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THE MORPHOLOGICAL VARIATION OF *MYZUS PERSICAE* (HEMIPTERA: APHIDIDAE) FROM PEACH AND TOBACCO IN SERBIA AND MONTENEGRO

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Abstract - Multivariate morphometric analysis was used to compare nine characteristics of 47 populations of *Myzus persicae* (Sulzer) originating from two host-plants, peach and tobacco, from 13 localities in 2004 and 34 localities in 2005, in Serbia and Montenegro. Multivariant discriminant analysis showed there to be a distinct discrimination between the populations from the peach and tobacco host-plants. The most important discrimination characteristics are the ultimate rostral segment length and processus terminalis length, which are greater in the aphids from tobacco than in those from peach. This is the first indication that in this part of Europe there are two subspecies: *M. persicae* (Sulzer) and *M. persicae nicotianae* Blackman.

Keywords: Aphids, green peach aphid, morphology, tobacco aphid, Serbia, Montenegro

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INTRODUCTION

Myzus persicae (Sulzer) (Hemiptera: Aphididae) is an extremely polyphagous aphid species that feeds on over 400 plant species from 40 different families, and it is a major pest to many crops (Blackman and Eastop, 2000). In Serbia, *M. persicae* was found on more than 40 herbaceous plants (Petrović, 1998). It has two overwintering strategies: holocyclic with *Prunus persicae* (L.) as the primary host, and anholocyclic on many secondary hosts (Blackman and Eastop, 2000; Petrović-Obradović, 2003). For very long time populations of *M. persicae* on tobacco, *Nicotiana tabacum* L., have been considered to be different from populations on other plants (Blackman and Eastop, 2007). Multivariate morphometric analysis has revealed that populations of *M. persicae* feeding on tobacco are morphologically distinct from those on other host-plants, and the tobacco-feeding form has been given the name *M. nicotianae* Blackman (Blackman, 1987). However, several studies using molecular methods have provided evidence that the aphids on tobacco are not distinct at the species level from other populations of *M. persicae* (Clements et al., 2000a; Clements et al., 2000b; Field et al., 1994; Margaritopoulos et al., 1998). Also, it was considered that *M. nicotianae* populations were permanently parthenogenetic until holocyclic populations were found in Greece on peach (Margaritopoulos et al., 2002). These data suggest that tobacco-feeding aphids cannot be considered a valid species, and Eastop and Blackman (2005) proposed that the tobacco-adapted form should be called *Myzus persicae* ssp. *nicotianae*.

The morphological differences between tobacco-adapted and non-tobacco-adapted forms were investigated mostly in Greece, Italy and Japan (Margaritopoulos et al., 2003; Margaritopoulos et al., 2007). One sample from Serbia was included in studies of the morphological variation within and between the populations of the group of *M. persicae* (Blackman, 1987). But there has not been detailed research into the morphology of populations of *M. persicae* from peach and tobacco in this part of Europe (Serbia and Montenegro). For these reasons it was deemed important to investigate.

MATERIAL AND METHODS

The aphid samples were collected from tobacco fields and peach orchards in Serbia and Montenegro (Fig. 1) in the spring and summer of 2004 and 2005. The infested leaves were placed in plastic bags. The bags were placed in isolated plastic containers containing ice packs and transferred to the laboratory. Apterous adult females from each sample were collected and preserved in tubes filled with ethyl alcohol (75%) until slide preparation. The aphids were mounted on slides according to the method of Blackman and Eastop (1984). Aphids from a total of 47 samples were measured, 17 of which were collected from peach (140 specimens) and 30 from tobacco (278 specimens). A minimum of four and maximum of 12 apterous adult parthenogenetic females were measured from each sample.

Fig. 1. Collection sites and host – plants of *Myzus persicae* in Serbia and Montenegro.

T – tobacco, P – peach, 1T – Male Pijace (MpiD05, MPi205D, MPi5D05, MPi6D05, K2d05), 2T – Mali Pesak (MpeKD05, Mpe2D05), 3T – Kanjiža (K1D05, K2D05), 4T – Čoka (CC5D04, CC8D04), 5T – Senta (S1D04, S3D04, S105D, S2D05, S305D, S4D05, S5D05), 6T – Ostojićevo (OD04), 7T – Vačica (VsD05), 8T – Kukujevci (K05D), 9T – Futog (FB05D), 10T – Beška (BsD04, Bs05D), 11T – Bački Vinogradi (BV05D), 12T – Mladenovac (MD04), 13T – Tuzi (T05D), 14T – Sukuruć (SuDo5), 15T – Vranj (V1D05, V3D05), 1P – Horgoš (HB04), 2P – Bela Crkva (BCB04, BCB05), 3P – Stara Pazova (SPBO4), 4P – Nova Galenika (NGB04), 5P – Galenika (GB05), 6P – Smederevo (SmB05), 7P – Topola (T05B), 8P – Radmilovac (Rdb05), 9P – Ritopek (RtB04, RtB05), 10P – Vinča (V1B05, V2B05, V3B05), 11P – Brestovik (B1B05, B2B05), Podgorica (PgB04)

Nine variables (characteristics, traits) were measured: the length of the third antennal segment (ant III), the length of the base of the sixth antennal segment (base VI), the length of the terminal process of the sixth antennal segment (pt), the length of the ultimate rostral segment (urs), the length of hind femur (hf), the length of second segment of the hind tarsus (ht2), the length of siphunculus (ls), the maximal width of the distal swollen part of the siphunculus (mws) and the length of cauda (lc). The methods of measurement are illustrated by Ilharco and van Harten (1987). All measurements were carried out with a phase contrast microscope (LEICA DMLS).

All variables that were used in the analyses followed normal distribution with homogeneity of variance. The data were tested for normality using Kolmogorov–Smirnov and Shapiro-Wilk tests.

When there are several dependent variables, multiple tests of (likely) correlated dependent variables (Tabachnik and Fidell, 1996) suffer from inflated Type I errors (Zar, 1999).

A multivariate analysis of variance (MANOVA) allows for the comparison of the population means of all variables of interest at the same time (multivariate response), rather than considering multiple responses as a suite of univariate responses (Zar, 1999). This reduces the magnitude of Type I errors. The statistical significance of MANOVA can be determined in a variety of ways. The most often used statistic test of Wilks' lambda was applied (Zar, 1999).

One-way MANOVA was used to examine the effects of the host-plant on *M. persicae* morphological variation. The fixed factors were the two host-plants (peach and tobacoo). When the MANOVA was statistically significant, subsequent

univariate ANOVAs were performed to elucidate which responses contributed to the significant multivariate response. For this purpose a Unequal N HSD post-hoc test was used. Additionally, to describe and interpret the effects from the MANOVA, a multivariate discriminant analysis (DA) was used following MANOVA. Discriminant analysis was employed on all the data in order to determine the relative importance of characteristics as discriminators between a priori groups and the relative positions of the centroids of those groups (Manly, 1986). In addition, canonical variables were computed. All statistical analyses were conducted using the Statistica 6 software package (StatSoft, Inc 2001).

All the aphid samples from tobacco are from peach-growing regions and were collected during summer, while samples 4P, 5P, 6P, 7P, 8P, 9P, 10P and 11P were collected in tobacco-free areas from spring to early summer.

RESULTS

Descriptive statistics of the quantitative traits of *M. persicae* are given in Table 1. The one-way MANOVA of *M. persicae* from the two host-plants revealed the significant effects of host-plant interaction on *M. persicae* morphological variation ($p<0.001$, Table 2). The univariate results for each of the traits are shown in Table 2.

Tab. 1. Mean values for nine morphological traits of 47 *Myzus persicae* populations from peach and tobacco (the measurement unit is μm).

Samples \ Characters	antIII	baseVI	pt	urs	hf	ht2	ls	mws	lc
BCB04	408,00	125,97	406,30	115,77	623,73	123,93	478,31	47,03	211,65
SPB04	375,94	120,21	404,36	122,40	654,69	130,41	453,17	46,92	209,83
RtB04	358,46	127,50	327,86	116,57	616,37	126,77	426,94	51,00	217,11
HB04	447,87	128,89	479,86	113,13	686,17	112,66	530,86	47,94	223,47
PgB04	374,00	115,60	387,60	114,03	559,30	119,00	414,80	45,90	180,20
NGB04	375,36	120,87	368,22	116,79	618,12	122,91	428,40	48,92	205,53
CC5D04	455,94	128,52	514,08	121,89	693,60	120,36	506,43	43,46	212,16
OD04	432,23	129,41	488,87	127,88	673,20	123,68	498,53	44,36	216,75
MD04	458,49	128,52	493,68	129,54	710,94	119,04	548,76	45,90	228,48
CC8D04	393,43	130,01	465,12	127,50	614,91	109,29	476,85	44,92	209,83
S1D04	455,60	137,70	515,95	130,05	746,30	129,20	561,85	44,33	258,40
S3D04	448,23	124,67	540,60	121,83	640,90	112,20	472,60	40,80	193,23
BsD04	465,12	133,62	540,09	122,40	713,49	123,67	537,54	47,60	230,01
BCB05	405,96	113,00	432,48	117,00	579,36	109,14	448,80	41,41	191,25
V1B05	387,60	123,79	390,51	119,64	598,16	120,21	432,77	46,77	198,17
SmB05	379,10	118,75	421,60	114,58	562,70	106,25	448,80	43,35	188,70
V3B05	375,87	107,50	379,44	121,00	557,94	116,79	414,12	46,82	198,90
V2B05	376,38	118,06	391,00	121,75	581,91	120,36	431,46	48,86	195,84

Tab. 1. Ctd.

Samples \ Characters	antIII	baseVI	pt	urs	hf	ht2	ls	mws	lc
RtB05	367,66	115,00	356,07	115,45	589,75	121,94	408,00	43,30	191,95
GB05	391,68	118,00	372,30	117,75	607,92	123,33	424,32	49,88	195,84
B2B05	389,87	123,33	396,67	121,94	598,40	123,53	454,47	48,17	213,07
T05B	363,12	120,25	387,60	117,00	584,46	121,38	442,68	47,50	195,84
B105B	403,92	124,50	401,88	122,25	610,98	111,69	447,78	47,43	204,00
Rdb05	385,05	121,88	397,80	124,38	576,30	114,25	441,15	45,14	206,55
K2do5	428,40	129,00	456,45	130,00	716,04	128,01	558,96	44,88	238,68
MPe2D05	477,36	135,00	492,69	132,00	756,84	123,42	571,20	46,92	263,22
K05D	429,42	122,75	411,57	129,00	695,64	120,70	531,93	45,25	235,05
S5D05	464,10	131,00	508,47	133,25	748,68	123,42	586,50	49,50	250,41
BV05D	454,92	131,75	451,86	130,00	720,00	117,30	555,90	46,41	243,78
K1D05	443,19	132,25	481,44	132,25	717,06	123,00	565,59	48,25	254,49
S205D	447,80	130,25	474,30	133,00	737,46	121,89	562,98	47,43	242,76
T05D	469,20	135,25	510,00	132,75	687,48	117,30	512,04	45,90	214,20
Bs05D	437,58	127,75	466,93	128,50	727,26	123,42	549,84	45,90	236,64
MPi205D	471,05	132,27	463,64	131,14	749,24	122,86	560,07	47,48	248,51
S305D	466,14	129,50	491,64	130,56	735,42	127,50	547,74	44,88	229,50
S1D05	451,35	131,88	473,03	129,06	729,30	124,95	566,10	45,64	249,90
SuD05	481,10	134,58	504,90	133,75	717,40	121,55	532,10	47,43	236,30
FB05D	438,60	128,13	481,91	130,63	712,73	126,23	541,88	46,79	247,35
MPi6D05	436,56	133,25	512,55	132,25	725,22	125,46	559,98	44,78	250,92
S4D05	456,45	131,00	502,86	131,39	741,54	122,40	570,18	45,49	241,23
VsD05	446,25	128,00	466,14	130,25	728,73	120,70	570,18	46,10	245,82
MPeKD05	470,22	132,50	489,60	131,00	755,82	129,54	552,84	46,41	252,96
MPi5D05	461,04	127,25	465,12	131,25	739,50	125,15	554,88	45,29	243,78
V3D05	462,06	126,94	463,08	129,25	708,90	118,12	521,22	47,63	240,72
V1D05	475,32	134,50	514,08	130,75	700,74	119,85	547,74	47,02	240,21
K2D05	472,26	135,50	488,47	129,13	761,94	126,08	579,36	47,74	256,53
MPiD05	472,26	129,75	495,27	131,50	742,56	126,99	559,98	47,84	251,94
All Grps	431,88	127,19	455,69	125,89	680,49	121,49	512,18	46,39	226,72

Tab. 2. Results of one-way MANOVA on the considered traits of *M. persicae* from two Host-plants (as factor), P-level: * = p<0.05; ** = p<0.01; ***= p<0.001.

Main effect F (23, 85)	Wilks' λ	F (9, 379)
Host-plant	0.205241	163.07***
Specific effects	Host-plant	
Characters	F (1, 387)	
antIII	323.97***	
baseVI	134.10***	
pt	379.41***	
urs	485.00***	
hf	571.35***	
ht2	18.41***	
ls	549.23***	
mws	3.78^{ns}	
lc	271.93***	

(hf) length of hind femur, (pt) length of processus terminalis, (urs) length of ultimate rostral segment, (ht2) length of second segment of hind tarsus, (ls) length of siphunculus, (antIII) length of third antennal segment, (mws) maximal width of distal swollen part of siphunculus, (lc) length of cauda, (baseVI) length of base of sixth antennal segment.

Results from the Unequal N HSD post-hoc test revealed that only the variable mws (maximal width of distal swollen part of siphunculus) is non-significant.

The result of the discriminant analysis showed there to be distinct discrimination between the populations from tobacco and peach based on the first canonical axis (CVA1) (Fig. 2).

The total correct percentage of the classification matrix of all the groups was high (97.368%).

From the standardized coefficients for the canonical variables it is evident that the first canonical variable (CVA1) describes 63.43 % of the total variability; the first and second together (CVA1+CVA2), 76.88 %; the first, second, and third, 84.31 %. The length of the urs (ultimate rostral segment) and length of the pt (processus terminalis) contribute most to this discrimination. The following characteristics contribute, but to a lesser extent: length of hf (hind femur) and length of ls (siphunculus) (Table 3).

The result of DA showed the most important and distinct discrimination to be between the populations from tobacco and peach based on the first canonical variable (Fig. 2).

Since the first and second discriminatory axes describe the bulk of variability (76.88%), discrimination is clearly evident in Fig. 2 between the populations of aphids from peach (left-hand grouping of centroids, numbers colored in blue) along the first discriminatory axis, and the populations from tobacco (right-hand grouping of centroids, numbers colored in black).

This function is a positive coefficient for the variables urs, pt, hf, and ls, but has negative weight for the variables mws and ht2 (Table 3).

Tab. 3. Summary of discriminant function analysis (DA) with standardized coefficients for canonical variables on the first (CVA1) and second (CVA2) canonical axes; (EV= eigenvalues and CP= cumulative proportions).

Characters*	CVA 1	CVA 2
hf	**0,446**	**-0,452**
pt	**0,501**	**0,673**
urs	**0,523**	0,052
ht2	-0,283	**-0,335**
ls	**0,412**	-0,085
antIII	-0,170	**0,414**
mws	-0,299	-0,062
lc	0,056	**-0,429**
baseVI	-0,214	-0,035
EV	6,898	1,463
CP	0,634	0,769

*Abbreviations as on Table 2.

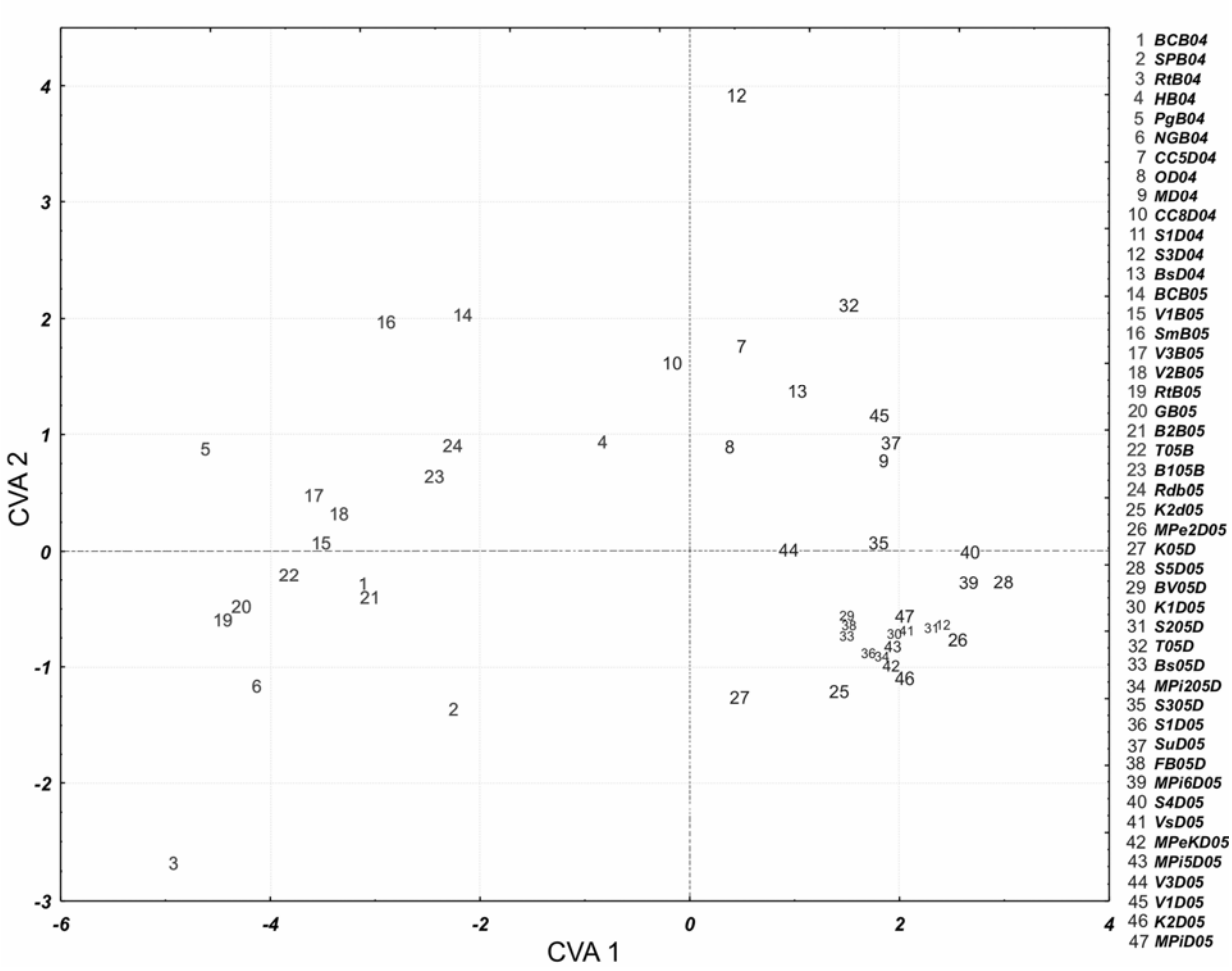

Fig. 2. Plots of scores (means of canonical variables) of the first two canonical axes (CVA1 and CVA2) of 47 populations of *Myzus persicae* from two different host plants and geographically distant areas in Serbia and Montenegro (numbers = population means = centroids) (blue numbers – samples from peach populations and black numbers – samples from tobaco populations).

Both subspecies are found in two basic color forms, green and red. The green form was dominant in the peach, while the red form was most numerous in tobacco. The aphid from peach is most often light-green, while the subspecies from tobacco is dark-red. The green form of aphid from tobacco is considerably darker than the green form from peach.

The symptoms caused by these aphids on the given hosts are also different. Both subspecies form dense colonies, most often on the reverse side of the leaf, but the peach aphid curls the peach leaves, whereas the tobacco aphid causes no deformations to tobacco leaves. Both subspecies are economically very significant pests.

DISCUSSION

In the present study, morphological variation was examined in *M. persicae* populations from peach and tobacco host-plants. As previous results have shown that morphological characteristics are stable after many years of feeding on the same secondary host (Blackman, 1987; Margaritopoulos et al., 2000), such characteristics were measured in the aphids collected from the field.

Nine characteristics were measured, and multivariate morphometric analysis revealed that aphids feeding on peach are morphologically distinct from those feeding on tobacco. The most important discriminative characteristics are the lengths of the ultimate rostral segment length and processus terminalis, which are greater in populations from tobacco.

Aphids with a longer last rostral segment, processus terminalis, hind femur, and siphunculus are tobacco aphids and on the basis of these characteristics it is easy to separate the aphids from peach and tobacco host-plants.

Canonical discriminant analysis clearly indicates that aphids from peach differ morphologically from those from tobacco, and that the differences are great enough for us to regard the populations from these two hosts in Serbia and Montenegro as separate subspecies: *M. persicae* (Sulzer) and *M. persicae nicotianae* Blackman.

All aphid samples from tobacco are from peach-growing regions, meaning that both subspecies exist at the same locations. The clones originating from peach were not found on tobacco plants. In southern Italy, tobacco-adapted and non-tobacco-adapted forms co-exist in the same region but they have different reproductive strategies. The tobacco-adapted form has no ability to reproduce sexually (Margaritopoulos et al., 2003). Holocyclic clones of the subspecies from tobacco were not found in Spain, Germany and France (Kephalogianni et al., 2002) But, in northern Greece and Japan, in the main peach-growing regions, the tobacco-adapted form has the ability to overwinter as diapause eggs on peach (Margaritopoulos et al., 2002, Margaritopoulos et al., 2007). In Serbia and Montenegro the life cycle is not known at the moment.

The present investigations indicate the existence of two subspecies that clearly differ morphologically. However, in order to determine the degree of separation of these subspecies and the possibility of their imminent divergence into two species (a question considered by Eastop and Blackman (2005)), further efforts need to be devoted to the study of the life cycle and research at the molecular level.

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МОРФОЛОШКА КАРАКТЕРИЗАЦИЈА *MYZUS PERSICAE* (HEMIPTERA: APHIDIDAE) СА БРЕСКВЕ И ДУВАНА У СРБИЈИ И ЦРНОЈ ГОРИ

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Мултиваријантна анализа варијансе коришћена је за поређење девет карактера код 47 популација *Myzus persicae* (Sulzer) са две биљке домаћина, бресквe и дувана. Узорци су узети са 13 локалитета у 2004. и 34 локалитета у 2005. години у Србији и Црној Гори. Мултиваријантна дискриминаторна анализа показала је да се ваши са дувана јасно одвајају од вашију са бресквe. Главни дискриминаторни карактери су дужина вршног сегмента рострума и дужина вршног дела шестог сегмента пипка који су значајно дужи код вашију са дувана у односу на ваши са бресквe. Ово су први подаци који говоре о постојању две подврсте у овом делу Европе: *M. persicae persicae* (Sulzer) i *M. persicae nicotianae* Blackman.