FOLIA FORESTALIA POLONICA
Seria B, zeszyt 29, 1998

COMPUTER-ASSISTED NUMERICAL CLUSTERING ANALYSIS OF VARIOUS STRAINS OF *SERPULA LACRYMANS* (WULFEN:FR.)SCHROETER APUD COHN*

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Eight strains of *Serpula lacrymans* were compared in relation to their previously determined factors (5 physiological and 10 toxicometrical). A numerical clustering analysis was used along with, as coefficient of similarity, "cos Q" after standardization of all results.

Key words: dry rot, wood preservatives, CCA, NaPCP, numerical analysis, *Serpula lacrymans*

INTRODUCTION

Computer-assisted numerical analysis is widely applied in microbiology for the classification, identification and characterization of bacteria and yeasts (Sneath and Sokal 1973, Szulga and Gnot 1977, Malashenko et al. 1980). More recently, there have been attempts to use this method in the taxonomy of filamentous fungi belonging to the subdivisions of Ascomycotina and Deuteromycotina (Bridge 1988). Some experiments were also carried out on the scope of application of the numerical method to Basidiomycotina. Wood-destroying fungi belonging mainly to this subdivision often occur only in the form of mycelium with little evidence of individual morphological characters and in the absence of fructification. For these reasons they create serious

* Paper presented on the IRG on Wood Preservation 23rd Annual Meeting in Harrogate, U.K.
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problems for the identification of particular species - and especially for their strains. The identification methods for pure cultures which are very useful for this purpose (Long and Harsch 1918, Nobles 1965, Stalpers 1978) have not been prepared in mathematical form. However, numerical analysis for Basidiomycetes has been applied by Proctor and Kendrick 1963 (Haplobasidion sp.), Kendrick and Weresub 1966 (different species) and Boisselier-Dubayle 1983, Kulkarni et al. 1986 and May and Royse 1988 (Pleurotus sp.).

The present document constitutes an attempt to apply the numerical analysis for comparison of 8 strains of the dry rot fungus *Serpula lacrymans* on the basis of the results of our previous investigations on physiological and toxicometric properties of their pure cultures (Thornton and Ważny 1986, Ważny and Thornton 1986, 1989a,b).

EXPERIMENTS

The numerical analysis applied for comparison of 8 strains of *Serpula lacrymans* (Wulfen:Fr.)Schroeter apud Cohn (see Pegler 1991 for current taxonomy) originating from England (FPRL 12C), Germany (FPRL 12E - Eberswalde 315), Poland (Warsaw III), Japan (HFP 7802) and Australia (DFP 16508, 16509, 16521 and 16522) applied in these countries as test organisms in investigations on the biology and the fungicidal value of wood preservatives.

Growth rates and dry mass of mycelium, decay capacity and final moisture content, reduction of the compression strength, toxic value for CCA and NaPCP preservatives in the agar-plate and agar-block tests by using both mass loss reduction and compression strength reduction criteria, were each taken into consideration. In total the effect of 15 factors, comprising 5 physiological and 10 toxicometrical, were investigated. In order to produce a numerical classification the first step was to choose a suitable coefficient which would describe similarity between strains. Among some coefficients available the "cos Q" coefficient was used, where Q is the angle between two vectors, each representing one strain (Sneath and Sokal 1973). Chosen coefficients of similarity between two strains "i" and "j" were described in the following formula:

$$\cos Q_{ij} = \frac{\sum_{k=1}^{m} x_{ik}\ x_{jk}}{\sqrt{\sum_k x_{ik}^2 \cdot \sum_k x_{jk}^2}}$$

where: x_{ik} - represents the value of the k^{th} factor in the i^{th} strain,

x_{jk} - the k^{th} factor in the j^{th} strain.

Before calculation of coefficients of similarity, numerical data should be standardized in order to avoid problems with the different units in which factors were originally measured. The standardization is done by subtracting the mean value from each data and dividing the result by the standard deviation. The standardizations are performed in the form of non-unit measurements with mean equal to zero and standard deviation equal to one.

After the standardization (Fig. 1-3), the matrix of similarity values for the "cos Q" coefficient was calculated and weighted where the pair grouping average method (Davis 1973) was used. Finally, three dendrograms (Fig. 4.) produced sets of data containing all measurements, and sets with physiological and toxicometric factors taken separately. The threshold level of similarity was an angle of 45°, which gives a coefficient value of 0.714.

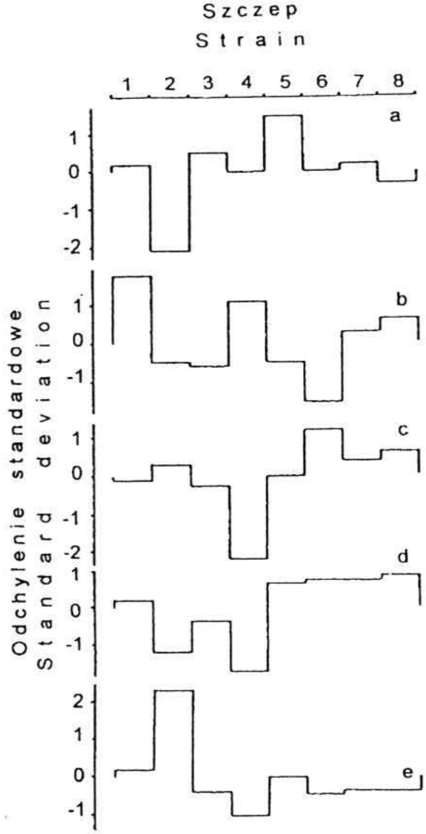

Fig. 1. Standardised values of physiological factors of various strains of *S. lacrymans*: 1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - growth rate, b - mycelium dry mass, c - decay capacity, d - final moisture content, e - reduction of the compression strength

Rys. 1. Wartości standaryzowane czynników fizjologicznych dla różnych szczepów *S. lacrymans*: 1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - szybkość wzrostu, b - sucha masa grzybni, c - szybkość rozkładu, d - wilgotność końcowa drewna, e - redukcja wytrzymałości na ściskanie)

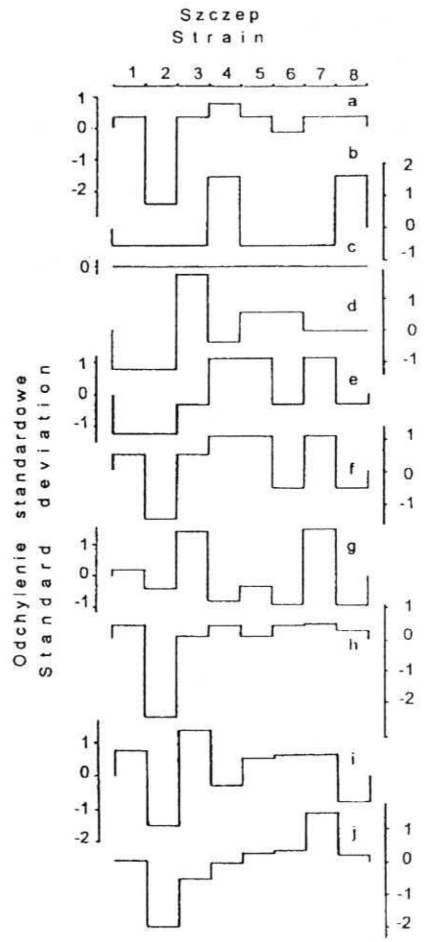

Fig. 2. Standardised values of toxicometric factors of various strains of *S. lacrymans*: 1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - toxic value of CCA in agar /ED_{50}/, b - toxic value of CCA in agar /ED_{100}/, c - toxic value of CCA in agar /LD/, d - toxic value of NaPCP in agar /ED_{50}/, e - toxic value of NaPCP in agar /ED_{100}/, f - toxic value of NaPCP /LD/, g - toxic value of CCA in agar-block /mass loss/, h - toxic value of NaPCP in agar-block /mass loss/, i - toxic value of CCA in agar-block /red. of strength/, j - toxic value of NaPCP in agar-block /red. of strength/

Rys. 2. Wartości standaryzowane czynników toksykometrycznych dla różnych szczepów *S. lacrymans:* 1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - wartość toksyczna ED_{50} dla CCA metodą agarową, b - wartość toksyczna ED_{100} dla CCA metodą agarową, c - wartość letalna LD dla CCA metodą agarową, d - wartość toksyczna ED_{50} dla NaPCP metodą agarową, e - wartość toksyczna ED_{100} dla NaPCP metodą agarową, f - wartość letalna LD dla NaPCP metodą agarową, g - wartość toksyczna dla CCA metodą agarowo-klockową na podstawie ubytku masy, h - wartość toksyczna dla NaPCP metodą agarowo-klockową na podstawie ubytku masy, i - wartość toksyczna dla CCA metodą agarowo-klockową na podstawie redukcji wytrzymałości, j - wartość toksyczna NaPCP metodą agarowo-klockową na podstawie redukcji wytrzymałości)

RESULTS

Results of comparative investigations of different factors of 8 *Serpula lacrymans* strains following the standardization of each factor have been presented graphically. In Fig. 1 the variability of particular physiological factors and in Fig. 2 the variability of particular toxicometric factors are presented. Fig. 3 illustrates separately the average of the standardized factors of the first and second group and the average for all the factors. These variabilities differ somewhat from the variability presented in per cent form (Ważny and Thornton 1991). In the scope of physiological factors (Fig. 1) the widest differentiation was shown by the growth rate, decay capacity and then mycelium dry mass and reduction of compression strength, the narrowest one was shown by the final moisture content.

Which regard to the toxicometric factors (Fig. 2) the widest differentiation was shown by the toxic value in relation to NaPCP determined by the agar-block method (mass loss criterion) and of CCA (strength reduction criterion). The further succession of differentiation comprised LD for NaPCP, ED_{50} of CCA and LD of CCA all in agar. A lack of any differentiation occurred for ED_{100} of CCA.

The complex differentiation of physiological factors (Fig. 3,a) was much lower than the differentiation of toxicometric factors (Fig. 3,b). The differentiation of standardized values for all the factors under study was relatively the narrowest (Fig. 3,c) for all under study.

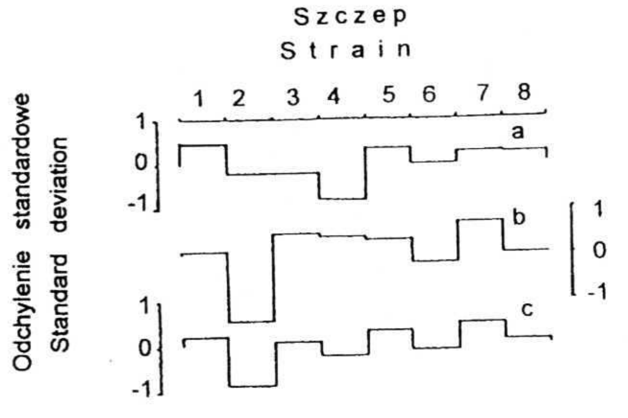

Fig. 3. Standardised values of complex factors of various strains of *S. lacrymans*:
1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - physiological factors, b - toxicometric factors, c - final complex factors)
Rys. 3. Wartości standaryzowane kompleksu czynników dla różnych szczepów *S. lacrymans*:
1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - czynniki fizjologiczne, b - czynniki toksykometryczne, c - wszystkie czynniki łącznie)

The numerical value of physiological factors of 8 *S. lacrymans* strains investigated (Fig. 4,a) has shown that they connect in three groups. The groups are decided according to whether or not the individual horizontal lines (each representing one strain) on Fig. 4, are connected at the precise position of the value of 0.714 on the scale of coefficient of similarity (see Materials and Methods for the reason why such a point represents the threshold level). The first group (Fig. 4,a) comprises the HFP 7802, DFP 16509, 16521 and 16522 strains, the second group FPRL 12E, Warsaw III and DFP 16508 strains, while the third constitutes the FPRL 12C strain only.

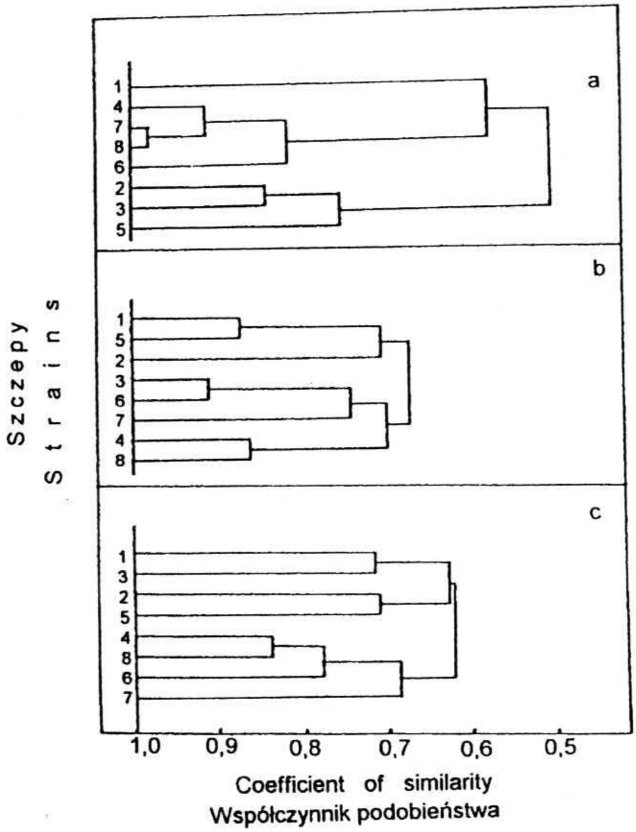

Fig. 4. Dendrograms of similarity of 8 strains of *S. lacrymans*:
1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7.DFP 16521; 8. DFP 16522;
(a - for physiological factors, b - for toxicometric factors, c - for the complex of all factors)
Rys. 4. Dendrogramy podobieństwa 8 szczepów *S. lacrymans:*
1. FPRL 12C; 2. FPRL 12E; 3. Warsaw III; 4. HFP 7802; 5. DFP 16508; 6. DFP 16509; 7. DFP 16521; 8. DFP 16522;
(a - dla czynników fizjologicznych, b - dla czynników toksykometrycznych, c - dla wszystkich czynników łącznie)

The analysis of toxicometrical factors of the above strains (Fig. 4,b) discerns two groups. The first consists of the FPRL 12E, FPRL 12C and DFP 16508 strains, while the remaining strains belong to the second group.

Analysis of all complex factors (Fig. 4,c) resulted in division of the investigated strains into four groups. The first group comprised the strains FPRL 12C and Warsaw III, the second group strains FPRL 12E and DFP 16508, the third HFP 7802, DFP 16509 and 16522. The fourth contained only the one strain DFP 16521.

Based upon the numerical analysis one can state that the investigated strains of *S. lacrymans* show in relation to a total of 15 physiological and toxicological factors a mutual similarity in 4 groups (Fig. 4,c). The first group comprises the English plus Polish strains, the second comprises the German plus one of the Australian strains, the third the Japanese plus two Australian strains and the fourth the remaining Australian strain. Incidentally, this separation indicates that real differences exist among the group of four strains that were isolated from widely separated buildings within the Australian state of Victoria (see Thornton 1989 for locations).

With respect to the physiological factors, the strains under study show similarity in three groups. Toxicometric factors, being of importance with regard to research on preservatives carried out in various countries, differentiated the *S. lacrymans* strains into two groups: the first of them comprises the English FPRL 12C, German FPRL 12E and Australian DFP 16508 strains, the second group containing the remaining five strains. The first group is interesting in that the German strain is included, although the data shows (Fig. 4,b) that the coefficient of similarity with the other two strains forming the group of three is only just higher than the threshold value of 0.714. The original data had led the authors to previously conclude that the German strain was more sensitive to preservatives than the other 7 strains (Ważny and Thornton 1986, 1989a,b).

RECAPITULATION

In conclusion, the numerical analysis has enabled the estimation of similarities and differences of the 8 *S. lacrymans* strains investigated on the basis of the 15 different, experimentally-determined factors.

Received in May 1998

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KOMPUTEROWA ANALIZA NUMERYCZNA RÓŻNYCH SZCZEPÓW *Serpula lacrymans* (Wulfen:Fr.) Schroeter apud Cohn

Streszczenie

W oparciu o wyniki badań 15 cech fizjologicznych i toksykometrycznych przeprowadzono analizę numeryczną 8 szczepów grzyba testowego *Serpula lacrymans* stosowanych w badaniach wartości grzybobójczych środków ochrony drewna.

Po standaryzacji uzyskanych danych określono matrycę podobieństwa tych wartości. Jako współczynnik korelacji zastosowano wartość "cos Q", gdzie Q stanowi kąt pomiędzy dwoma wektorami. Opracowano dendrogramy dla badanych wszystkich czynników łącznie oraz oddzielnie dla czynników fizjologicznych i toksykologicznych.

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