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CORRELATION BETWEEN BEAN SEED SURFACE LIPIDS AND *ACANTHOSCELIDES OBTECTUS* SAY DEVELOPMENT

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Abstract: The influence of bean seed surface lipids on infestation of seeds by *Acanthoscelides obtectus* Say was investigated. The experiments were performed in dual-choice bioassays on three bean varieties: Blanka, Bor and Longina. The collected data for natural and solvent washed seeds concerned the number of ovipositions, embryo mortality, lack of seed-boring activity, dead larvae inside seeds and developed insects. The results clearly indicated that bean seed surface lipids are involved in all infestation stages, and could be used to distinguish resistant and non-resistant varieties of been. Chemical analyses revealed the following groups of surface lipids: wax esters, long chain primary alcohols, n-alkanes, sterols, fatty acids, squalene, aldehydes, monoacylglycerols, ketones and fatty acid esters. Quantitative composition of surface lipids was analysed using selected chemometric procedures to determine correlation with bioactivity. Cluster analysis of surface lipid composition enabled to distinguish resistant and non-resistant varieties. Fatty acids and monoacylglycerols were found to deter bean weevil infestation, while alkan-1-ols acted as attractants.

Key words: bean, *Phaseolus vulgaris*, seed surface lipid composition, *Acanthoscelides obtectus*, bioassays of infestation, chemometrics

INTRODUCTION

Insects are responsible for significant loss of grain and bean seeds during storage. This can be reduced by mechanical or chemical treatments. Both procedures

are costly and the latter is potentially hazardous to the environment. A better solution of the problem is to use natural plant resistance to insect pests. Plant natural resistance to herbivorous insects is a result of integration of numerous chemical and non-chemical factors, including plant nutritional quality and the presence of different monomeric and polymeric chemical components (Hedin 1983).

Chemical composition of the plant or seed surface is one of the factors which play an important role in plant-insect interaction (Espelie et al. 1991). The surface is a site where an insect makes the first contact with the plant. Specific surface lipid components have been shown to enhance or deter herbivory (Eigenbrode and Espelie 1995; Szafranek and Szafranek 2001). Organic solvent extracts containing surface lipids have also been demonstrated to have semiochemical activity (Winiecki et al. 1994). There were several reports implying that surface lipids affect oviposition (Städler 1986). As yet, little has been done about bean seed resistance to bean weevil infestation (Brzostek and Ignatowicz 1992; Nietupski et al. 2001). It has been found however, that some bean varieties are not preferred for oviposition. Experimental observations of larval mortality rate were related to seed testa hardness and toxic barriers (Thiéry 1984). It was suggested that a-amylase inhibitors, arcelin and phytohemagglutinin, play a role in bean resistance (Mirkov 1994). It was also shown that seed coat quality may influence the population dynamics of *A. obtectus,* because the removal of semiochemicals from the seed surface by washing with chloroform caused larvae to reject treated seeds (Thiéry et al. 1994).

The present work aims to develop a procedure for determining the effect of stimulants of bean seed surface on oviposition and biting by *Acanthoscelides obtectus*.

MATERIALS AND METHODS

Three bean varieties: Blanka, Bor and Longina, were chosen for the study according to their resistance established earlier (Ciepielewska and Fornal 1993; Fornal et al. 1993). Light petroleum (bp. 40–60°C) was applied for extraction to test the involvement of surface waxes in bean weevil infestation. *P. vulgaris* L. seeds (cultivars Blanka, Bor and Longina) were obtained from a seed and plant breeding farm (Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa "Torseed" S.A., Toruń, Poland) from the 1998 harvest. The experiment, in which the development of bean weevil was studied, was conducted at the turn of 1999 and 2000. The geometrical features of investigated bean seeds are presented in Table 1.

Bioassays. Dual – choice bioassays in 10 replications for each variety were conducted. For each variety, natural and solvent extracted seeds were tested. Five natural and five extracted seeds were placed in two glass dishes in one container

Table 1. Geometrical features of investigated bean seeds (average values)*

Bean variety	No of seeds	Area (mm^2)	Perimeter (mm)	Length (mm)	Width (mm)	Circularity	Elongation
Blanka	23	148.76	45.28	16.43	10.95	0.9	1.5
Bor	32	49.73	27.37	10.88	4.56	0.83	1.94
Longina	29	106.23	39.12	15.39	8.5	0.87	1.82

*Measurement was done using Laboratory Universal Computer Image Analysis LUCIA ver. 4.80

together with five couples of adult insects. Individuals of bean weevil used in the experiment originated from a mass breeding population maintained at the Department of Phytopathology and Entomology of the UWM in Olsztyn. One-day old individuals were settled on bean seeds. The insects were removed after 10 days when the females laid the eggs. The experiments were performed at constant temperature 27 ± 2°C and relative humidity 75 ± 2%. The following data were determined: number of eggs (1), number of dead embryos (2), number of larvae that did not penetrate into the seeds (3), number of dead larvae inside the seeds (4) and number of developed beetles (5).

Chemical analysis. Seed surface lipids were extracted with light petroleum (bp. 40–60°C) for 1 min and extracts were concentrated under vacuum at 25°C. Concentrated extracts were subjected to qualitative and quantitative analysis by high performance liquid chromatography (HPLC-LLSD), gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). HPLC gradient separation (from 100% n-hexane to 100% of a mixture of methylene chloride and acetone 85:15 V/V) was performed on a LP6A Shimadzu liquid chromatograph, equipped with a silica gel Econosil Silica 5 Micron column (250 mm × 4,6 mm, Alltech) and a laser light scattering detector. The HPLC separated fractions were GC and GC-MS analysed as the native compounds and after derivation. Methyl esters and silyl ethers and esters were used. GC analyses were performed using 8000 TOP CE gas chromatograph equipped with a 30 m DB-1 HT capillary column at temperature programme of 4°C/min between 40°C and 380°C. Similar GC conditions were used for GC-MS runs. Quantitative bean seed lipid composition results were evaluated by chemometric methods. After transformation and autoscaling, the data were analysed by cluster method (Massart and Kaufman 1983) and principal component (PC) routines (Mardia et al. 1979; Otto 1999).

RESULTS AND DISCUSSION

The bean beetle *Acanthoscelides obtectus* is regarded as a monophagous species that can complete its development both in maturing and in stored seeds. Females deposit batches of eggs either in the cavity of mature pods or on stored bean seeds. Both pods and seeds are thought to have their surface covered with chemicals which control the insect behaviour. In the dual-choice assays differences between naturally coated and light petroleum washed seeds were found, although they were not confirmed statistically (Table 2).

Relative oviposition rate (K_1/E_1) divides bean varieties into two groups characterised by higher (above one, Blanka variety) and lower (below one, Bor and Longina varieties) values. This suggests the presence of chemical compounds on the surface with attractant (Blanka) and deterrent (Bor, Longina) activities for oviposition. The dead embryo ratio (K_2/E_2) for Blanka variety (1.09) is different from those for Bor (0.61) and Longina (0.53) cultivars. Higher value of this ratio means negative effects of the surface lipids on hatching. Seeds of cv. Bor bean had the smallest surface area among all the bean cultivars tested (Table 1). Their average surface area was 49.73 mm^2, while seeds of cv. Longina had an average surface area of 106.23 mm^2 and the surface area of cv. Blanka seeds was 148.79 mm^2. Al-

Table 2. Dual–choice assays of *Acanthoscelides obtectus* at different development stages

Bean variety		Different development stages of *Acanthoscelides obtectus*														
		oviposition			dead embryos			not penetrating larvae			dead larvae found in seeds			developed beetles		
		K_1[1]	E_1[2]	K_1/E_1	K_2	E_2	K_2/E_2	K_3	E_3	K_3/E_3	K_4	E_4	K_4/E_4	K_5	E_5	K_5/E_5
Blanka	n	657	563	1.16	214	195	1.09	208	155	1.34	212	193	0.11	23	20	1.15
	%	100	100	–	32.5	34.6	–	31.7	27.5	–	32.3	34.3	–	3.5	3.6	–
Bor	n	265	472	0.56	71	115	0.61	95	153	0.62	91	192	0.47	8	12	0.66
	%	100	100	–	26.8	24.4	–	35.8	32.4	–	34.3	40.7	–	3.1	2.5	–
Longina	n	351	521	0.67	80	150	0.53	141	233	0.6	127	111	1.14	3	27	0.11
	%	100	100	–	22.8	28.8	–	40.1	44.7	–	36.2	21.3	–	0.9	5.2	–

[1]K – number of eggs found on natural seeds
[2]E – number of eggs found on solvent extracted seeds

though the small surface area of cv. Bor seeds could have had some effect on the number of eggs laid by female beetles of *A. obtectus*, it did not influence the numbers of bean weevil at the remaining developmental stages examined.

Non-penetrating larvae ratio (K_3/E_3) tends to be similar, i. e. higher for Blanka (1.34) and lower for Bor (0.62) and Longina (0.60) varieties. Higher K_3/E_3 ratio means negative effect of surface lipids on locomotory and boring behaviour of larvae. However, boring activity of bean weevil larvae has been found to be successful in 90% cases for freshly matured seeds, compared to 20% in older seeds, and even less than 10% in artificially dried ones (Stamopoulos and Huignard 1980; Thièry 1982). This represents the influence of physical characteristic of seed testa on boring activity of larvae.

The number of dead larvae found in seeds were very similar for Blanka seeds but varied for seeds of Bor variety. This characteristic is controlled by seed toxicity alone. In the final test, developed beetles ratio (K_5/E_5) was found to be higher for Blanka (1.15) and lower for Bor (0.66) and Longina (0.11) varieties. To correlate the results of bioassays of insect development with seed surface lipid composition, relevant qualitative and quantitative analysis were performed. Chemical analysis of bean seed surface lipids provided the following group of chemicals: wax esters, long chain primary alcohols, n-alkanes, sterols, fatty acids, squalene, aldehydes, monoacylglycerols, ketones and fatty acid esters (Table 3).

The lipids of bean seed surface consist mostly of wax esters of long chains alcohols and fatty acids (84.5%, 86.6% and 69.2% of the total extracts of the cultivars Bor, Longina and Blanka, respectively) with even and odd numbers of carbon atoms, ranging from C_{38} to C_{58}. Primary alcohols are the second major group of compounds, and their percentage in total extract was 8.4; 11.2; and 22.4 in the cultivars Longina, Bor and Blanka, respectively. The most abundant are alcohols with even numbers of carbon atoms, although those with odd numbers are also significant. n-Alkanes (3.0% in Longina cultivar; 2.2% in the Bor and 6.5% in the Blanka), sterols (0.8%; 0.5% and 0.9%, respectively), fatty acids (0.6%; 0.7% and 0.1%), squalene (0.3%; 0.3% and 0.4%), aldehydes (0.2%, 0.3 % and 0.6%) and monoacylglycerols (0.1%; 0.2% and 0.01%) were found to be less significant compounds.

Table 3. Chemical composition of bean seed surface lipids [ng/cm^2]

Group of compounds	Blanka variety	Bor variety	Longina variety
A) Wax esters	2154.40	1482.80	1713.30
B) Alkan-1-ols	698.50	197.60	165.80
C) n-Alkanes	201.60	39.40	58.90
D) Squalene	12.10	4.60	6.00
E) Sterols	27.10	8.80	16.50
F) Fatty acids	1.00	11.60	12.40
G) Aldehydes	18.30	5.20	3.20
H) Ketones	0.60	0.07	0.10
I) Monocylglycerols	0.05	4.00	0.60
J) Fatty acid isopropyl esters	1.00	0.30	0.40
K) Fatty acid methyl esters	ca. 0.05	0.30	0.40
L) Fatty acid ethyl esters	ca. 0.05	0.10	ca. 0.05

Aldehydes present in bean seed waxes with 22 to 31 carbon atoms showed a similar distribution in Longina and Blanka varieties. Aldehydes ranging from C_{18} to C_{28} were found in cv. Bor's waxes. Components with low-molecular weight (C_{18}–C_{22}) comprise half of the total aldehyde concentration in Bor. The surface waxes of the three cultivars differ in the relative composition of low-weight fatty acids and n-alkanes. Beside the groups of lipids mentioned above, there were also ketones, isopropyl, methyl and ethyl esters of fatty acids, which made up less than 0,1% in each group.

The plant-insect interaction is usually a complex phenomenon, in which specific compounds at appropriate relative concentrations in mixtures of precisely determined composition are involved. Thus, to correlate chemical compounds with the bioactivity observed, chemometric methods such as cluster and principal component routines need to be used. Wax esters, long chain primary alcohols, n-alkanes, sterols, fatty acids, squalene, aldehydes, monoacylglycerols, ketones and fatty acid esters concentrations found on the seed surfaces were chosen as the properties (variables) in the matrix data. Cluster analysis based on Ward's method (Massart and Kaufman 1983) for varieties is presented in Figure 1. Bean variety classification based on seed surface lipid composition clearly distinguished Blanka from Longina and Bor varieties. This is in full agreement with the bioassay results, but no information about chemicals involved was obtained. For such correlation, principal component analysis has to be done.

Principal component (PC) analysis classifies the surface lipids of bean seeds by factors. Eigenvalues of surface lipid data matrix show two factors, which explain more than 96 % of total variance. Thus the variance seems to be described by these two factors only (Fig. 2). The slope reaches nearly zero after the second factor.

As it can be seen from Figure 3, the three objects (varieties) are completely separated. The projection of the objects on component 1 separates Blanka (non-resistant variety) from Longina and Bor (resistant varieties).

The importance of variables can be established from the plots of PC loadings. Figure 4 shows the loading plot of the first two PC of the surface lipids as a projection of the features on the principal components. There are three separated clusters

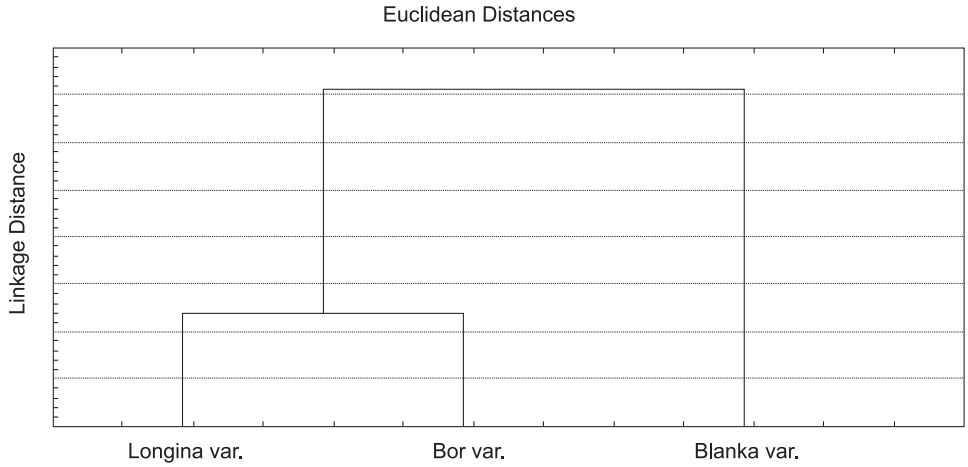

Fig. 1. Cluster analysis of Blanka, Bor and Longina bean varieties based on seed surface lipid composition

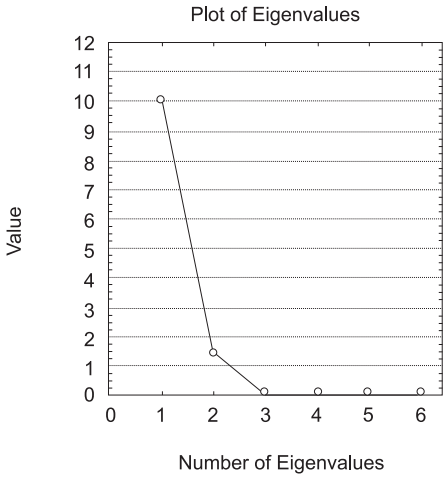

Fig. 2. Scree-plot for the factors

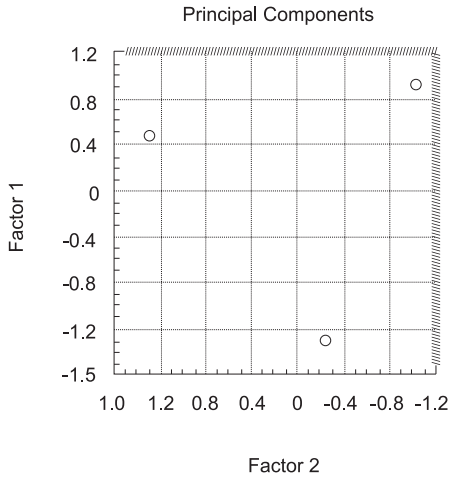

Fig. 3. Principal component scores for surface lipids data of bean varieties

of the variables strongly correlated within the groups. Thus, only one representative of a group can be chosen for correlation studies. Consequently, alkan-1-ols, fatty acids and monoacylglycerols were chosen as indicator groups because their concentrations differ significantly in non-resistant and resistant varieties.

The non-resistant variety features high levels of long chain alcohols but low concentration of fatty acids and monoacylglycerols, contrary to the resistant varieties, where the alcohols were found to be lower but fatty acids and monoacylglycerols higher in concentration. It seems that alkan-1-ols are attractants but fatty acids and

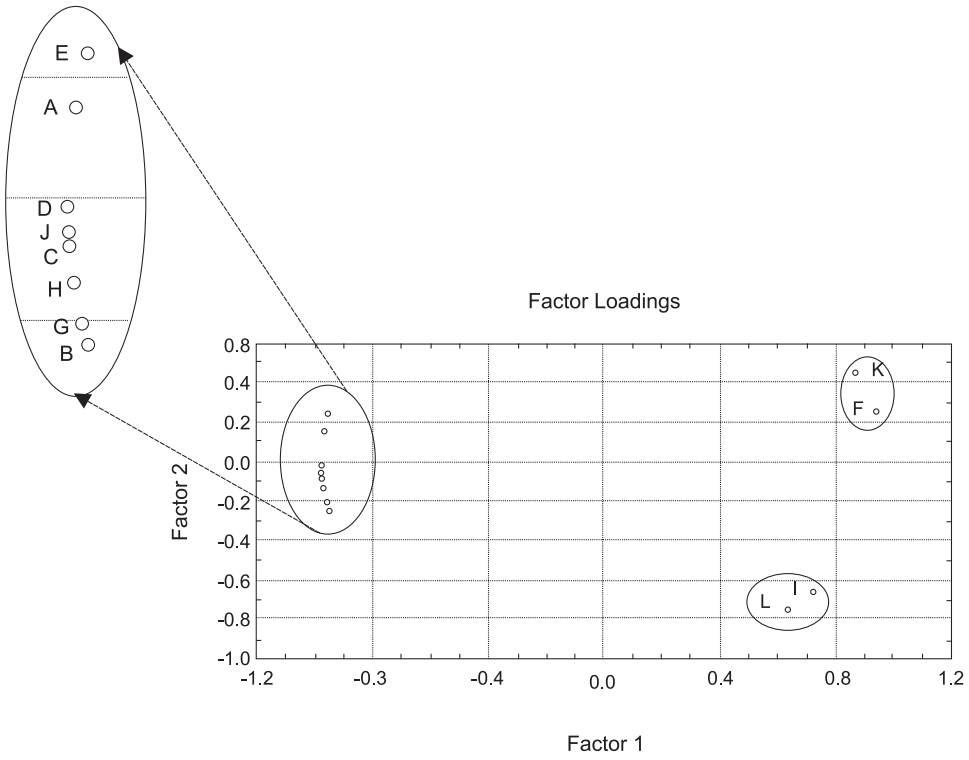

Fig. 4. Grouping of the variables in factors 1 and 2 (see Table 3)

monoacylglycerols are deterrents. The conclusion needs to be confirmed by direct behavioural studies using more varieties.

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POLISH SUMMARY

KORELACJA POMIĘDZY SKŁADEM WARSTWY WOSKÓW POWIERZCHNIOWYCH NASION FASOLI A ROZWOJEM STRĄKOWCA FASOLOWEGO (*ACANTHOSCELIDES OBTECTUS* SAY)

W przeprowadzonym doświadczeniu badano wpływ warstwy wosków powierzchniowych nasion fasoli na rozwój strąkowca fasolowego. Eksperyment przeprowadzono z wolnym prawem wyboru na nasionach 3 odmian fasoli: Blanka, Bor i Longina. Badano rozwój szkodnika na nasionach kontrolnych i wymywanych eterem naftowym, określając liczbę jaj złożonych przez samice, śmiertelność embrionów, larw niepodejmujących wgryzania, śmiertelność stadiów wewnątrz nasion i liczbę chrząszczy potomnych. Uzyskane wyniki wskazują, że woski powierzchniowe nasion fasoli mogą wpływać na poszczególne stadia rozwojowe strąkowca fasolowego, a tym samym mogą być czynnikiem różnicującym odmiany fasoli odporne i nieodporne na żerowanie *A. obtectus* Say. Analizy chemiczne wykazały, że w skład warstwy wosków powierzchniowych nasion fasoli wchodzą: estry kwasów tłuszczowych, długołańcuchowe alkohole pierwszorzędowe, n-alkany, sterole, kwasy tłuszczowe, skwaleny, aldehydy, monoacyloglicerole i ketony. Stwierdzono, że kwasy tłuszczowe i monoacyloglicerole działają odstraszająco na strąkowca fasolowego, natomiast niektóre alkohole są atraktantami.