However no indication of the scale of the aggregation was provided. Thus, a simple random sampling pattern may not be adequate for this disease.

The autocorrelation technique outlined has great potential as a method for obtaining detailed information of the spatial and temporal development of disease in crops. There is no reason for the observations to be restricted to visual disease assessments. With the introduction of quantitative immunological diagnostic kits (Miller & Martin, 1988; Unger & Wolfe, 1988) there may soon be need to devise protocols for sampling strategies appropriate to their use. The transect technique could provide a method by which such information could be obtained.

4. THE RELIABILITY OF VISUAL ASSESSMENTS OF DISEASE SEVERITY

4.1 The problem

There are many convincing models describing disease epidemics with respect to time, or in relation to the yield losses they cause. Since a large number of these models have been deduced from experiments in which disease severity has been estimated visually, there is some danger that observers may become complacent of their ability to make such estimates. Polley & Thomas (1991) point out some essential features of good disease assessment:

"Undertaking disease assessments at a single location has the advantage of ensuring uniformity of methodology and consistency of assessment standards. This greatly increases the accuracy of comparisons of disease levels between regions and from year to year. Use of training programmes for assessing disease severity such as DISTRAIN (Tomerlin & Howell, 1988) have recently become available and will further improve accuracy (*sic*)."

Currently there is little information available to show that, even taking the precautions suggested by Polley & Thomas, observers are able to attain acceptable levels of accuracy and precision. Furthermore, empirical tests of training programmes have yet to be undertaken to discover what knowledge and experience are needed by an observer before improvements in assessment reliability are achieved.

The following features relate directly to the reliability of visual disease severity assessments:

1.   The precision of individual observers and their consistency over different sampling dates,
2.   The comparability of assessments made by different observers,
3.   The type and extent of training needed to ensure similar results from different observers.

4.2 Procedures

During the study several experiments were done to address these aspects of the problem. It is beyond the scope this document to consider the experimental approaches in detail, but necessary to present an outline (for further details see Parker, 1991) .

Nine experienced observers from LARS were tested for the accuracy and precision of their severity assessments of *S. tritici* on seven occasions. Not all observers participated in every test. On a single occasion five of the same observers were tested for the reliability of assessments of powdery mildew.

For each test approximately 30 leaves, collected from field plots, were used. The disease severity range was typical of that found in the field: for *S. tritici* this was approximately 0.1-50% and for mildew 0.1-10%.

Objective disease assessments were obtained by tracing disease lesions onto acetate sheets from which their area was measured using an Optomax V image analyzer. Leaf areas were measured directly by the same machine. Subjective disease assessments were those provided by visual estimates of disease severity.

Precision, accuracy and bias of an observer on a given occasion can be described by using a least squares regression of subjective estimates on objective severity. The regressions can be compared across observers, or across occasions for the same observer. The existence of statistically significant differences between sets of data can be determined by comparing the variability remaining when each data set is fitted by a separate regression, when each has a common slope or intercept, or when all are constrained to have the same slope and intercept.

In practice logarithmically transformed data were used, both to give an even spacing to widely varying amounts of disease, and because almost all observers had linear regressions of subjective on actual severity after this transformation.

The ratio between the difference in the regression mean square for a given regression model and the residual mean square when each set of data was fitted separately provided F values that could be used to test various hypotheses. For example, did all observers perform as well on all occasions?, were the slopes for all observers constant between different occasions? *etc.*

The accuracy of disease severity estimates is determined by the closeness of the slope to unity and the intercept to zero (Fig. 10). The precision of the estimates is indicated by the coefficient of determination that is the percentage of variation accounted for by the regression (%VAF) as:

%VAF=100(1-(residual mean square)/(total mean square))

A perfectly precise assessment would provide a %VAF of 100%.

4.3 Results

None of the observers studied in this investigation achieved perfectly accurate assessments for *S. tritici* or powdery mildew on winter wheat. As an example, Fig. 10 shows the comparison with a perfectly accurate assessment of one observer's assessment regression for *S. tritici*.

The percentage of variation accounted for by the fitted regression for each date was high for all the observers on most occasions, suggesting that they were scoring to some determinant related to actual severity. Fitting a common slope, intercept or line to the observers' data on each occasion showed that the regression coefficients differed between individual observers, as shown for example in Fig. 11.

Similar analysis for individual observers over several dates indicated that they were not consistent in their estimates across occasions. This is illustrated in Fig. 12.

The apparent changes in observers perceptions across assessment dates can cause
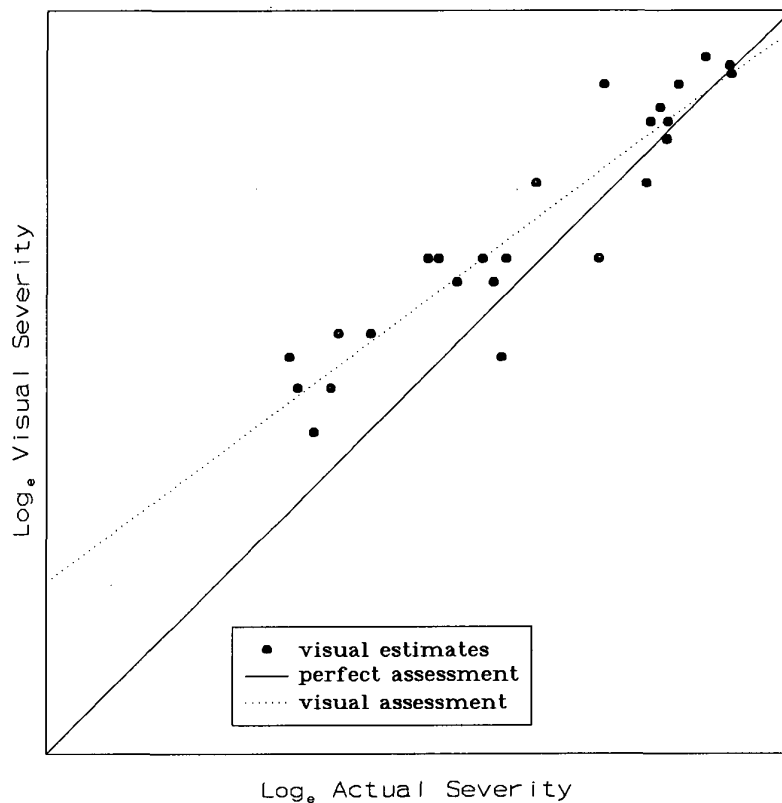
47

Fig. 10. Comparison of a perfectly accurate assessment with the fitted *S. tritici* assessment regression for an experienced observer on one occasion in April 1987

alarming discrepancies. For example, in July 1987 one observer was scoring 25% actual disease at 16%, but during June 1988 the same severity was being scored by the same observer at around 40%.

To summarize, experiments with real leaves indicated that:

1. Observers were not accurate in their estimates of disease severity.

2. However, the precision of an observers estimate was, in general, high during an individual set of assessments.

3. Unfortunately the size and form of the inaccuracy differed between observers.

4. Worst of all, the size and form of the inaccuracy was not consistent for an individual observer over time.
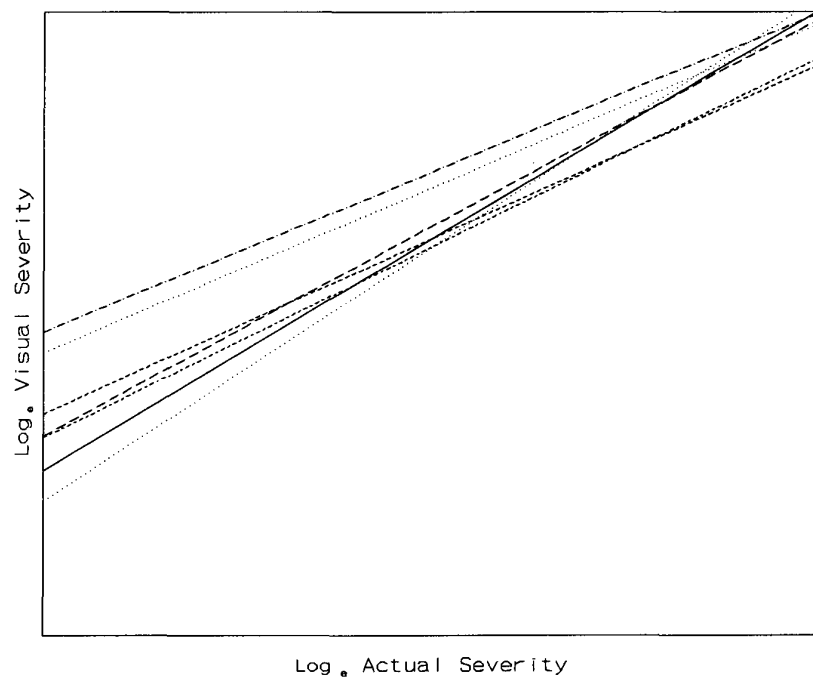
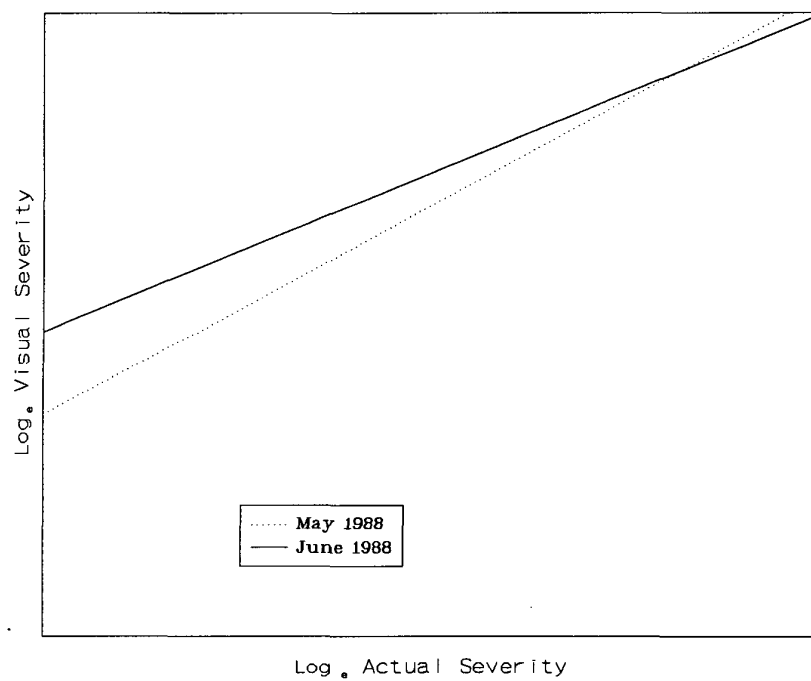**Fig. 11.** Comparison of 7 observers estimating the severity of *S. tritici* on a single occasion in June 1988

May 1988
June 1988

Fig. 12. Comparison of 2 assessments of *S. tritici* made 1 month apart by an experienced observer

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4.4 Need we be concerned?

In experiments in which the intensity of disease is compared between different treatments there will be concern if estimation errors lead to real differences between treatments being missed or spurious differences being detected. The potential for such errors is likely to be greatest where more than one observer is responsible for the assessments and may not be eliminated by the usual practice of blocking on observers. To investigate these problems further, a small trial for which leaves of all layers could be measured accurately was required. Conveniently, a glasshouse experiment to select mildew isolates resistant to a common fungicide seed treatment was available in which differences in disease levels in response to three doses of the fungicide were large. On the whole, observers were able to detect these gross differences, but the combined effects of lack of accuracy and precision were found to lead to the treatment responses remaining undetected for leaf layer 2 (Table 11).

This is not usually cause for serious concern where assessments of the kind used for primary screening of fungicides are made, or in the underlying investigation in this study where resistant isolates were being sought. However, the implications can otherwise be immense: for example in studying detailed epidemiology of pathogens, or in the accurate determination of recommended field rates of new fungicides.

4.5 What causes assessment illusions?

Three groups of model leaves of different area were used to investigate the reasons for observers illusions in disease assessment, and to enable groups of observers from other establishments to be studied. The model leaves were made of stiff cardboard and prepared using three templates traced from 3rd fully emerged leaves of winter wheat (*cv* Longbow) at different growth stages. Sixty leaves were made for GS39 and these were divided into two sets denoted A and A_1. Thirty leaves were prepared for GS30/glume primordia and for GS22-30/double ridge. Using a Rotring pen disease patterns typical of *S. tritici* were drawn in black ink on the models.

Table 11. Comparison by t-test of the severity of powdery mildew on individual leaf layers with respect to different rates of Ferrax applied as a seed treatment. The plants were glasshouse grown seedlings (cv Halcyon) and were assessed at GS12/13

1. Observer estimates of severity

	Leaf 1		Leaf 2		Leaf 3	
Control	15.6	a	65.3	a	7.6	a
FR[a]	13.5	a	22.7	b	6.3	b
2FR[b]	7.0	b	14.3	b	2.6	c

2. Objective measurement of disease severity

Control	Leaf 1		Leaf 2		Leaf 3	
FR	6.8	a	19.6	a	14.5	a
2FR	6.8	a	7.2	b	2.6	b
	2.1	b	3.6	c	1.3	c

[a] Ferrax applied at field rate & [b] at twice field rate

Four important conclusions were drawn from this part of the investigation.

Firstly, there was no relationship between the assessment regression intercept and leaf area for most observers. Nor was the slope of the assessment regression linked to leaf area. This is not therefore the explanation for differences between observers estimates on different occasions when examining real leaves.

Secondly, neither accuracy nor consistency of disease severity estimates appear to be improved by experience. This should not be surprising because when making disease assessments observers are not provided with any feedback about their success. It is known that this impedes learning.

Thirdly, it was found that the model leaves were generally assessed more precisely than real leaves. This has important implications for training. The use of standard area diagrams as tests in training programmes might instil unjustified confidence in

the ability of an observer. Moreover, there must be some concern that disease assessment keys which provide diagrams of high contrasting black lesions against a light coloured background are fundamentally flawed.

The fourth point concerns the consistency of observers' precision. Work described earlier showed that plant pathologists should be concerned about the lack of accuracy and consistency with which disease severity assessments are made. If the precision of the disease estimate is high an observer will at least be able to distinguish small differences in disease severity, and thus treatment response on each assessment occasions. This poses a dilemma for researchers making disease assessments because rapid assessments were the least precise. Conventional wisdom recommends that as many observations as possible are made to provide the best estimate of mean and variance. Yet, if observers are obliged to make vast numbers of observations they are likely to make them rapidly.

The relationship between precision and speed of assessment needs further investigation. If the relationship proves to be strong it is clear that observers should be given generous allocations of time to make assessments. However, this is a luxury that is unlikely to be available. The need for treatment replication and large sample size which are inherent to good experimental design conspire to force observers to make large scale assessments. Further investigation may show that by requiring observers to make large numbers of estimates within strict time constraints, researchers risk sacrificing the reliability of their data.

4.6 Could better training help?

The effectiveness of the DISTRAIN computer programme (Tomerlin & Howell, 1988) was also investigated, albeit briefly.

Use of the programme did not successfully train two novice observers to provide accurate disease assessments. Moreover, there was no evidence that the assessment precision of the two observers was any better than achieved by observers trained by more traditional methods.

Comparisons of the effectiveness of three levels of training, namely: none, conventional training by a colleague and use of key, and DISTRAIN, were also made. Observers trained by one of the most widely used training protocols (*i.e.,* by colleague & assessment key), were no more reliable than observers with no training and no experience.

Although observers trained by DISTRAIN were not accurate in their assessments, it was encouraging to find that the regression models describing the observers were the same shortly after training. However, this comparability was short-lived: by three weeks after training the two observers were no longer comparable.

The comparability of the observers' assessments shortly after training may have been a consequence of chance. However, if the improved comparability was really due to the training provided by DISTRAIN, perhaps the programme could be used regularly or prior to severity estimates being made by observers engaged in the same experiment. Further work is necessary to confirm this.

4.7 Methods of disease assessment not reliant on visual severity estimates

The assumption is normally made that visual disease symptoms are highly correlated with the amount of fungus infecting the plant. Whilst the logic behind this is immediately appealing for diseases such as powdery mildew, the same is not necessarily true for pathogens that are more invasive of the host tissue. To test this fundamental assumption with respect to infections of *S. tritici*, a chemical method was used for estimating the amount of pathogen present in experimentally inoculated juvenile leaves of glasshouse grown winter wheat plants (*cv* Longbow).

The chemical assay used was based on that described by Ride & Drysdale (1971,1972). The assay relies on the fact that chitin is not found in plant tissue but is a principal component of the cell walls of fungi.

No strong correlation was evident between the visual symptoms of disease severity

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measured objectively by image analysis and the amount of chitosan extracted from the leaf (Fig. 13). The variance of the visual severity measurements was stabilized by means of a cube root transform. The least squares regression of these transformed data against chitosan weight confirmed the evidence of the plot; although association was detected by the regression ANOVA ($p < 0.001$) the coefficient of determination of 17.5% indicated that the fit was very poor.

Conventional wisdom recommends that *Septoria* diseases on cereal leaves be assessed only as the area covered by pycnidia. While this may effectively preclude senescence not attributable to the effects of the pathogen being included in the assessment, it may underestimate the extent of pathogen development in the leaf due to hyphal growth. Despite this it is impossible to argue with any conviction that the current method of assessing such diseases should be altered to include adjacent senescence. Investigation has shown that the disease is generally overestimated in any case. This overestimation may to some extent ameliorate the deficiencies in the protocol with respect to unseen disease.
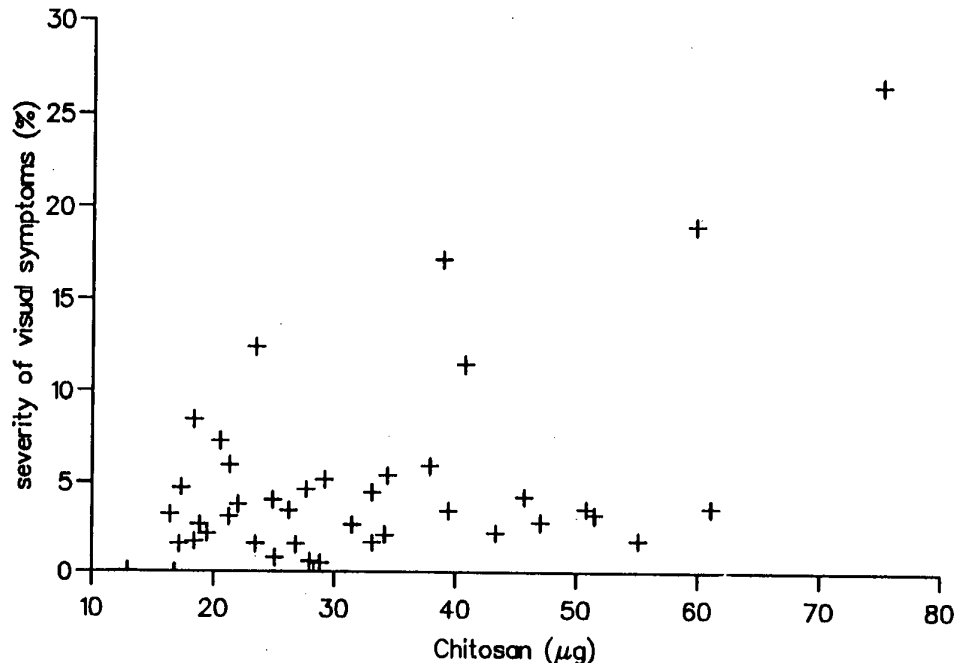

Fig. 13. Relationship between the percentage severity of visual symptoms of *S. tritici* measured objectively on individual leaves and the weight of chitosan extracted from the leaf as a measure of the amount of fungus actually infecting the leaf.

5. A NEW KEY FOR FOLIAR DISEASE ASSESSMENTS AND AN AMENDED SCALE FOR STEM BASE DISEASE ASSESSMENTS

5.1 An alternative disease assessment key for foliar disease

Although published disease assessment keys were made available to observers for the assessment tests for real disease, their use was never imposed. This was partly because it is impossible to guarantee that such an imposition is being followed, but primarily because there seemed little point in insisting on the use of keys if the majority of observers do not use them in the real situation. Evidence provided by the disease monitoring survey, and casual observation of the assessment tests suggested that assessment keys are not widely used. The reason for this may be that observers are generally sceptical of the effectiveness of such keys.

An obvious drawback of assessment keys is the need for considerable interpolation by the observer. The keys are prepared as generic leaves usually drawn to a scale smaller than the real leaf. The figurative lesions provided by the keys attempt to represent the typical pattern of disease on a real leaf for a range of severity. But in a sample of diseased leaves the typical pattern will probably not be seen and the severity on each leaf will be at a level between those identified by the key. Thus the observer is expected to interpolate from the assessment key factors of scale, lesion pattern and severity before giving an estimate of the disease severity on the real leaf. Complicating the problem still further for general field assessments is the need for more than one key to account for the variety of diseases present in the crop.

The observation that observers are able to assess disease on the high contrast model leaves more accurately than on real leaves must provoke some concern that the design of conventional assessment keys is flawed.

To address some of these problems a new key has been developed, a prototype example of which is shown in Fig. 14. The key is simple in design and based on a leaf divided into 1% sectors. It is proposed that future versions could be smoothed to provide a more realistic leaf shape. The principal advantages of the new key are:

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1. that it reduces the need for observers to interpolate by avoiding the need for typical disease patterns and example severities

2. that it is not reliant upon high contrast diagrams which observers apparently find easier to discriminate between than real disease severities

3. that it avoids the need for a number of different keys for assessments of more than one disease.

In the longer term it is envisaged that the key could be incorporated into a computer software package. By providing simple information to the program such as the average length and breadth of the leaves to be assessed the generic leaf could be recalculated and the resultant image printed to a suitable hardware device. Thus providing the opportunity to obtain a key relevant to the crop variety, leaf layer and growth stage for every disease assessment done.

Unfortunately the key has not yet been tested for its effectiveness in improving the disease estimates of observers. However the design appears to have an intuitive appeal to a number of plant pathologists experienced in making disease assessments.

5.2 An amended scale for the assessment of stem base diseases

A scale applicable for use in rating stem base disease severity throughout the season has not been reported in the literature. Thus stem base diseases may be measured using unrelated schemes during the period of an investigation. For example Hoare (1987) used three protocols for the measurement of eyespot severity. Winter assessments were done using a 0-5 scale, and in spring a scale running from 4 (lesion penetrated to the 4th leaf sheath) to 7.4 (lesion penetrated through all 6 leaf sheaths and girdling stem completely). A complicated 'disease score', having limits between 0-100%, was calculated from May onwards. Stem lesions were graded 0 to 4 depending on stem girdling:

0 = no lesion on stem
1 = stem 1/4 girdled
2 = stem 1/2 girdled

3 = stem 3/4 girdled

4 = stem fully girdled

The disease score (*ds*) was determined as:

$$ds = \%\text{tillers } 4 + 0.75(\% \text{ tillers } 3) + 0.5(\% \text{tillers } 2) + 0.25(\% \text{ tillers } 1)$$

Clearly this provides an underestimate of disease present in the sample if a significant proportion are infected only on the leaf sheaths. Such infections must have a detrimental effect on the plant by interfering with the vascular transport system, but in the protocol described above this effect is not recognized. A modification of the method was therefore made to rectify the defect in the scale making it appropriate for use throughout the season.

The assumption is made that, on average, each plant will have 13 leaves. However, of these only 10 will be at risk of infection when the diseases become active in spring, the other 3 having sloughed-off due to senescence. Hence we can attach notional severities with respect to the whole plant for infection of both the stem and the 10 leaves at risk:

Disease severity							
5%	**10%**	**15%**	**20%**	**25%**	**50%**	**75%**	**100%**
leaf 9	leaf 7	leaf 5	leaf 3	Stem 1/4 girdled	stem 1/2 girdled	stem 3/4 girdled	stem fully girdled
leaf 10	leaf 8	leaf 6	leaf 4	Flag leaf leaf 2			

No account is made of the vertical spread of disease along the stem. However, for the purposes of most assessments this is not really of interest. The adverse effects of stem base disease are a consequence of the weakening of the stem (potentially causing lodging), and the interruption of the flow of water and nutrients between the roots, leaves and ear. Both symptoms are made more severe by increase in the girdling of the disease around the stem.

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Based on leaf 3 (from flag) of cv Longbow
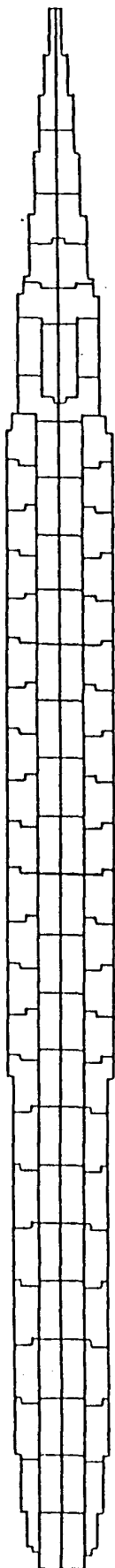
Each sector is equal to 1%

Fig. 14. Prototype of a new assessment aid proposed to avoid some of the disadvantages associated with the use of conventional disease assessment keys.

RECOMMENDATIONS

The recommendations of this study fall into two areas, both of which must be acted upon if the maximum benefit from disease monitoring in winter wheat is to be achieved. The first area contains considerations and advice that are applicable now, whilst the second area highlights the questions that require further experimental research.

1. Improving disease monitoring

(a) Disease monitoring is widely practised. However, the accuracy of this monitoring is probably not optimized by most practitioners. Clearly defined monitoring protocols need to be described that can provide information appropriate to the requirements of the industry. These protocols must be widely adopted and to achieve this they must be well publicized.

(b) A random sampling pattern is probably adequate for monitoring winter wheat. However, where conditions are favourable to the development of mildew, a W shaped sampling pattern will probably provide the most reliable information about the level of disease in the crop.

(c) Sample size has a large influence on the reliability of the information obtained about the disease status of a crop. Despite this there is rather laissez faire attitude adopted towards this factor by many involved in disease monitoring. On the field scale the inspection of anything less than 50 plants is probably of very limited value. Sample sizes greater than this are more accurate.

(d) The assessment of disease severity (*i.e.,* the percentage of leaf area with symptoms) is a difficult task even for experienced observers. Currently those involved in disease monitoring receive, at best, a very limited form of training in making assessments. This suggests that most observers are probably unaware of the factors that can lead to grossly inaccurate measurements. Disease assessment aids currently available are not widely used. Better assessment aids are needed that have practical appeal to users, so that they will be used as a matter of routine.

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(e) There is evidence that DISTRAIN, a computer based training aid, improves the comparability of the disease assessments made by different observers. However, the programme may have to be used on a regular basis, possibly before each assessment, if it is to confer this improvement. The use of such a programme in plant pathological research would be possible. The benefits of comparable disease assessments out-weigh the cost of the time needed to implement such a training programme.

2. Further work

(a) The present study has provided a powerful technique for the experimental study of disease development in cereal crops. Using this technique comprehensive information about the major pathogens of winter wheat could be collected. This would enable reliable and efficient monitoring protocols to be devised. More widely, the information obtained would also improve greatly our knowledge of the epidemiology of these pathogens.

(b) A new assessment aid, devised through this study, is untested for its effectiveness in improving visual assessments of diseases of winter wheat. The key was designed with regard to the aspects of disease assessment implicated to be responsible for assessment errors. A similar aid, but for use with another crop system, has been shown to provide improvements in disease assessment reliability.

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SURVEY OF THE USE OF DISEASE ASSESSMENTS IN CONTROL STRATEGIES

1. **JOB DESCRIPTION** (please tick)

   Farmer/Farm Manager

   ADAS Advisor

   Independent Advisor

   Sales Advisor

   Field Trials Officer

   Research Scientist .... HSO or above

   .... below HSO

2. **HOW OFTEN DO YOU MAKE VISUAL ASSESSMENTS OF CEREAL DISEASES?**

   Often   Occasionally   Rarely   Never

3. **WHAT IMPORTANCE DO YOU THINK SHOULD BE PUT UPON SUCH ASSESSMENTS WHEN MAKING DISEASE CONTROL DECISIONS?**

   Much importance   Some importance · No importance

4. **HOW WOULD YOU RANK THE FOLLOWING INFLUENCES ON DECISIONS ABOUT DISEASE CONTROL ACTIONS? NUMBER THE MOST IMPORTANT 1, THE SECOND 2 ETC.**

   Tank mix convenience

   Weather conditions

   Growth stage of crop

   Disease present in crop

   Visual disease symptoms at ADAS threshold level

5. **HOW ACCURATELY DO YOU THINK YOU CAN RECOGNIZE DISEASES WITHIN CEREAL CROPS?**

   Very accurately   Fairly accurately   Inaccurately

6. HOW ACCURATELY DO YOU THINK YOU CAN ESTIMATE THE AMOUNT OF DISEASE ON A CEREAL LEAF?

Very accurately   Fairly accurately   Inaccurately

7. WHICH DISEASES DO YOU MOST COMMONLY ENCOUNTER IN YOUR CEREAL CROPS?

8. WHICH OF THESE DO YOU THINK IS THE MOST IMPORTANT TO ECONOMIC LOSS?

9. DOES THE IMPORTANCE OF THE DISEASE SPECIFIED BY YOU IN QUESTION 8 INFLUENCE THE RANKING OF CONSIDERATIONS IN QUESTION 4; IF SO HOW?

10. WERE YOU TAUGHT TO SCORE DISEASE SEVERITY?   Yes   No
IF YES, WHAT MANNER DID THIS TRAINING TAKE? (please tick)

Diagrammatic assessment keys
By a colleague
By a formal training course
Other (please specify)

11. DO YOU USE AN ASSESSMENT KEY?

Always   Occasionally   Never

12. WHAT TYPE OF ASSESSMENT KEYS DO YOU USE *E.G.*, ADAS: YELLOW RUST

13. HOW MANY PLANTS DO YOU NORMALLY ASSESS FROM A FIELD? ....

14. WHERE IN THE FIELD ARE THE PLANTS YOU ASSESS SITUATED? (please tick)

(i) All from the same area

(ii) At random throughout the field

(iii) From along a particular walk pattern; if so what type:

/

X

W

(iv) Other (please specify)

15. HOW OFTEN DO YOU ASSESS A FIELD DURING A TYPICAL SEASON?

16. ARE THESE ASSESSMENTS DONE AT SPECIFIC GROWTH STAGES, IF SO WHICH?

17. PLEASE USE THIS SPACE IF THERE ARE ANY SPECIFIC POINTS YOU WISH TO MAKE ABOUT ANY OF THE QUESTIONS IN THIS SURVEY, OR FOR ANY GENERAL COMMENTS ABOUT SAMPLING AND MONITORING DISEASE.