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A CORRELATION BY CHEMOMETRIC METHODS OF *SOLANUM TUBEROSUM* EPICUTICULAR WAX COMPONENTS AND PLANT INFESTATION WITH *LEPTINOTARSA DECEMLINEATA* (SAY)

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Abstract: The group composition of epicuticular waxes of the five Polish varieties of *Solanum tuberosum* were studied by HPLC, GC, and GC/MS. Alkanes, alcohols (primary and secondary), fatty acids and ketones (terminal and internal) were identified and quantified. The quantitative compositional results were analysed by chemometric methods such as cluster and principal component analysis. A correlation was found between Colorado potato beetle infestation and quantities of specific chemical groups in epicuticular waxes.

Key words: chemometrics, *Solanum tuberosum*, epicuticular waxes, plant infestation, *Leptinotarsa decemlineata* S.

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a pest of potato plants (*Solanum tuberosum)* causing serious damages in the crops. There are potato varieties that show some degree of resistance to *L. decemlineata* infestation (Flanders et al. 1992). This host-plant resistance can be incorporated as a useful component of an integrated pest management. According to chemical and open-field assays, many groups of compounds are involved in plant-insect interaction as the attractants or deterrents. A strong attractant for Colorado potato beetle is a „green leaf" odour which is perceived by many insect receptors (Wei-Chun and Visser 1978). However it is released by many plants not only potato. It is a general believe that glycoalkaloids are the strongest deterrents for potato defoliation by *L. decemlineata* larvae. Strong toxicity for human beings reduces the significance of these compounds in breeding searching for new varieties.

The layer of surface lipids present on the plant leaf surface called epicuticular waxes plays also an important role in the insect-plant interactions (Woodhead and Chapman 1986, Eigenbrode and Espelie 1995). Leaf surface chemicals can affect insect behaviour for example settling, moving, feeding and ovipositing. It can be assigned by the single compound or by the mixtures being complicated by synergism. The studies of the chemical influences of epicuticular wax compounds on insect behaviour are rather difficult because of rich wax compositions.

The epicuticular wax compositions vary with plant species and varieties. Differences in the wax compositions can be correlated with different resistance of cultivated plants using the methods of chemometrics what otherwise would be impossible.

We present here the results of qualitative and quantitative studies of epicuticular waxes of five Polish potato varieties and the correlation of these with open-field assays on the infestation by *L. decemlineata*. The correlation is performed by chemometric methods such as cluster (Massart and Kaufman 1983) and principal component routines (Mardia et al. 1979).

MATERIALS AND METHODS

Plants of *Solanum tuberosum* were grown from certified tubers in the field. The 3–4 week old plants were harvested and immediately transferred to the laboratory. The potato leaves were removed from stalks and weighted. The surface area of the sample of leaves was determined by photocopying, weighting the exposed area and taking the ratio to the known weight of 1 cm^2 of the paper. The epicuticular waxes were obtained by dipping the intact leaves into CH_2Cl_2 for 10 s and taking care not to immerse any cut part of the leaves in the solvent. Evaporation of the solvent to approx. 1 ml under reduced pressure at room temperature gave the wax sample, which was then separated into the various compound classes by HPLC.

A HPLC chromatograph with gradient elution system from Shimadzu (LC-6A pumps, SCL-6B system controller) equipped with a Knauer valve injector (4 µl sample loop) and light-scattering detector (Stołyhwo et al. 1983) were used. The separations were performed on a silica gel column (Econosil Silica 10 Micron, Alltech, 250 mm × 10 mm id.). Binary gradient elution with eluent A (light petroleum, b.p. 40–60°C) and eluent B (acetone: methylene chloride (15:85 by vol)) was applied with the linear gradient formation programmed from 100% of eluent A to 100% of eluent B during 20 min and then held at 100% of eluent B for 10 min. Total flow was maintained at 3.5 ml/min. A 1:12 splitter was applied to the column eluent, one part going to the detector and the rest being collected as fractions. The lipid classes were identified by a comparison of their retention times with those of commercial standards (Sigma, Aldrich).

The HPLC fractions were subjected to GC/FID and GC/MS studies as native and modified samples. TMSi (trimethylsilyl) derivatives were prepared from the lipid samples using a mixture of N,O-*bis*(trimethylsilyl)acetamide and trimethylchlorosilane (Aldrich) (85:15, by vol) in a vial with a teflon-lined septum at 70°C for 30 min. Fatty acid methyl esters were prepared from fatty acids using diazomethane in diethyl ether solution.

GC/FID analyses were carried out on a CE Instruments gas chromatograph GC 8000 Top with a flame-ionization detector using a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 0.25 μm film of EC-1 as the stationary phase (Alltech). The column temperature was programmed from 80°C to 320°C at a rate of 4°C/min and then held for 30 min for determining the TMSi derivatives of lipid samples or from 40°C to 320°C at a rate of 4°C/min and then held for 30 min for determining the native samples. The temperatures of injector and detector were 330°C. The injection split ratio was 1:30 and argon carrier gas at a pressure of 95 kPa was used. Relative proportions of the compounds measured were determined by measuring peak area. The quantitative analyses were carried out with *n*-tetracosane (Fluka) and *n*-tricosanoic acid methyl ester (Applied Science Lab.) as internal standards on the native and TMSi modified extracts of the potato epicuticular waxes.

Mass spectra (70eV) were measured on a TRIO 2000 mass spectrometer connected with a Hewlett-Packard 5890 gas chromatograph (GC/MS) equipped with the same column and under the same chromatographic conditions as mentioned for GC/FID. Helium was used as a carrier gas. The ion source was maintained at 270°C. Analysed compounds were identified by comparison of their mass spectra with published data.

The quantitative results of chemical group compounds of epicuticular waxes were analysed by chemometric methods after autoscaling for equal significance of variances (Kowalski and Bender 1972). Cluster analysis and principal component routines were used. EXCEL, STATISTICA and MATLAB softwares were applied.

According to quantitative analysis results, the concentrations of the six compound groups as the properties (variables) were assigned for five cases (potato varieties) which gave data vector matrix. Cluster analysis classified the varieties (cases) on their similarities (Massart and Kaufman 1983) in n-dimensional space. Principal component analysis was used to reduce the dimensions of the space for correlations (Mardia et al. 1979).

RESULTS AND DISCUSSION

Leaf epicuticular waxes of five Polish commercial potato varieties, Aster, Mila, Perkoz, Maryna, and Ibis were analysed by HPLC, GC and GC/MS. Homologous series of the compounds were identified and quantified as a sum in the groups (Tab. 1). The analysis of the group compositions will be reported separately. Chemical group quantitative results for alkanes, alcohols (primary and secondary), fatty acids and ketones (terminal and internal) were chosen as the properties (variables).

Cluster analysis based on Ward's method (Massart and Kaufman 1983) for varieties is presented in figure 1. The analysis distinguishes the cases with the maximal distance between Aster and Mila varieties, with the remaining three appearing between. This directly correlates with the beetle infestation. Open-field assay of *L. decemlineata* infestation on Polish potato varieties shows differences between varieties: Aster (the ratio of dead beetles to all beetles and larvae = 17), Perkoz (2.2), Maryna (0.77), Ibis (0.59) and Mila (0.16) (Roztropowicz 1994). But no conclusion could be drawn about chemicals involved in the interaction. Herein, the correlation

Table 1. The main chemical compound groups in the epicuticular waxes of potato leaves [in ng×cm^{-2} of leaf surface]

Potato variety	A*	B	C	D	E	F
ASTER	600	220	250	120	9	15
MILA	1600	270	220	90	40	110
PERKOZ	315	37	18	7	0.5	16
MARYNA	1050	84	107	3	0.5	13
IBIS	1500	128	13	2	0.5	0.05

* where letters stay for: A – alkanes, B – alkane-1-ols, C – fatty acids, D – methylketones E – internal ketones, F – alkane-2-ols

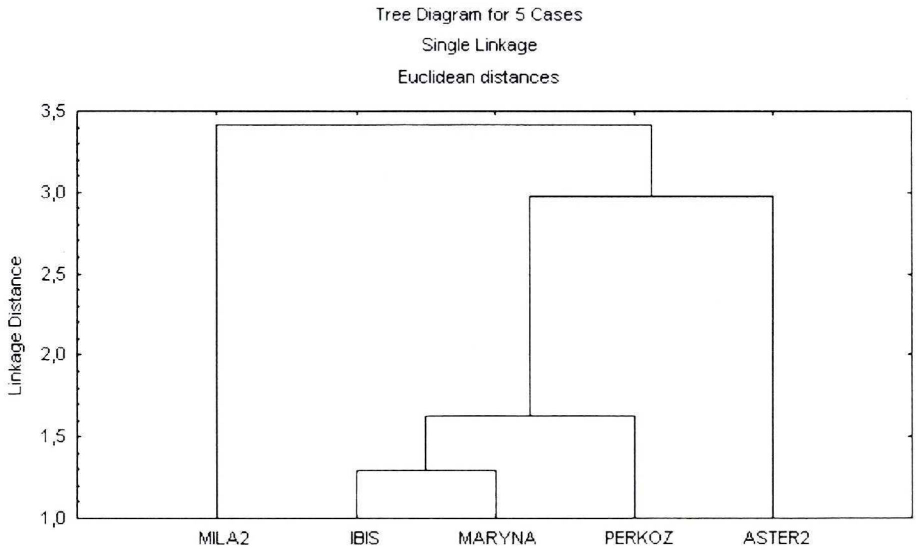

Fig. 1. Cluster analysis of five Polish potato varieties

Table 2. Eigenvalues and variances of data matrix of epicuticular wax component compositions. Extraction: principal components

Factors	Eigenvalues	% total variance	Cumulative eigenvalues	Cumulative % of variances
1	4.012369	66.87282	4.012369	66.87282
2	1.255176	20.91960	5.267546	87.79243
3	0.507070	8.45116	5.774615	96.24358
4	0.130147	2.16911	5.904762	98.41270
5	0.047619	0.79365	5.952381	99.20635

methods of n-variables in n-dimensional space are needed. Factor analysis is a solution of dimension reduction for a correlation. Table 2 contains eigenvalues and variances related to factors of the data set studied. Eigenvalues of the potato leaf waxes data matrix show three factors that stay for 96.2% of total variances. Thus it seems to be described by three factors only.

Group classification of the cases (potato varieties) in a similar way as degree of infestation was obtained in a plot of eigenvector 1 vrs 2 (Fig. 2). Factor 2 gives the maximal distance between resistant and non-resistant varieties. From the coefficients (loading) of the features in the factor 2, principal components such as fatty acids and methylketones were found to be important in Colorado beetle - potato ecology. However, these two groups appear in waxes of two varieties, Aster and Mila, both with diametrically different degrees of infestation. One methylketone, tridecanon-2, has been found to be toxic for *L. decemlineata* larvae (Kennedy et al.

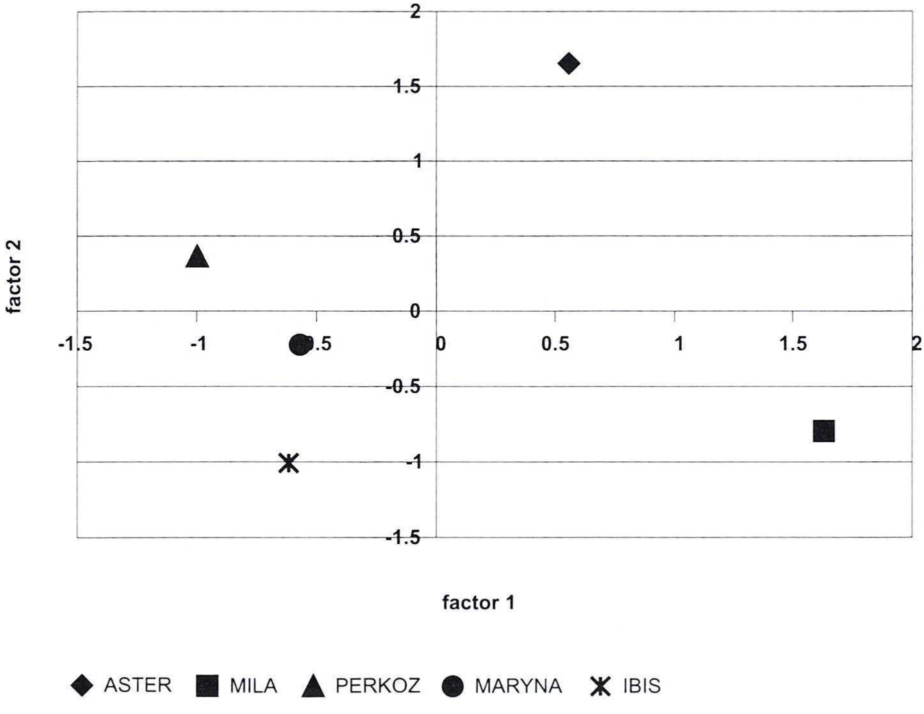

Fig. 2. Classification of potato varieties in factor 1 and 2

Table 3. Principal components in variables (properties) of epicuticular wax components. Factor loadings unrotated. Extraction: principal components

Compound groups	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
alkanes	0.449820	-0.795133	-0.396330	0.054256	-0.024624
alkane-1-ols	0.942821	0.038403	-0.257735	-0.139904	0.001943
fatty acids	0.843349	0.438739	-0.117583	0.286001	0.023019
methylketones	0.820021	0.524314	-0.104689	-0.146022	-0.061024
internal ketones	0.917419	-0.237272	0.267377	-0.052872	0.161202
alkane-2-ols	0.834140	-0.312681	0.432768	0.041417	-0.129496

1985). Similar compounds but with longer carbon chains were found in potato waxes. But it seems to us that specific composition of the waxes is responsible for the effect. Final conclusion about chemicals involved in ecology can be drawn after direct behavioural and antenographic analysis with the natural extracts and pure synthetical chemicals.

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POLISH SUMMARY

CHEMOMETRYCZNA ANALIZA ZALEŻNOŚCI MIĘDZY SKŁADEM WOSKÓW EPIKUTYKULARNYCH POLSKICH ODMIAN ZIEMNIAKA *SOLANUM TUBEROSUM* A ICH PODATNOŚCIĄ NA ŻEROWANIE STONKI ZIEMNIACZANEJ *LEPTINOTARSA DECEMLINEATA* SAY

Woski epikutykularne liści 5 odmian ziemniaka jadalnego zostały wyekstrahowane dichlorometanem i poddane analizie chromatograficznej HPLC z laserowym, aerozolowym detektorem promieniowania rozproszonego. Gradientowa elucja pozwoliła uzyskać wyniki jakościowej i ilościowej analizy następujących grup związków : n-alkanów, alkoholi (pierwszo- i drugorzędowych), kwasów tłuszczowych i ketonów (o grupach C=O w końcowym i wewnętrznym położeniu). Ilościowa zawartość grup związków chemicznych posłużyła do zbudowania macierzy danych. Po transformacji i autoskalowaniu dane zostały wykorzystane w analizie wiązkowej i głównych składników. Klasyfikacja chemometryczna składów wosków epikutykularnych odmian ziemniaka odpowiadała ich odpornościom na zasiedlanie przez stonkę ziemniaczaną.