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# Analysis of the spatial spread of esca in some Tuscan vineyards (Italy)

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**Summary.** Four vineyards in Tuscany [GTFI at Gambassi Terme, cv. Sangiovese; CBSI-1, 2, 3 at Castelnuovo Berardenga, cv. Sangiovese (1), cv. Trebbiano (2) and various cultivars (3)] were examined for esca over periods of 4 (CBSI-1, 2, 3) or 6 years (GTFI). A high level of discontinuity in the symptoms expression of each diseased plant was observed from year to year. The cumulated disease incidence, calculated by counting all plants exhibiting symptoms at least once during the entire 4 to 6 year test period, was 49.09% at Gambassi Terme and an average of 13% for the 3 vineyards at Castelnuovo Berardenga. Analysis of the field data by three indices of dispersion (Lloyd's index of patchiness, variance-to-mean ratio and Morisita's index) and ordinary runs tests showed occasional aggregation of diseased vines only in vineyards with higher disease incidence (GTFI and CBSI-3). The results of two-dimensional distance class and correlation analyses (2DCLASS and 2DCORR) indicated a significant spatial correlation of infected-infected plant pairs in the GTFI vineyard both along and across columns. For CBSI-3 (19 columns, one cultivar for column), however, the results indicated a tendency for infected vines to be aggregated along columns. The remaining 2 vineyards (CBSI-1 and 2) consistently exhibited a random spatial pattern of diseased vines. This result suggests that the down-column contagion found for CBSI-3 may merely be a byproduct of cultivar dependent susceptibility to disease. On the whole, the results obtained suggest that in the vineyards examined esca was spread by airborne spores from distant and/or internal sources rather then by contaminated pruning tools along the vine columns.

**Key words:** grapevine, esca, epidemiology, spatial pattern.

## Introduction

Over the last few years, under the impulse of a real esca emergency, numerous studies on this disease have been carried out. The results so far, while they have elucidated some aspects of the aetiology, epidemiology and physiology of the disease, have also demonstrated the extreme complexity of esca. For example, various factors seem to justify the hypothesis that the three most important fungi linked to esca, *Fomitiporia punctata* (*Fop*), *Phaeoacremonium chlamydosporum* (*Pch*) and *Phaeoacremonium aleophilum* (*Pal*) acting either together or one after another, are responsible for at least three different syndromes: brown streaking in rooted cuttings, Petri grapevine decline (or black goo) and esca, which in turn is subdivided into: young esca, white rot and esca proper (Graniti *et al.,* 1999; Graniti *et al*., this issue). However, there are many points pertinent to this hypothesis and other aspects of the disease that still await clarification. For example, it is still not possible to predict ex-

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actly how long a time period after infection must elapse before external symptoms appear (primarily chlorosis and necrosis of the leaves). The initial onset of symptom-expression is highly variable and infected plants may show or not show any external symptoms in successive years for a number of years (Hewitt, 1957; Mugnai *et al*., 1996, 1999). For some infected plants years with symptoms alternate irregularly with asymptomatic years. In such a haphazard epidemic it is simply impossible to tell when a particular infected plant actually became infected. For all of these reasons, we chose to estimate the real incidence of esca in a vineyard by cumulating observed incidence over a number of years. It is hoped that by using this approach all infected vines, including those newly infected, will eventually be identified (Mugnai *et al.,* 1996; Surico *et al.,* this issue).

Two other still open questions are the primary introduction of the disease into the vineyard and the secondary spread. According to the above-mentioned complex hypothesis on the nature of esca and its origin, the disease may be spread by infected propagation material. Alternatively the vine plant can be infected with *Pch*/*Pal* and/or *Fop* as soon as it begins to be pruned but the classic symptomatology of esca (esca proper, as defined by Graniti *et al.,* 1999) generally does not appear until the plant is mature (8-10 years or older). As for the secondary spread of esca in the vineyard, it is usually stated that esca spreads more easily along columns, being transmitted by pruning tools. In that case a striped pattern of diseased plants will be seen along columns. If the disease were introduced from a source outside the field, on the other hand, there would be a random or uniform pattern; while spread from internal sources would tend to produce an aggregated or clustered pattern of diseased plants.

It is well known that spatial analysis may be used to provide information on inoculum sources and the spread of plant pathogens. During the last 50 years much time and effort has been expended by epidemiologists on quantifying and interrelating measurements of the spatial properties of diseased plants in a plane. Besides simple indices of dispersion or aggregation, more complex forms of statistical analyses have also been developed. These are, for example, geostatistics, spatial-spatiotemporal autocorrelation analyses and others (Matherson, 1963; Gray *et al*., 1986; Reynolds and Madden, 1988; Nelson, 1995, 1996; Gibson, 1997). In the present study, different forms of statistical analysis, some simple and some more complex, were applied to interpret the spatial pattern of esca-diseased vines in four vineyards in Tuscany. The results obtained are reported in this paper.

## Materials and methods

### Vineyards and data collection

Vineyard GTFI at Gambassi Terme (Florence) and vineyards CBS-1, 2 and 3 at Castelnuovo Berardenga (Siena) were surveyed for incidence of esca; the findings are described in a separate paper (Surico *et al.,* this issue). One of the vineyards that were examined in that study, SCFI, at San Casciano Val di Pesa (Florence), had to be excluded from the statistical analysis here because of the great number of vacancies and the high disease incidence.

The data on esca incidence were used to generate two-dimensional maps of the spatial patterns of diseased vines (see Figures 2, 3, 4 and 5). These maps show, for each vineyard, all the vines that presented external symptoms at least once during the survey period of 4 years (1995-1998) for CBS-1, 2 and 3 and 6 years (1993-1998) for GTFI. To facilitate application of the statistical analyses (other than the ordinary runs analysis), some rows at the top and/or bottom, and in some cases the outermost column were removed from the original, irregularly shaped maps and the disease incidence calculated according to the new, rectangular maps.

### Data analysis

To examine aggregation of adjacent vines and more complex spatial relationships over longer distances we used three different indices of dispersion: Morisita's index ($I_\delta$) (Morisita, 1959), Lloyd's index of patchiness (LIP) (Lloyd, 1967) and the variance-to-mean ratio (VM) (*in* Campbell and Madden, 1990), as well as ordinary runs analysis (Madden *et al*., 1982) and two forms of two-dimensional distance class analysis (Gray *et al*., 1986; Nelson and Campbell, 1993; Ferrandino, 1996, 1998).

To compute the indices of dispersion, the vineyards were divided into quadrats of different sizes, with increasing numbers of vines depending on

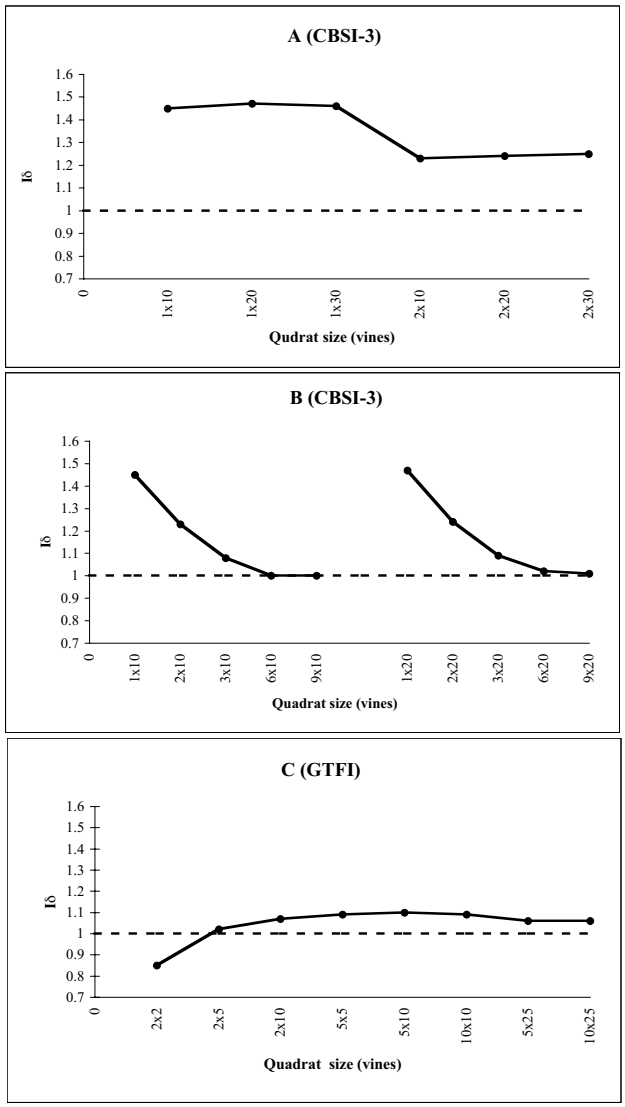


Fig.1. Morisita's index of dispersion plotted against a series of quadrat sizes with increasing numbers of vines. A and B, Castelnuovo Berardenga; C, Gambassi Terme. Value 1 indicates a random pattern.

the configuration of the different vineyards. The quadrats were laid out along the columns or along the rows. Index values less than 1 indicate a uniform pattern, a value of 1 indicated a random spatial pattern, and values greater than 1 indicate an aggregated pattern (Campbell and Madden, 1980).

Ordinary runs analysis, a one-dimensional form of spatial autocorrelation especially suited for detecting the spread of a disease between adjacent plants in one direction (along rows or columns), was performed separately for each column of each vineyard and by joining adjacent columns in each vineyard to form a single continuous column. A nonrandom pattern (i.e. aggregation) of diseased plants was assumed for a particular column or for joined columns if the observed number of runs was less than expected at $P$=0.05 (for $-Z_u$ values greater than 1.64). A run is defined as a sequence of one or more like elements preceded and followed by unlike elements. If diseased plants in a column result from a pathogen spreading from plant to plant there will be a clustering of infected plants and a clustering of healthy plants, and thus few runs. If the pathogen does not spread from plant to plant there will be a random mixing of diseased and healthy plants, and thus a greater number of runs.

Two-dimensional distance class analysis (2DCLASS) was used to characterise the spatial pattern of plants with esca disease symptoms. The 2DCLASS software program developed by Nelson and Guzman (1997) was used to analyse the data from each vineyard under study.

For spatial autocorrelation analysis the X, Y spatial location of grapevine plants in each vineyard were used as input data, and the number of [X, Y] distance classes [the number of horizontal (X) and vertical (Y) unit moves that separate a pair of plants from each other] in which the observed standardised count frequency (SCF) was significantly greater than or less than expected (confidence limit on the level of significance $P \leq 0.05$ and $P \geq 0.95$ respectively) under the null hypothesis of randomness was calculated. Expected count frequencies were determined by 400 computer simulations. Data sets were interpreted as having nonrandom spatial patterns if the total number of significant SCFs was 5% or more of the total distance classes. Two or more adjacent [X, Y] distance classes with SCFs significantly greater than or less than expected formed a cluster. The core cluster was defined as a group of adjacent [X, Y] distance classes with SCFs significantly greater than expected that formed a discrete group contiguous to the origin (distance class [0, 0]). Reflected clusters on the other hand were contiguous, discrete groups of [X, Y] distance classes with SCFs significantly greater than expected and discontiguous with the origin, the core cluster, or both. Row effects represented the maximum number of significant and

adjacent SCFs per data set in an X direction, column and diagonal effects in a Y and a diagonal direction respectively. Edge effects were interpreted as significant if more than 15% of the distance classes in the edge effect region (Xmax and Ymax of the distance class analysis matrix) had SCFs values that were significantly greater than expected. For within-row and across-row effects adjacent to the [X, Y] distance class [0, 0], a value of 1 was added to the total number of adjacent and significant SCFs.

The spatial pattern of esca-diseased vines was also subjected to two-dimensional correlation analysis (2DCORR), an approach developed by Ferrandino (1996, 1998), which is based on the same principles as 2DCLASS in that it considers interplant orientation and distance inside the field, but uses a different procedure to calculate the "expected" infected pairs (I-I) in each distance-orientation class. With 2DCORR the expected values are calculated analytically using the observed data, not stochastically as with 2DCLASS. 2DCORR reduces the probability of falsely claiming significant deviation from random behaviour and so in the final results the appearance of "reflected clusters" is reduced, and the detection of short range correlations ("core clusters") is enhanced. To reduce the risk of considering false positive results (TYPE1 ERROR) due to the many comparisons (n) that are necessary to execute these types of analysis, Ferrandino also applied the significance test at higher levels of confidence $\alpha=1-(1-0.05)^{1/N}$ instead of $\alpha=0.05$: using N = the total number of comparisons, one obtains the Bonferroni confidence limit; if N is the number of comparisons at the same distance or less than the distance class being tested, we obtain the proximal confidence limit.

To identify a spatial correlation between infected plants when the 2DCORR analysis results are symmetrical to the origin**,** it is possible to calculate for each distance r ( r=1, 2, 3 …n plants) the corresponding fraction of the total number of infected plants [It(It-1)/2]. Each of the values obtained is then compared with the expected value calculated with 2DCORR analysis for each of the distance classes. In general, if infected plants really tend to cluster, then a large fraction of the overall number of infected plants will be found to be located at small distances. The difference between observed and expected infected pairs is then calculated at each distance ($\Delta$) and the $\Delta$max value is evaluated with the Kolmogorov-Smirnov test (level of significance $\alpha=0.05$). If this value is statistically significant the corresponding distance r is the measure of the distance, in all directions, of the correlation between infected plants.

## Results

The observed range of disease symptoms varied greatly over all the vineyards and included plants with only foliar symptoms (chronic esca), plants with foliar symptoms and part of the canes with wilted bunches, wilted plants (acute esca), and dead plants.

Cumulated disease incidence (%) was 49.09, 9.93, 12.01 and 17.55 for GTFI, CBSI-1, 2 and 3 respectively. GTFI, the vineyard most affected, with 244 diseased plants out of 497, had a block of esca-infected vines roughly located in the first 10 rows of vines (Fig. 2, middle). There was an even larger block of infected plants from row 27 to 50. A lower number of more sparsely distributed diseased vines was identified in the middle upper part of the vineyard (from approximately row 11 to 26). In CBS-3, which had 1031 standing vines, the 181 diseased vines were more or less uniformly distributed through the vineyard with only small clusters of contiguous diseased vines along some columns or rows (Fig. 5, middle). No recognisable patterns of esca-diseased vines were found at all in CBS-1 (49 diseased vines out of 493) (Fig. 3, top) or 2 (56 diseased vines out of 466) (Fig. 4, middle).

Ordinary runs analysis of the esca incidence data in GTFI found that columns two (29 out of 51 plants infected, 15 runs) and eight (28 out of 58 plants infected, 22 runs) contained a nonrandom distribution of esca-diseased plants ($P \leq 0.05$). The probability of a nonrandom distribution of infected plants in the remaining columns on the other hand was greater than 0.05 (Table 1). The same proportion of columns with a nonrandom distribution of infected plants was found in CBSI-3: here there were 4 columns with nonrandom infections, 3, 5, 12 and 18, out of a total of 19 columns. Of these, column 3 had 18 infected plants out of 73, 21 runs; column 5, 20 infected plants out of 73, 23 runs; column 12, 13 infected plants out of 70, 18 runs; and column 18, 3 infected plants out of 72, 5 runs. Two columns with a nonrandom distribution

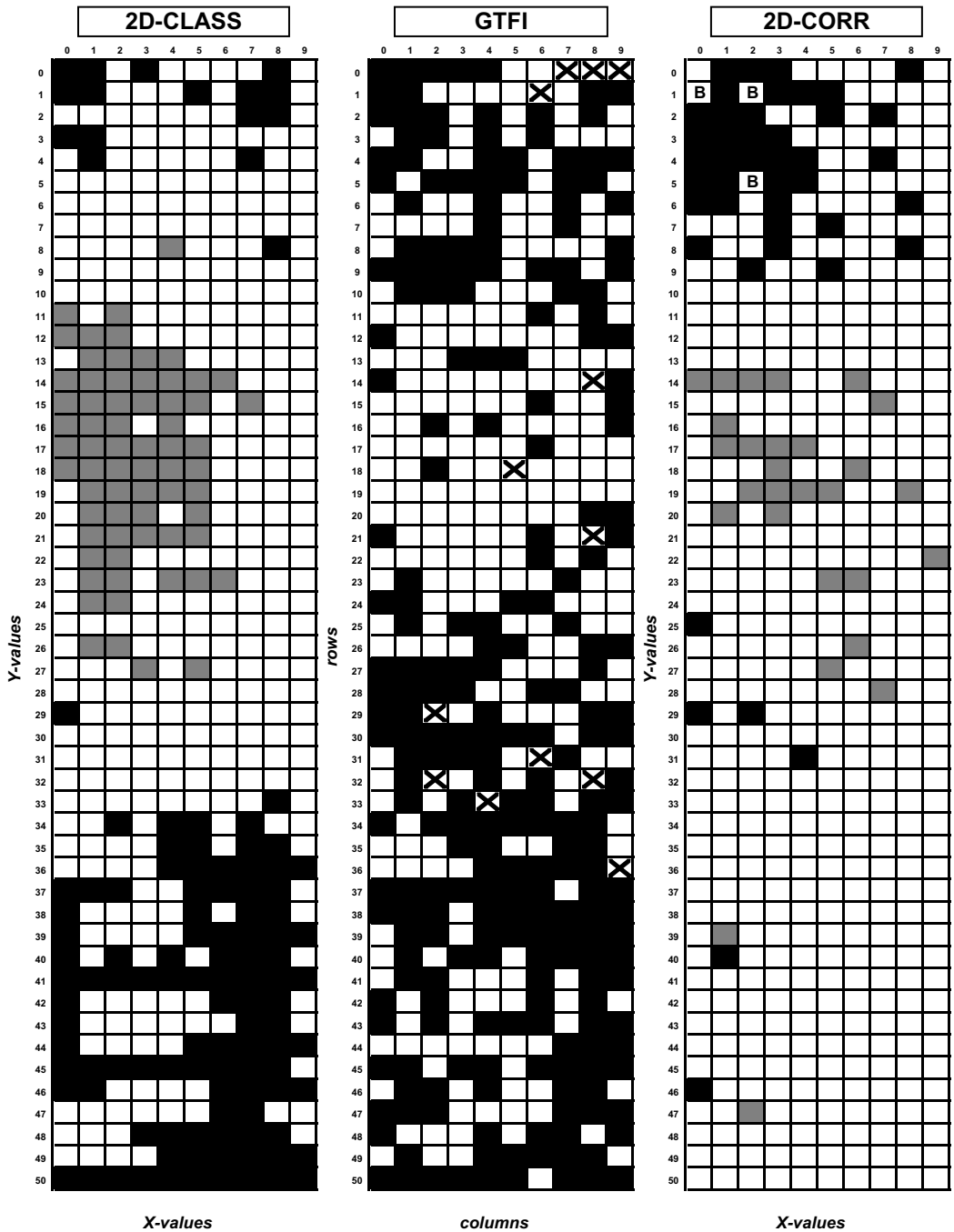


Fig. 2. Middle: spatial pattern of esca in a vineyard at Gambassi Terme, Tuscany. Black squares, white squares and squares with "**X**" indicate position of esca-diseased vines, healthy plants and missed vines before 1993 respectively. Left: proximity pattern matrix from two dimensional distance class analyses (2DCLASS). Black and gray squares indicate [X, Y] distance classes with greater ($P \leq 0.05$) and less ($P \geq 0.95$) then expected standardized count frequencies (SCF) respectively. White squares indicate [X, Y] distance classes with SCFs as expected. Right: two dimensional correlation analysis (2DCORR) of esca-diseased vines of vineyard GTFI. Black and gray squares indicate significantly greater and less than expected observed pair counts respectively. Squares with "B" indicate significance at the Bonferroni level of probability ($P \leq 0.00196$). White squares indicate observed pair counts as expected.

Table 1. Ordinary runs ($Z_U$) analyses of the aggregation of esca infected vines in four Tuscan vineyards.

| Location | Test period | Incidence[a] % | | No. of columns tested | Proportion of columns with a significant $Z_U$[d] | |
|---|---|---|---|---|---|---|
| | | V=1[b] | V=2[c] | | V=1[b] | V=2[c] |
| Gambassi, GTFI | 1993-1998 | 48.8 | n.d.[e] | 10 | 0.20 | n.d.[e] |
| C. Berardenga, CBSI-1 | 1995-1998 | 9.77 | 13.53 | 19 | 0.105 | 0.052 |
| C. Berardenga, CBSI-2 | 1995-1998 | 11.46 | 16.76 | 19 | 0.105 | 0.105 |
| C. Berardenga, CBSI-3 | 1995-1998 | 16.40 | 29.42 | 19 | 0.210 | 0.421 |

[a] Cumulated disease incidence.
[b] Missed vines before the beginning of the test period recorded as symptomless.
[c] Missed vines before the beginning of the test period recorded as diseased.
[d] Number of columns with a significant aggregation / number of columns tested.
[e] Not determined because of the low number of vacancies.

occurred in CBSI-1 (6 and 11) and in CBSI-2 (10 and 19) but these columns had very few diseased vines (from 1 to 5) and therefore few runs. Aggregation of esca-diseased vines along columns was thus detected by ordinary runs analysis in all the vineyards [due to the small number of vines along rows, less than 20 in all the vineyards examined, the ordinary runs analysis was not performed along rows, but only along columns]. However, the percentage obtained in the tests along columns indicated that aggregation was low even in the two vineyards with the highest disease incidence, GTFI and CBSI-3. CBSI-3 showed a higher proportion of columns with a nonrandom distribution of infected plants only when missed plants before the first year of observations were considered in the analysis to be diseased (Table 1). Aggregation in all the vineyards was also observed when adjacent columns were combined to form one continuous column, but this was probably because of the large sample size with combined columns, as was found by Madden *et al*. (1987). These findings indicated that in general esca-diseased vines did not greatly enhance the esca-infection risk of immediately adjacent vines along columns.

At a higher level in the hierarchy of spatial analysis the clustering of esca-diseased vines was also analysed by three indices of dispersion, $I_\delta$, LIP and VM, using quadrats of different sizes. It should be stated that Upton and Fingleton (1985) found that values of $I_\delta$ and LIP were numerically similar when calculated for the same data.

For CBSI-1 and CBSI-2 the values of these indices exceeded unity, but not to a level of significance, and only on a few of the quadrat sizes tested. This indicated a random or uniform distribution of diseased plants in these vineyards (Tables 2 and 3). In GTFI and CBSI-3, on the other hand, there was a stronger indication of aggregation for most of the quadrat sizes tested, in general the larger ones where the average disease incidence was higher than 0.16 (CBSI-3) or 0.47 (GTFI) (Tables 4 and 5). Quadrat sizes with aggregated vines were laid out along columns in CBSI-3; along both columns and rows in GTFI.

$I_\delta$ values were plotted against the series of quadrat sizes used to analyse the incidence of esca (Fig. 1). When compared with Morisita's standard curves for different distributions (Morisita, 1959), the shape of the curves obtained at CBSI-3 was consistent with a contagious distribution of vines with small clumps of diseased vines but a random distribution within clumps. At Gambassi Terme on the other hand (GTFI) the distribution of diseased vines was also contagious but with larger clumps; here the within-clump distribution was uniform. Finally, in CBSI-1 and 2 the distribution of diseased vines was random or uniform (curves not shown).

2DCLASS analysis detected nonrandomness in GTFI, CBSI-2 and CBSI-3 (Table 6, Fig. 2, 4 and 5). The number of distance classes with significantly greater than expected SCFs under a random spatial distribution ranged from 6.21 to 22.74% and tended to increase from vineyard to vineyard as the disease incidence increased. Proportions of distance classes with lower than expected SCFs were smaller overall, ranging from 0.01 in CBSI-2 to 0.13

Table 2. Indices of dispersion ( LIP, VM, $I_\delta$) and $\chi^2$ goodness-of-fit to Morisita's index ($I_\delta$) for esca infected grapevine at Castelnuovo Berardenga (Siena, Italy) (CBSI-1).

| Quadrat size (vines) | Diseased plants per quadrat (mean) | No. of plants per quadrat | Indices of dispersion | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LIP[a] | VM[b] | $I_\delta$[c] | $I_\delta$ | | |
| | | | | | | $\chi^2$ | df[d] | *P* |
| [e] 2 x 2 | 0.38 | 4 | 1.35 | 1.13 | 1.36 | 131.50 | 116 | -[f] |
| → 3 x 2 | 0.56 | 6 | 0.99 | 0.99 | 0.74 | 65.91 | 77 | - |
| → 6 x 2 | 1.13 | 12 | 1.07 | 1.08 | 1.07 | 41.09 | 38 | - |
| → 9 x 2 | 1.69 | 18 | 0.88 | 0.79 | 0.88 | 19.82 | 25 | - |
| ↓ 2 x 13 | 2.44 | 26 | 1.20 | 1.50 | 1.20 | 25.54 | 17 | - |
| ↓ 3 x 13 | 3.67 | 39 | 1.01 | 1.06 | 1.01 | 11.64 | 11 | - |
| ↓ 6 x 13 | 7.33 | 78 | 1.11 | 1.84 | 1.10 | 9.18 | 5 | - |
| ↓ 9 x 13 | 11.00 | 117 | 1.06 | 1.64 | 1.04 | 4.91 | 3 | - |
| ↓ 2 x 26 | 4.89 | 52 | 1.00 | 0.99 | 1.00 | 7.95 | 8 | - |
| ↓ 3 x 26 | 7.33 | 78 | 0.93 | 0.47 | 0.94 | 2.36 | 5 | - |
| ↓ 6 x 26 | 14.67 | 156 | 1.00 | 0.98 | 1.00 | 1.95 | 2 | - |
| ↓ 9 x 26 | 22.00 | 234 | 0.97 | 0.36 | 0.98 | 0.36 | 1 | - |

[a] LIP, Lloyd's index of patchiness; [b] VM, variance-to-mean ratio; [c] $I_\delta$, Morisita's index. Values of LIP, VM, and $I_\delta$ not significantly different from 1 ($0.95>P>0.05$) indicate that the pattern of diseased plants was indistinguishable from random. Values >1 indicate rejection of a random pattern of diseased plants in favour of an aggregated pattern.
[d] Degrees of freedom.
[e] Horizontal arrow: quadrats laid out along rows; vertical arrow: quadrats laid out along the columns.
[f] -, $P>0.05$.

Table 3. Indices of dispersion ( LIP, VM, $I_\delta$) and $\chi^2$ goodness-of-fit to Morisita's index ($I_\delta$) for esca infected grapevine at Castelnuovo Berardenga (Siena, Italy) (CBSI-2).

| Quadrat size (vines) | Diseased plants per quadrat (mean) | No. of plants per quadrat | Indices of dispersion | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LIP[a] | VM[b] | $I_\delta$[c] | $I_\delta$ | | |
| | | | | | | $\chi^2$ | df[d] | *P* |
| [e] 2 x 2 | 0.48 | 4 | 0.69 | 0.85 | 0.68 | 98.61 | 116 | -[f] |
| → 3 x 2 | 0.72 | 6 | 0.96 | 0.97 | 0.96 | 74.93 | 77 | - |
| → 6 x 2 | 1.43 | 12 | 0.99 | 0.98 | 0.99 | 37.32 | 38 | - |
| → 9 x 2 | 2.15 | 18 | 0.96 | 0.92 | 0.96 | 22.93 | 25 | - |
| ↓ 2 x 13 | 3.11 | 26 | 0.85 | 0.52 | 0.85 | 8.93 | 17 | - |
| ↓ 3 x 13 | 4.67 | 39 | 1.04 | 1.18 | 1.03 | 13.00 | 11 | - |
| ↓ 6 x 13 | 9.33 | 78 | 1.00 | 1.01 | 1.00 | 5.07 | 5 | - |
| ↓ 9 x 13 | 24.67 | 117 | 1.05 | 1.76 | 1.04 | 5.28 | 3 | - |
| ↓ 2 x 26 | 6.22 | 52 | 0.94 | 0.63 | 0.95 | 5.07 | 8 | - |
| ↓ 3 x 26 | 9.33 | 78 | 0.99 | 0.88 | 0.99 | 4.43 | 5 | - |
| ↓ 6 x 26 | 18.67 | 156 | 1.04 | 1.84 | 1.03 | 3.68 | 2 | - |
| ↓ 9 x 26 | 28.00 | 234 | 1.03 | 1.78 | 1.01 | 1.78 | 1 | - |

[a] LIP, Lloyd's index of patchiness; [b] VM, variance-to-mean ratio; [c] $I_\delta$, Morisita's index. Values of LIP, VM, and $I_\delta$ not significantly different from 1 ($0.95>P>0.05$) indicate that the pattern of diseased plants was indistinguishable from random. Values >1 indicate rejection of a random pattern of diseased plants in favour of an aggregated pattern.
[d] Degrees of freedom.
[e] Horizontal arrow: quadrats laid out along rows; vertical arrow: quadrats laid out along the columns.
[f] -, $P>0.05$.

in GTFI. In CBSI-1 (Fig. 3) the disease incidence (<10%) was too low for 2DCLASS analysis to be applied. For this vineyard and for CBS-2 and 3, 2DCORR revealed a random distribution of infected plants.

In GTFI significant distance classes were clustered near the origin (core cluster) (Fig. 2). The core cluster was 4 and the [X, Y] coordinates were [0, 0-1] and [1, 0-1]. The relatively small core cluster indicated that diseased vines grew in close proximity to each other. Additional evidence for non-uniform distribution of disease was presented by a large group of distance classes with significantly lower than expected SCFs ($P \geq 0.95$) within the distance class matrix (Fig. 2). The approximate [X, Y] coordinates for this group were [0-5, 11-24]. However, the highest densities of distance classes with significantly positive SCFs were located farther from the origin and demonstrated the presence of discontinuous proximity patterns. The coordinates for these groups were [7, 1-2], [8, 0-2], [0, 3], [1, 3-4] and [0-9, 33-50]. The shape of this last cluster was amorphous; the others were rectangular and column-oriented. [X, Y] distance classes [0-9, 50] indicated possible significant edge effects.

Significant column effects were detected in CBSI-3 (Fig. 4). The distance classes [0, 0-17 ], [ 0, 19-21], [0, 24], [0, 32], [0, 35], [0, 38], [0, 42], [0, 52], [0, 55] and [0, 57] here indicated that diseased plants tended to occur, sometimes in close proximity, along given columns. Eight groups of two to six significant [X, Y] distance classes furthest from the origin were consistent with the presence of numerous relatively small clusters of diseased plants. Fifty-four distance classes, grouped in 11 clusters, with SCFs significantly lower ($P \geq 0.95$) than expected provides additional evidence for the presence of small clusters of infected plants in this vineyard.

2DCORR confirmed the results of 2DCLASS and provided additional and clearer information. In the proximity pattern matrix for GTFI, 33 distance classes with significantly higher than expected pair counts were located near the origin, thus indicating a strong aggregation of contiguous plants. The remaining 14 distance classes with significantly higher than expected pair counts were sparsely distributed within the distance class matrix. Moreover, the Delta page (not shown), in which the deviation between observed and predicted infected pairs is plotted against the

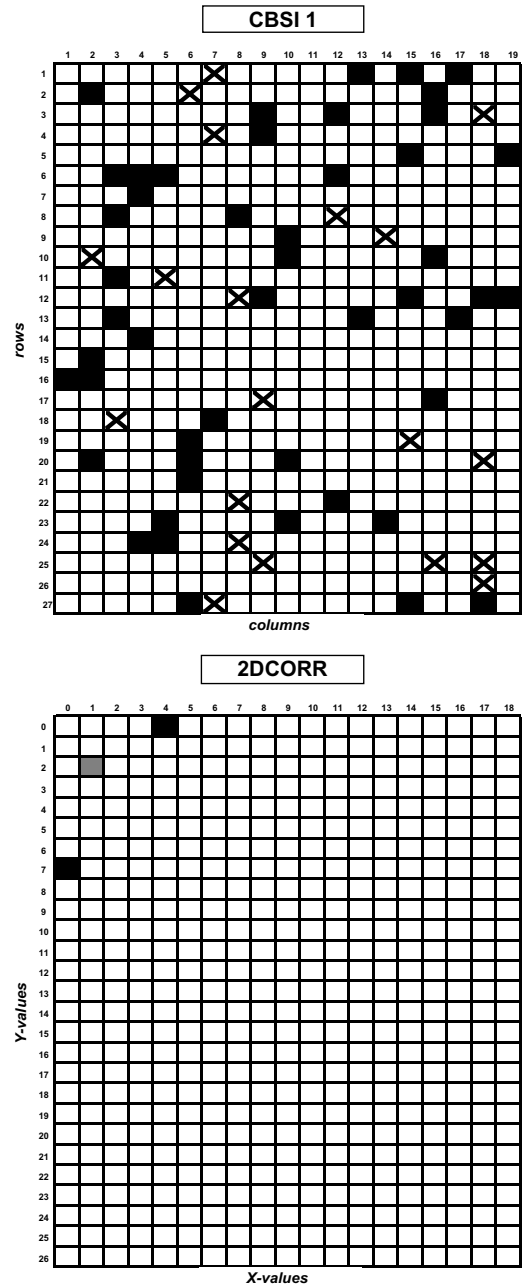


Fig. 3. Top figure: spatial pattern of esca in a vineyard at Castelnuovo Berardenga, Tuscany. Black squares, white squares and squares with "**X**" indicate position of esca-diseased vines, healthy plants and missed vines before 1995 respectively. Bottom figure: two dimensional correlation analysis (2DCORR) of esca-diseased vines of vineyard CBSI-1. Black and gray squares indicate significantly greater and less than expected observed pair counts respectively. White squares indicate observed pair counts as expected.

Table 4. Indices of dispersion ( LIP, VM, $I_\delta$) and $\chi^2$ goodness-of-fit to Morisita's index ($I_\delta$) for esca infected grapevine at Gambassi Terme (Florence, Italy) (GTFI).

| Quadrat size (vines) | Diseased plants per quadrat (mean) | No. of plants per quadrat | Indices of dispersion | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LIP[a] | VM[b] | $I_\delta$[c] | $I_\delta$ | | |
| | | | | | | $\chi^2$ | df[d] | *P* |
| [e] 2 x 2 | 1.88 | 4 | 0.85 | 0.72 | 0.85 | 88.9362 | 124 | -[f] |
| → 5 x 1 | 2.4 | 5 | 0.94 | 0.85 | 0.94 | 84.5745 | 99 | - |
| ↓ 2 x 5 | 4.7 | 10 | 1.02 | 1.10 | 1.02 | 54.1489 | 49 | - |
| → 5 x 2 | 4.7 | 10 | 1.03 | 1.15 | 1.03 | 56.2766 | 49 | - |
| ↓ 2 x 10 | 9.4 | 20 | 1.07 | 1.65 | 1.07 | 39.5745 | 24 | - |
| 5 x 5 | 11.7 | 25 | 1.10 | 2.17 | 1.09 | 41.1702 | 19 | * |
| → 5 x 10 | 23.5 | 50 | 1.11 | 3.52 | 1.10 | 31.6808 | 9 | * |
| 10 x 10 | 47.0 | 100 | 1.11 | 6.45 | 1.09 | 25.7872 | 4 | * |
| ↓ 5 x 25 | 58.8 | 125 | 1.07 | 5.46 | 1.06 | 16.3872 | 3 | * |
| ↓10 x 25 | 117.5 | 250 | 1.13 | 15.83 | 1.06 | 15.8340 | 1 | * |

[a] LIP, Lloyd's index of patchiness; [b] VM, variance-to-mean ratio; [c] $I_\delta$, Morisita's index. Values of LIP, VM, and $I_\delta$ not significantly different from 1 ($0.95>P>0.05$) indicate that the pattern of diseased plants was indistinguishable from random. Values >1 indicate rejection of a random pattern of diseased plants in favour of an aggregated pattern.
[d] Degrees of freedom.
[e] Horizontal arrow: quadrats laid out along rows; vertical arrow: quadrats laid out along the columns.
[f] -, $P>0.05$; *, $P<0.01$.

Table 5. Indices of dispersion ( LIP, VM, $I_\delta$) and $\chi^2$ goodness-of-fit to Morisita's index ($I_\delta$) for esca infected grapevine at Castelnuovo Berardenga (Siena, Italy) (CBSI-3).

| Quadrat size (vines) | Diseased plants per quadrat (mean) | No. of plants per quadrat | Indices of dispersion | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LIP[a] | VM[b] | $I_\delta$[c] | $I_\delta$ | | |
| | | | | | | $\chi^2$ | df[d] | *P* |
| [e] 2 x 2 | 0.65 | 4 | 1.05 | 1.03 | 1.05 | 277.0 | 269 | -[f] |
| → 3 x 2 | 0.97 | 6 | 0.91 | 0.91 | 0.91 | 163.4 | 179 | - |
| → 6 x 2 | 1.94 | 12 | 0.94 | 0.89 | 0.94 | 79.6 | 89 | - |
| → 9 x 2 | 2.92 | 18 | 0.93 | 0.80 | 0.93 | 47.5 | 59 | - |
| ↓ 1 x 10 | 1.62 | 10 | 1.45 | 1.73 | 1.45 | 184.8 | 107 | * |
| ↓ 2 x 10 | 3.24 | 20 | 1.23 | 1.74 | 1.23 | 92.5 | 53 | * |
| ↓ 3 x 10 | 4.86 | 30 | 1.08 | 1.39 | 1.08 | 48.6 | 35 | - |
| ↓ 6 x 10 | 9.72 | 60 | 1.00 | 1.04 | 1.00 | 17.6 | 17 | - |
| ↓ 9 x 10 | 14.58 | 90 | 1.00 | 1.00 | 1.00 | 11.0 | 11 | - |
| ↓ 1 x 20 | 3.24 | 20 | 1.48 | 2.55 | 1.47 | 135.1 | 53 | * |
| ↓ 2 x 20 | 6.48 | 40 | 1.25 | 2.60 | 1.24 | 67.7 | 26 | * |
| ↓ 3 x 20 | 9.72 | 60 | 1.09 | 1.97 | 1.09 | 33.49 | 17 | * |
| ↓ 6 x 20 | 19.44 | 120 | 1.02 | 1.49 | 1.02 | 11.94 | 8 | - |
| ↓ 9 x 20 | 29.17 | 180 | 1.01 | 1.39 | 1.01 | 6.95 | 5 | - |
| ↓ 1 x 30 | 4.86 | 30 | 1.47 | 3.28 | 1.46 | 114.8 | 35 | * |
| ↓ 2 v 30 | 7.92 | 60 | 1.27 | 3.60 | 1.25 | 61.0 | 17 | * |

[a] LIP, Lloyd's index of patchiness; [b] VM, variance-to-mean ratio; [c] $I_\delta$, Morisita's index. Values of LIP, VM, and $I_\delta$ not significantly different from 1 ($0,95>P>0,05$) indicate that the pattern of diseased plants was indistinguishable from random. Values >1 indicate rejection of a random pattern of diseased plants in favour of an aggregated pattern.
[d] Degrees of freedom.
[e] Horizontal arrow: quadrats laid out along rows; vertical arrow: quadrats laid out along the columns.
[f] -, $P>0.05$; *, $P<0.01$.

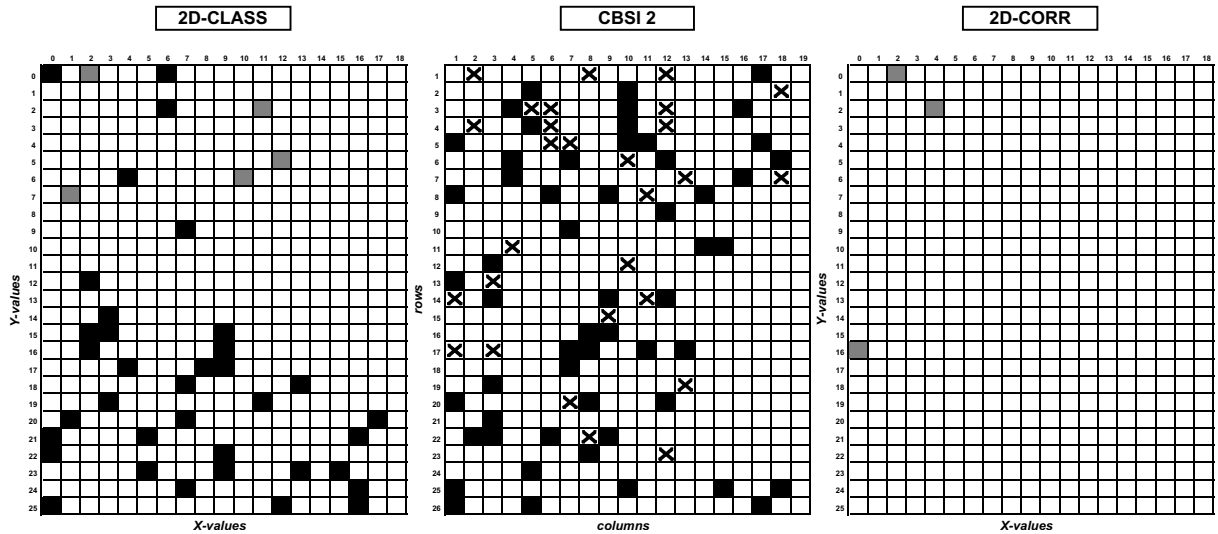


Fig. 4. Middle: spatial pattern of esca in a vineyard at Castelnuovo Berardenga, Tuscany. Black squares, white squares and squares with "**X**" indicate position of esca-diseased vines, healthy plants and missed vines before 1995 respectively. Left: proximity pattern matrix from two dimensional distance class analyses (2DCLASS). Black and gray squares indicate [X, Y] distance classes with greater ($P \leq 0.05$) and less ($P \geq 0.95$) than expected standardized count frequencies (SCF) respecti-vely. White squares indicate [X, Y] distance classes with SCFs as expected. Right: two dimensional correlation analysis (2DCORR) of esca-diseased vines of vineyard CBSI-2. Black and gray squares indicate significantly greater and less than expected observed pair counts respectively. White squares indicate observed pair counts as expected.

Table 6. Spatial statistics from two-dimensional distance class analysis (2DCLASS) for esca symptoms in four vineyards in the provinces of Florence and Siena, Tuscany, Italy.

| Vineyard | Test period | | No. of infected vines | Disease[a] incidence | Total No. SCF[b] | Significance[c] | | Pattern[d] | Cluster size | | Total No. of clusters[g] | Significant column effect[h] | Significant edge effect[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | from | to | | | | SCF+ | SCF- | | Core[e] | Reflected[f] | | | |
| GTFI | '93 | '98 | 244 | 49.09 | 510 | 116 | 67 | A | 4 | 35.6 | 3 | + | + |
| CBSI-1 | '95 | '98 | 49 | 9.93 | n.p.[e] | | | | | | | | |
| CBSI-2 | '95 | '98 | 56 | 12.01 | 494 | 35 | 5 | A | 1 | 3.16 | 6 | - | - |
| CBSI-3 | '95 | '98 | 181 | 17.55 | 1159 | 72 | 72 | A | 23 | 3 | 8 | + | - |

[a] Number of esca-diseased vines/total number of vines.
[b] Standardised count frequencies.
[c] Number of [ X,Y] distance classes with SCF significantly greater (SCF+) or less (SCF-) than expected.
[d] Proximity pattern: A, aggregated (5% ≤ number of significant SCFs ≤ 80%).
[e] Number of adjacent, significant distance classes that form a discrete group contiguous with the origin.
[f] Average number of adjacent, significant distance classes that form discrete groups discontiguous with the origin, the core cluster, or both.
[g] Number of reflected clusters.
[h] Maximum number of significant and adjacent SCFs in the two dimensional distance class analysis matrix along the Y direction. Column effect was considered to be significant if more than 15% of the distance classes in a Y direction had SCFs values that were significantly greater than expected. +, significant column effect; -, non-significant column effect.
[i] Maximum number of significant and adjacent SCFs in the outermost row and column of the two dimensional distance class analysis matrix. The edge effect was considered to be significant if more than 15% of the distance classes in the edge effect region had SCFs values significantly greater than expected. +, significant edge effect; -, non-significant edge effect.
[e] n.p., test not performed because of low disease incidence.

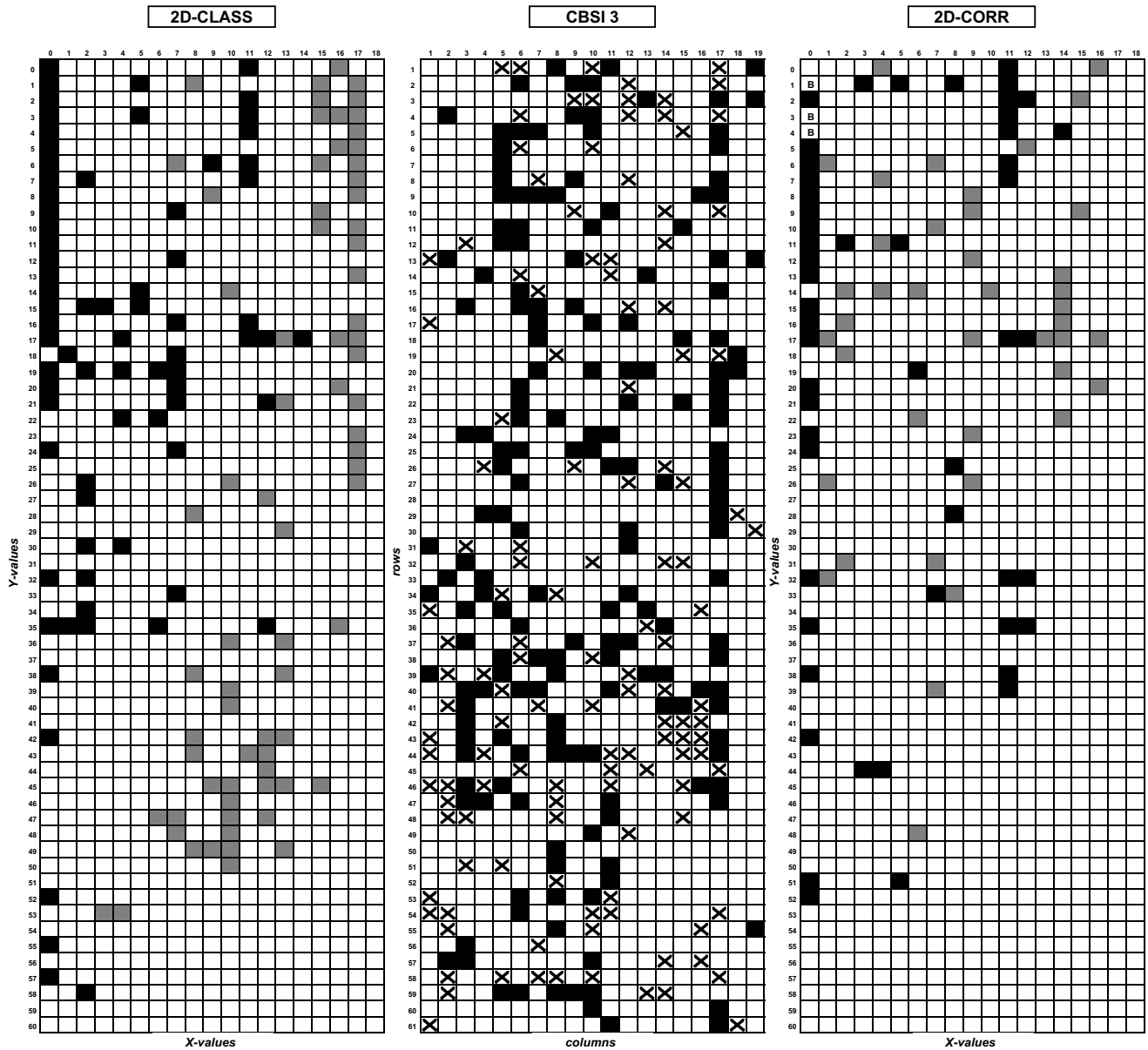


Fig. 5. Middle: spatial pattern of esca in a vineyard at Castelnuovo Berardenga, Tuscany. Black squares, white squares and squares with "**X**" indicate position of esca-diseased vines, healthy plants and missed vines before 1995 respectively. Left: proximity pattern matrix from two dimensional distance class analyses (2DCLASS). Black and gray squares indicate [X, Y] distance classes with greater ($P \leq 0.05$) and less ($P \geq 0.95$) then expected standardized count frequencies (SCF) respectively. White squares indicate [X, Y] distance classes with SCFs as expected. Right: two dimensional correlation analysis (2DCORR) of esca-diseased vines of vineyard CBSI-3. Black and gray squares indicate significantly greater and less than expected observed pair counts respectively. Squares with "B" indicate significance at the Bonferroni level of probability ($P \leq 0.000863$). White squares indicate observed pair counts as expected.

distance r expressed as number of plants, showed that the spatial correlation among diseased plants extended to a distance of 10 plant lengths in all directions. In the case of CBSI-1, 2 and 3, 2DCORR analysis yielded no significant spatial correlation among diseased plants. However, in CBSI-3 the plotting of Delta showed a tendency for diseased plants to be within 8 plant-spacings of each other, while the probability page indicated that most of this correlation was down-column (data not shown).

## Discussion

Of the vineyards surveyed for esca in this study, CBSI-1, 2 and 3 were monitored for four years, GTFI for six years in succession. In spite of this, it proved impossible to trace the progress of esca in these four vineyards over time, mainly because it is at present not possible to know precisely when a plant becomes infected, which may be a long time before it begins to present external symptoms, and even then these symptoms do not appear consistently every year. To have an accurate picture of the true rate of esca infection in a vineyard we are thus forced with the available data to draw up maps plotting all vines that have shown esca symptoms, whether chronic or acute, at least once during a selected observation period. Ideally such maps will show the total cumulated esca data over the lifetime of the vineyard, from its establishment until the last survey year. Since the number of years covered by the present survey was fairly high in relation to the age of the vineyards, which ranged from 12 to 23 years, we believe we identified most if not all esca-infected plants.

Within these accepted limitations, some statistical procedures were applied to the mapping data to extract preliminary information regarding the spread of esca. In addition, since the three vineyards at Castelnuovo Berardenga were only twelve years old in 1995, it was possible to offer some speculations on how the disease was first introduced (primary spread). It also seemed necessary to determine whether the data supported the view that secondary spread occurred along the columns by means of infected pruning tools. It is still recommended that viticulturists should mark esca-infected plants and prune them last, disinfect all pruning tools frequently, and protect pruning wounds with a healing varnish or a dressing containing a broad-spectrum fungicide.

The results of the statistical analyses show that there was aggregation of infected plants in GTFI, where esca incidence was about 50%, and also, though to a somewhat less degree, in CBSI-3, where esca incidence was 17.5%. In CBSI-1 and CBSI-2, on the other hand, infected plants were mostly spatially isolated. In these two vineyards, which each comprised 19 columns, there were only 6 infected plant pairs and one contiguous group of three infected plants along columns in CBSI-1, and 5 infected pairs plus one group of four infected plants along columns in CSBI-2. All the other infected plants, 34 in CSBI-1 and 42 in CBSI-2 were spatially isolated in the vineyard. The statistical analyses revealed a nearly uniform distribution of infected plants for these vineyards. In fact the ordinary runs analysis and the values of the indices of dispersion indicated only infrequent along-column aggregation of immediately adjacent vines with esca, and 2DCLASS and 2DCORR analysis substantially confirmed these results.

It may be supposed that in CBSI-1 and CBSI-2 esca was still at an initial stage and was introduced with infected propagation material or with inoculum introduced from outside. It is easy to foresee that when the incidence of esca begins to increase here there will be an increase in the aggregation of infected plants. Such a development is suggested by what occurred in GTFI and in CBSI-3, where cumulated esca-incidence was higher (49.09 and 17.5% respectively). In GTFI infected plants were aggregated within 10 plant-spacings of each other in all directions, not along any particular axis, and 70% of distance classes with significantly higher than expected pair-counts were contiguous to the origin, signifying that infected plants tended to grow in proximity to each other. However, even in this vineyard none of the analyses detected a preferential spread of the disease from plant to plant along columns. Moreover, infected plants formed two large groups, one at the high end and one at the low end. GTFI stretches from east to west for a distance of some 61 m. The terrain slopes about 7% for the first 12 m, 15% in the central portion and 5% for the last 12 m. Almost half the healthy plants (47%) were in middle upper part of the vineyard where the slopes are steeper, as is shown by the group of

distance classes with non-significant SCFs with coordinates [0-5, 11-24] in the 2DCLASS matrix. This could indicate that the vineyard slope has an effect on esca symptom expression, or, more precisely, that water accumulates differently at different parts of the slope. A greater incidence of esca where the slopes are less steep was also observed in other vineyards (data not shown).

Vineyard CBSI-3 was an interesting case. It consisted of 19 columns, and in each column a different cultivar was grown. Here there was a strong tendency, detected by all analytical procedures, for infected vines to be aggregated along columns. For example, ordinary runs analysis revealed that the aggregation of infected plants was particularly strong in columns 3 and 5 ($Z_u$=-2.27 and -2.09 respectively) and became still stronger when plants which died before 1994 were recorded as diseased. It should be noted however that in column 17, for example, grown with cv. Semillon, a high percentage of infected vines was found: 30 infected vines out of 72, or 41.66%, consisting of 6 vines spatially isolated and the remaining 25 in contiguous groups of from 2 to 6 vines. Nevertheless, the ordinary runs analysis for this column indicated that there was a random distribution of infected plants [on the other hand, the analysis indicated aggregation within columns even when there were only one or three infected vines]. All in all the data for each column and the statistical procedures seemed to indicate not so much a spread of esca along columns by pruning tools as a greater susceptibility of given cultivars, in this case 'Semillon', 'Pinot bianco' and 'Riesling italico' — although it must also be said that the 'Pinot bianco' vines in row 11 showed a lower incidence of esca.

It therefore seemed that the disease spread mainly by airborne spores from external and internal sources. This is consistent with recent findings on the spread of spores of *Fop* and species of *Phaeoacremonium* ( Larignon, 1999; Cortesi *et al*., 2000). Basidiocarps of *Fop* are rarely produced on the living trunks of esca-infected vines and are only occasionally encountered on dead vine trunks or branches left for a long time in the field or at the edge of vineyards after pruning (Mugnai *et al*., 1999). Most commonly the basidiocarps of *Fop* are produced on the trunks of very old vines with esca, and they grow first of all on live standing trees and slash of hardwoods and conifers (Larsen and Cobb-Poulle, 1990). It therefore seems most likely that *Fop* inoculum sources from outside the vineyard have a greater role in the spread of esca among vines. As for *Pch* and *Pal,* the two other fungi implicated in esca, it is likely that conidia are produced in abundance by them during the saprobic phase on the outer surface of living vines, and on dead vinewood and other plant debris (Larignon, 1999). The chlamydospores of both these fungi may also persist in the soil. Inoculum is thus available to infect living plants and is introduced by injuries caused by pruning, grafting or in other ways. Another very real possibility is that these fungi are already present in the propagation material derived either from infected mother plants or from scions or rooted cuttings that have themselves become infected through cuts and other wounds during preparation and storage. Primary infection with esca may thus occur through the introduction of *Pch* / *Pal*-infected material into the vineyard and/or through conidia and then basidiospores being released from external sources and disseminated by air currents (for the specific role of *Pch, Pal* and *Fop* in esca see Graniti *et al*., this issue). The random distribution of diseased vines in CBSI-1 and 2 seems to confirm both these hypotheses on the spread of esca. However, we should also bear in mind that the three vineyards at Castelnuovo Berardenga were not uniform with regard to grape cultivar and rootstock. In the analyses performed we mainly ignored this situation simply because there was reason to believe that the different grape cultivars and rootstocks were all equally susceptible to esca. Vineyards SCFI, CBSI-1, CBSI-2 and CBSI-3 were also surveyed in 1999, and some vineyards planted in 1997 have likewise been surveyed for two years now. It is hoped that the new data will confirm earlier findings and will enable us to refine our knowledge on esca spread.

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## Literature cited


Campbell C.L. and L.V. Madden, 1990. Spatial Aspects of Plant Diseases Epidemics II: Analysis of Spatial Pattern. *In*: Introduction to Plant Disease Epidemiology, J. Wiley & Sons, New York, NY, USA, 289-328.

Cortesi P., M. Fischer and M.G. Milgroom, 2000. Population diversity of *Fomitiporia punctata* from grapevine and spread of esca disease. *In*: IOBC/wprs Bulletin, Working Group "Integrated Control in Viticulture", 1-4 March 1999, Firenze, Italy, (in press).

Ferrandino F.J., 1996. Two-dimensional distance class analysis of disease-incidence data: problems and possible solutions. *Phytopathology*, 86, 685-691.

Ferrandino F.J., 1998. Past nonrandomness and aggregation to spatial correlation: 2DCORR, a new approach for discrete data. *Phytopathology*, 88, 84-91.

Gibson G.J., 1997. Investigating mechanisms of spatiotemporal epidemic spread using stochastic models. *Phytopathology*, 87, 139-146.

Graniti A., G. Surico and L. Mugnai, 1999. Considerazioni sul mal dell'esca e sulle venature brune del legno della vite. *Informatore Fitopatologico*, 49(5), 6-12.

Gray S.M., J.W. Moyer and P. Bloomfield, 1986. Two-dimensional distance class model for quantitative description of virus-infected plant distribution lattices. *Phytopathology*, 76, 243-248.

Hewitt W.B., 1957. Some manifestations of black measles of grapevines. *Phytopathology*, 47, 16 (abstract).

Larignon P., 1999. Esca disease from an European perspective. *In*: Black goo - Occurrence and Symptoms of Grapevine Declines. IAS/ICGTD Proceedings 1998 (L. Morton ed.), International Ampelography Society, Fort Valley, VA, USA, 43-55.

Larsen M.J. and L.A. Cobb-Poulle, 1990. *Phellinus* (Hymenochaetaceae). A survey of the world taxa. Fungiflora, Oslo, Norway.

Lloyd M., 1967. Mean crowding. *Journal of Animal Ecology*, 36, 1-30.

Madden L.V., R. Louie, J.J. Abt and J.K. Knoke, 1982. Evaluation of tests for randomness of infected plants. *Phytopathology*, 72, 195-198.

Madden L.V., R. Louie and J.K. Knoke, 1987. Temporal and spatial analysis of maize dwarf mosaic epidemics. *Phytopathology*, 77, 148-156.

Matherson G., 1963. Principals of Geostatistcs. *Economic Geology and Bulletin of the Society of Economic Geologists*, 58, 1246-1266.

Morisita M., 1959. Measuring of the dispersion of individuals and analysis of the distributional patterns. *Memoirs of the Faculty of Science*, *Kyushu University*, Ser. E (Biol.), 2(4), 215-235.

Mugnai L., R. Imbriani and G. Surico. Indagine sulla diffusione e gravità del 'mal dell'esca' in alcuni vigneti della Toscana. *Informatore Fitopatologico*, 46(6), 50-56.

Mugnai L., A. Graniti and G. Surico, 1999. Esca (black measles), and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*, 83, 404-418.

Nelson S.C. and C.L. Campbell, 1993. Comparative spatial analysis of foliar epidemics on white clover caused by virus, fungi and a bacterium. *Phytopathology*, 83, 288-301.

Nelson S.C., 1995. Spatiotemporal distance class analysis of plant disease epidemics. *Phytopathology*, 85, 37-43.

Nelson S.C., 1996. A simple analysis of disease foci. *Phytopathology*, 86, 332-339.

Nelson S.C. and P.R. Guzman, 1997. Two-dimensional and spatiotemporal distance class analysis. *In:* Exercises in Plant Disease Epidemiology (L.J. Francl and D.A. Neher, eds) APS Press, St. Paul, Minnesota, USA.

Reynolds K.M. and L.V. Madden, 1988. Analysis of epidemics using spatio-temporal autocorrelation. *Phytopathology*, 78, 240-246.

Upton G. and B. Fingleton, 1985. Spatial Data Analysis by Example, Vol. 1: Point Pattern and Quantitative Data, J. Wiley & Sons, New York, NY, USA, 410 pp.