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The sensitivity of the ordinary runs test for evaluating the spatial pattern of infected plants

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Abstract. *The influence of disease incidence, clumping of infected plants, and size of the sampling unit on the sensitivity of the ordinary runs test was simulated in order to identify the optimum sampling profile for investigating the spatial pattern of plant diseases. A simulation programme was written to generate plant populations with random and clustered spatial patterns displayed in quadrats of 50 by 200 plants. 600 and 1200 independent populations were generated for random and clustered patterns, respectively. 12 levels of disease incidence were simulated within the range 0.01-0.95. For every population, the simulation performed a sampling procedure with 9 sizes of the sampling unit (20, 30, 40, 50, 60, 80, 100, 120 and 150 plants). In each case the simulation was based on 1000 samples of continuous series of plants. In order to evaluate the sensitivity of ordinary runs test to the degree of aggregation, plant populations were simulated with two additional values of clumping power, for two levels of disease incidence. When a random pattern was simulated, the probability of rejecting the null hypothesis was almost unaffected by the size of the sampling unit and slightly decreased with disease incidence. When clustered patterns were generated, the probability of error clearly decreased both with disease incidence and size of the sampling unit. The probability of error was also affected by the degree of aggregation. As expected, the higher the clumping power the higher the probability of rejecting the null hypothesis. The implications of the sensitivity of the runs test on the design of sampling schemes are discussed.*

Introduction

Taylor (1984) stated that "spatial distribution is one of the most characteristic ecological properties of species". A simple but important principle in biological sciences is that organisms are not equal in number at all locations and that organisms are not equally associated with others of the same population at all locations (Madden 1988). Epidemics of plant disease vary both in time and space (Campbell et al. 1984, Madden et al. 1987a, 1987b and 1988). It is widely accepted by plant pathologists and epidemiologists that the spatial component of plant disease epidemics is as important as the temporal component (Madden et al. 1987b).

Pathogen movement can result in heterogeneous distribution of the disease within the affected crop (Madden 1988, Reynolds et al. 1988, Reynolds and Madden 1988, Campbell and Madden 1990, Madden et al. 1990a). As Campbell and Madden (1990) states, "*spatial pattern* in plant pathology can be defined as the arrangement of disease entities relative to each other". Spatial patterns may be classified into three categories, namely 1) uniform, 2) random, and 3) clustered or clumped (Madden and Campbell 1986, Southwood 1978). In a uniform pattern there is a regular arrangement of infected and healthy plants. "Random" means that all distinguishable arrangements of infected and healthy plants are equally possible, or, in other words, that in every point in the crop there is the same probability of a plant being infected. In a clustered pattern, every plant in the field does not have an equal probability of being infected so that a diseased plant increases the probability of nearby plants being infected (Campbell and Madden 1990).

Information on the spatial pattern of pathogens and plant diseases can be useful in various ways. The identification of disease patterns in the field can be used to develop an efficient sampling plan. Generally, greater aggregation requires larger sample sizes. The analysis of spatial patterns enables a better understanding of the dynamics of virus disease epidemics and determination of the mechanics of disease spread (Madden and Campbell 1986). For example, a random pattern of infected plants would suggest that the pathogen does not spread within the stand, i.e. no secondary transmissions occur. This observation would draw the attention of researchers to certain types of analytical models to describe the dynamics of disease epidemics (van der Werf et al. 1988, van der Werf and Riesbos 1990). Although most plant disease epidemic models assume a random pattern of disease, the need for incorporating aggregation into the appropriate equations has been pointed out by Madden (1988). Considering that studies of spatial pattern are relevant for disease epidemics, researchers should know the statistical properties of the tests they use to make spatial characterizations.

A frequently used method to evaluate the spatial distribution is the ordered sequence of plants (Madden and Campbell 1986). This type of tests was used to study the relationship between epidemic characteristics and spatial distributions, like the interaction between initial aggregation and initial disease level. Although ordered sequence techniques usually have a straight-forward interpretation, some disadvantages were identified. Madden et al. (1982) evaluated ordinary runs, original doublets, and corrected doublets for detecting the spatial pattern of plant virus diseases through simulation of random and clustered patterns. They found that the ordinary runs test was the most satisfactory in terms of misclassification chance. No clear relationship was found between frequency of rejection of the null hypothesis of randomness and disease incidence. However, both in their field and simulation data, the sampling unit always consisted of 100 plants. The question remains whether sampling size can affect the robustness of the ordinary runs test. In the field, various biological processes, like differential dispersal rates or secondary transmissions, could certainly give rise to different degrees of aggregation or "clumping powers". Madden et al. (1982) simulated clustered spatial patterns by assigning a single conditional probablity of a plant becoming infected on the infectious status of the immediately preceding one (p=0.75). This means that only one clumping power was simulated. We do not know whether this is a representative figure, neither do we know if such clumping power affects the outcome of the test. If there were plant diseases with different aggregation trends, ordinary runs test might have differential sensitivity to them. In this paper we explore the influence of incidence, clumping power of infected plants, and size of the sampling unit on the sensitivity of the ordinary runs test in order to determine the consistency of the test under different circumstances.

Ordinary runs test

As an example, consider the following pattern of 10 symbols representing a crop row with diseased (+) and disease-free (-) plants: + - - - + + - - + +. There are five runs in this example. Reading left to right, the runs are '+' , '- - -' , + +', '- -' and ' + +'. Given a random sequence of diseased (+) and healthy (-) plants, the expected number of runs (E(U)), can be calculated as

$$E(U) = 1 + \frac{2m\,(N - m)}{N}$$

where m: number of diseased plants, N: total number of plants in the sample, and U: number of runs. The standard deviation of U is given by

$$s(U) = \sqrt{2m(N-m)\frac{2m(N-m)-N}{N^2(N-1)}}$$

The observed number of runs will be less than E(U) if there is clustering of diseased plants and this can be tested using a standard normal deviate calculated as

$$Z = \frac{U - E(U)}{s(U)}$$

A value of Z < -1.64 indicates clustering (P < 0.05) (Campbell and Madden 1990).

Materials and Methods

Simulation study

A simulation program was written to generate plant populations with random and clustered spatial patterns displayed in a grid of 50 X 200 plants. For standard-grown corn crops, the area of simulated populations represents aproximately 0.18 hectares (near 1000 m^2). The source code list (QuickBasic 4.5) is available upon request to DEG.

Random pattern was simulated by setting the same probability of each plant in the grid becoming diseased, independent of any other plant in the population. In this case, the simulation algorithm started by the random selection of a coordinate pair (x, y) and asked whether a diseased plant should exist at that location. The algorithm used the simple rule of comparing a random number (RN, range 0-1) with 0.5; if RN < 0.5, then a diseased plant would occur at (x, y); otherwise, it would not. The procedure was repeated until the proportion of diseased plants in the simulated field reached the selected incidence value for the simulation (see below).

The representation of a clumped pattern of diseased plants through simulation is a more complicated process as it can be achieved in different ways. Basically, it depends on the distribution, shape, size and number of patches of diseased plants and the distribution and density of diseased plants within each patch. The simulation of aggregated patterns used the most parsimonious set of assumptions, where patches of diseased plants were located at random (according to a uniform probabilistic distribution) within the 50 X 200 simulated 'arena', patch size (square shape) varied randomly (uniform distribution) between 25 (=5 X 5) and 225 (=15 X 15) plants and diseased plants were located within the patch according to a 'clumping power rule' and the particular incidence value simulated. The clumping of diseased plants within the patch was determined by the probability (p) of a plant being diseased if the previously simulated plant was infected. If p were 0.5 the arrangement of diseased plants within the patch would be strictly random, so 'clumping powers' of 0.6, 0.75 and 0.9 were selected for the study. The algorithm first randomly selected the location of a diseased plant patch and its size; afterwards, using the same procedure as in the random pattern procedure, it selected the location of diseased plants within the patch comparing a random number with the clumping power (p), so that if RN <p, a diseased plant would occur. The procedure was iterated until the proportion of diseased plants in the simulated population reached the selected incidence value.

The simulation scheme defined four types of diseased plant populations: one type with infected plants arranged according to a strictly random pattern and three types with infected plants arranged according to a clustered pattern, with clumping powers of 0.6, 0.75 and 0.9. Within each type, 12 disease incidence levels (within the range 0.01 - 0.95) were simulated. Each incidence was in turn replicated 50 times. Thus, the simulation study comprised 2400 (50 X 12 X 4) populations. Whithin each population replicate, the simulation performed a sampling procedure with 9 sampling unit sizes (20, 30, 40, 50, 60, 80, 100, 120 and 150 plants). For each size class the program took 1000 independent (with reposition) samples. Each sample consisted of a continuous series of plants along a row. The row and first plant of the series were selected at random. When the location of the first plant did not allow taking a complete sample (i.e. was placed near a border) a new location was assigned.

Once the whole set of simulations had been run and all the samples had been taken, the program calculated the frequency of cases (out of 1000 samples) in which the null hypothesis of randomness was rejected (significance level a=0.01). The average frequency (over the 50 replicates) of rejections of the null hypothesis was calculated for each combination of the simulated variables:

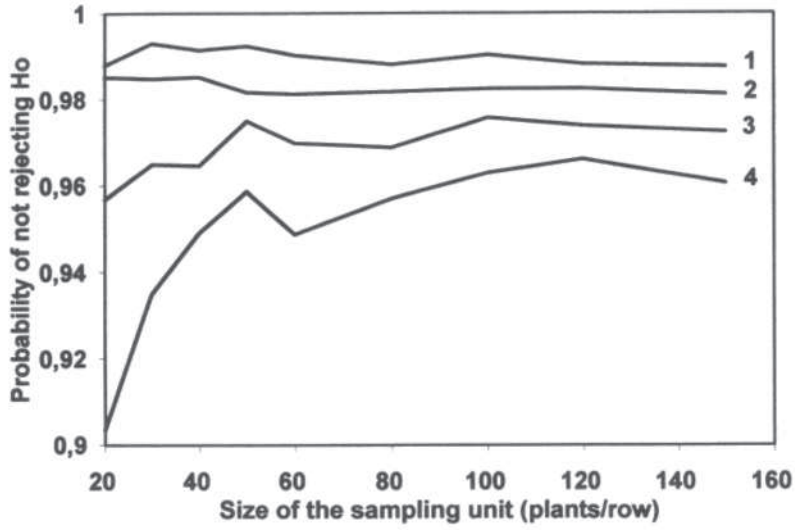

Figure 1. Probability of correct identification of the spatial pattern by the ordinary runs test in relation to the size of the sampling unit (number of plants per sample), when the sampled population has a random arrangement. Probabilities were estimated as the relative frequency of not rejecting the null hypothesis of randomness. Lines 1 to 4 represent disease incidence levels y=0.9, y=0.5, y=0.2, and y=0.01, respectively.

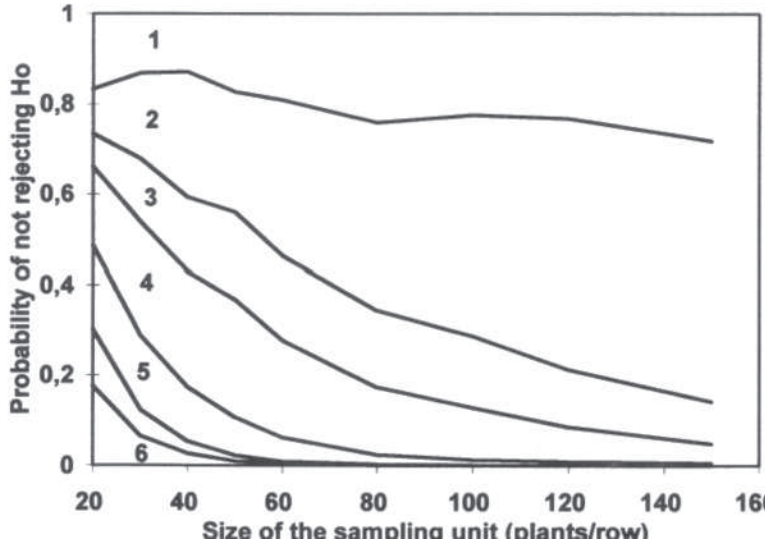

Figure 2. Probability of incorrect identification of the spatial pattern by the ordinary runs test in relation to the size of the sampling unit (number of plants per sample), when the sampled population has an aggregated arrangement (p=0.75). Probabilities were estimated as the relative frequency of not rejecting the null hypothesis of randomness. Lines represent the simulation outcomes for different sampling unit sizes and disease incidences. Lines 1 to 6 represent incidence levels (y)= 0.05, 0.2, 0.3, 0.5, 0.7 and 0.9.

spatial pattern (two levels), disease incidence (12 levels), 'clumping power' (three levels) and sampling unit size (nine levels). The null hypothesis (H_0) of the runs test states that a number of observed runs is not significantly lower than the expected number of runs obtained from a randomly distributed population, and the alternative hypothesis (H_1) states that the observed number of runs is significantly lower than the expected number of runs obtained from a randomly distributed population. Under this simulation study, H_0 will be true when a random pattern of diseased plants is simulated, whereas H_1 will be true when a clumped distribution is simulated. As in every hypothesis test, there are two possible wrong decisions when H_0 is evaluated: to reject H_0 when H_0 is true (Type 1 error, with probability α; in the present case estimated as the proportion of H_0 rejections out of 50 replicates when a simulated random pattern is analyzed), and not to reject H_0 when it is false (Type 2 error, with probability β; in the present case, estimated as the proportion of not rejection of H_0, out of 50 replicates, when a simulated clumped pattern is analyzed). It is worth noting that $1-\beta$ represents the power of the test.

Results

Random simulated pattern data. The probability of Type 2 error (no rejection of the null hypothesis of random pattern) was not affected by the size of the sampling unit, except for a small influence for levels of disease incidence of 0.2 or lower, and slightly increased with disease incidence (Fig. 1). The highest and lowest probabilities of rejecting the null hypothesis when a random pattern was simulated were 0.095 and 0.003 (9.5% and 0.3%), respectively.

Clustered simulated pattern data. The probability of Type 2 error decreased both with disease incidence and size of the sampling unit (Fig. 2). The probability of Type 2 error was also affected by the clumping power. As expected, the higher the clumping power, the lower the probability of not rejecting the null hypothesis (Fig. 3).

Discussion

Our results on simulations of random spatial patterns are similar to those obtained by Madden et al. (1982). However, there were important divergences among these studies when the results of the runs test were compared with simulated clustered patterns. Indeed, while the frequency of misclassifications estimated by Madden et al. (1982) was 5 %, our estimations ranged between 0 % and 80 %, depending on disease incidence, 'clumping power', and sample size. The procedure followed by Madden and coworkers to generate clustered patterns automatically produces a distribution of disease incidences from 30% to 70%. Then we constrained the comparison to those levels of disease incidence and length of the sampling unit actually shared by both studies. Fig. 3 shows that in our simulation study the probability of Type 2 error obtained for incidences from 0.3 to 0.7 and a sampling unit of 100 plants, ranged from aproximately 0% to near 13%, similar to the result obtained by Madden et al. (1982).

Field data on the spatial pattern of Rio Cuarto Corn Disease in Argentina were obtained using the ordinary runs test (Trumper et al. 1996). During the 1990-91, 1992-93 and 1993-94 growing seasons, the null hypothesis of randomness was rejected in 10%, 30% and 0% of the samples, respectively, yielding a general rejection frequency of 11 %. During the 1990-91 season, rejection of the null hypothesis occurred along a wide range of incidence values. When data sets from the last two seasons were pooled, neither incidence nor the length of sampling unit affected the results of the runs test significantly, but the samples identified as clumped corresponded to long sampling units.

According to the findings of Madden et al. (1982), if Río Cuarto Corn Disease were randomly distributed, no samples should be misclassified as clumped, but in the field work by Trumper and coworkers (1996) the null hypothesis was rejected in 11 % of the cases. In contrast, if the disease had a clumped spatial pattern, the spatial pattern would be missclasified as random in no more than 5 % of

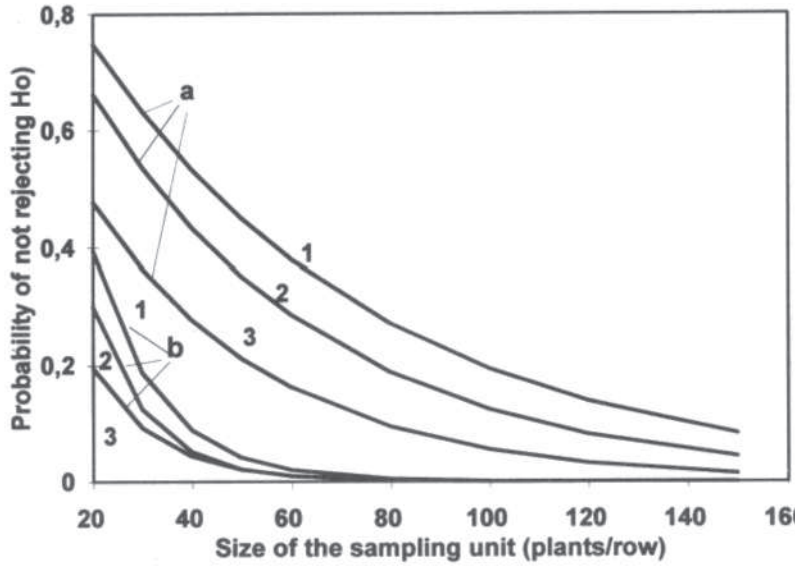

Figure 3. Effect of the clumping power (p) and incidence (y) on the probability of not rejecting the null hypothesis of randomness (runs test). Probabilities were estimated from the simulation study (see text for full explanation). Lines a and b correspond to incidence levels y=0.3 and y=0.7, respectively. Lines l, 2 and 3 correspond to clumping powers p=0.6 p=0.75 and p=0.9, respectively.

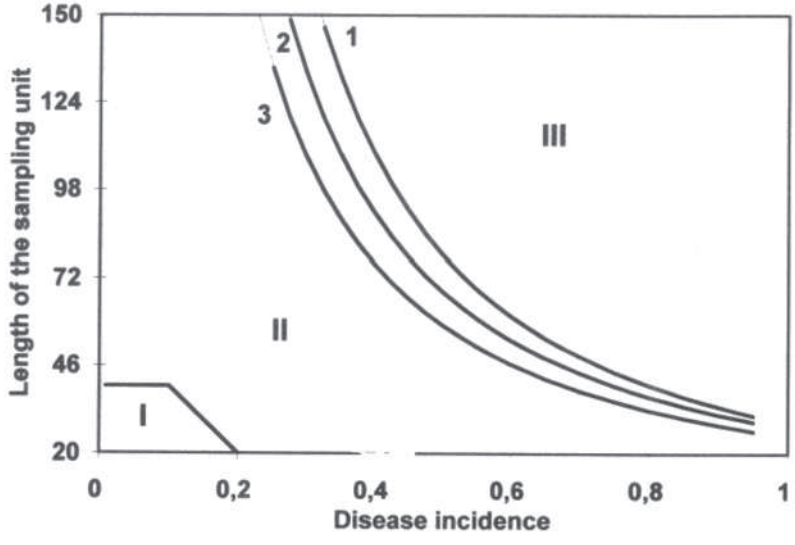

Figure 4. Combination of disease incidence and length of the sampling unit delimiting sectors of different α and β probabilities. Four isoclines of probability of error below 0.05 were calculated from the simulation data, one of them under the assumption of random spatial pattern of diseased plants and the other three under the assumptions of 3 different clumping powers (p=0.6, 0.75 and 0.9). Sectors I through III represent α and $\beta > 0.05$, $\alpha < 0.05$ and $\beta > 0.05$, and $\alpha < 0.05$, $\beta < 0.05$, respectively.

the cases, while in the field work this frequency was 89%. Field results on the spatial pattern of Río Cuarto Corn Disease show much more agreement with the hypothesis of randomness.

The frequencies of random cases in the field data (89%; Trumper et al. 1996) were very close to those obtained in our simulation of random patterns (90-99.9%) and clearly higher than those obtained in the simulation of clustered patterns (0-80%). The first observation indicates that the spatial pattern of Río Cuarto Corn Disease falls just within the limits of randomness. The second one, strongly suggests that the disease has a random spatial pattern.

The results of this simulation experiment should be considered cautiosly because of restrictions imposed by the simulation algorithm used (especially on the representation of the aggregation pattern, as other rules are possible giving similar spatial arrangements). However, the different pieces of evidence pointing into the same direction reinforce the conclusions to make them reliable.

Implications for sampling schemes

The simulation of random spatial distribution of diseased plants showed that, when disease incidence is low, there is no point in increasing the size of the sampling unit because this has almost no influence on the outcome of the runs test. For very low levels of disease incidence, sampling units of more than 40 plants seem a sensible choice. If the disease had a clustered spatial pattern, then it becomes crucially important to select the size of the sampling units according to the expected disease incidence. For very low disease incidence (0.05 to 0.1), increasing the length of the sampling unit does not increase the reliability of the test accordingly. In this case it seems convenient to distribute the time budget available for sampling many small units rather than a few large ones. When disease incidence is high, the slope of the relation between the probability of error and the size of sampling unit (Fig. 3) is steeper at short sampling units. Thus, significant reductions in the probability of error could be gained with small increases in the length of the sampling unit. However, the longer the sampling unit, the lower the slope of the curves will be. So it seems appropriate not to break the time budget into too small sampling units because they have a high probability of error associated. Neither should the units be too long as only very slight improvements would be achieved. The ideal size of the sampling unit lies somewhere in between.

Obviously, if there is interest in studying the spatial pattern of a plant disease using the ordinary runs test, whether this pattern is random or clustered is unknown before making any sampling. So, how can the researcher decide on the best sampling scheme? It is frequently recommended that every sampling program of any living organism or remains of its activity, should be based on knowledge about the mean and the variance of the population under study. These parameters can only be estimated by preliminary sampling (Southwood 1978). In the case of plant diseases, the preliminary sampling would provide the researcher with an estimation of disease incidence, which would help in selecting the most convenient size of the sampling unit. Obviously, it would not be wise to rely on this preliminary sampling to decide whether the disease has a random or clustered pattern and select the best sampling unit accordingly. When the spatial pattern is random, disease incidence has only a very slight impact on the performance of the runs test. On the contrary, for clustered patterns, the outcome of the runs test is crucially dependent on disease incidence. Consequently, it seems appropriate to decide upon the size of the sampling unit assuming the spatial pattern is clustered and having preliminary estimates of disease incidence.

We have already mentioned that the probability of Type 2 error depends on incidence and size of the sampling units and that for a given disease incidence, increasing sample size reduces the probability of Type 2 error. From a more general viewpoint, reliability of the test can be delimited identifying the conditions under which α and β fall below 0.05.

Figure 4 is a phase diagram of disease incidence and size of the sampling units delimiting three sectors given by different probabilities of type I and type II errors (α and β, respectively). In sector I, the probabilities of type errors I and II (α and β) are greater than 0.05. In sector II, α is <0.05, but β is > 0.05. Only in sector III are α and $\beta < 0.05$. Thus, if the researcher is interested in investigating the spatial pattern of a plant disease using ordinary runs test, sampling units should be selected as to fall within sector III.

Although the simulation study presented here provides a better understanding of the sensitivity

of ordinary runs test, another source of uncertainty remains which seems much more difficult to tackle. We showed that the probability of error is strongly affected by the clumping power of the infected plants. The lower the aggregation force, the less reliable the test is rendered. Unfortunately, preliminary sampling would not assist in estimating this clumping power, which could only be determined through either specific experimental studies or direct estimation in the field, of the conditional probability of a diseased plant being adjacent to another diseased one.

Figure 4 summarizes the conclusions of this paper: The higher the disease incidence the smaller the sample size required for $\beta < 0.05$ and the higher the disease incidence the smaller the impact of the clumping power of the infected plants. The recommendation that derives from these statements is to select sampling units long enough to fall within sector 111.

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