

# Discrimination and comparison of three wheat aphid species based on isoenzyme patterns (Homoptera: Aphididae)

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## ABSTRACT

The electrophoretic patterns of the isoenzymes esterase (EST), glutamate oxalacetate transaminase (GOT) and malate dehydrogenase (MDH) were studied at three different ontogenetic moments (nymphs, apterous and alate adults) in *Sitobion avenae*, *Rhopalosiphum padi* and *Schizaphis graminum* collected from Passo Fundo, Rio Grande do Sul State. The latter species was also studied from two other Brazilian localities: Jaguariúna, São Paulo and Dourados, Mato Grosso do Sul. Nine electromorphs were found for EST, 12 for GOT and five for MDH. Generally nymphs showed a larger number of electromorphs. Some electromorphs were limited to a single species, therefore electrophoresis could be used to distinguish aphid species despite their parthenogenetic mode of reproduction. Populations of the same species may also be differentiated by this technique.

The populations in Dourados and Jaguariúna were more similar to each other than to the Passo Fundo population at all stages. *S. avenae* and *R. padi* were more similar to each other than to *S. graminum*, which presented different isoenzymatic patterns for each ontogenetic stage. *S. avenae*, *R. padi* and *S. graminum* can be differentiated by the clustered ontogenetic pattern observed for GOT, MDH and EST, respectively. Electrophoresis would be a useful tool for species identification for aphid control on wheat.

## INTRODUCTION

The aphids, *Schizaphis graminum*, *Sitobion avenae* and *Rhopalosiphum padi* are considered generalists, attacking wheat in southern Brazil, especially in the State of Rio Grande do Sul. The nature of the genetic diversity of aphids remains unknown and variability estimates are based on quantitative characteristics easily influenced by founding effects and

environmental variance. Service and Lenski (1982) found evidence that aphid-host plant interactions maintain genetic diversity in aphid populations.

Comparisons of aphid genotypes have traditionally been made by the analysis of easily identifiable external characteristics. Nevertheless, the determination of profiles of soluble proteins and the isoenzyme composition of various enzymes, through electrophoresis, has made it possible to establish a complementary system for the discrimination of genotypes and for the study of aphid species complexes, migration and other ecological and taxonomic problems (Steiner *et al.*, 1985a,b; Lazzari, 1992). Furthermore, electrophoresis has allowed for the identification

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of subspecific taxa (host races, subspecies and biotypes; Berlorcher, 1980).

## MATERIAL AND METHODS

*S. graminum*, *S. avenae* and *R. padi* were collected from the city of Passo Fundo (28°15'S; 52°24'W), State of Rio Grande do Sul. *S. graminum* was also collected at Jaguariúna (22°41'S; 46°59'W) State of São Paulo and Dourados (22°09'S; 54°50'W) State of Mato Grosso do Sul. These aphids were collected and maintained on BR-35 wheat, placed inside clear plastic boxes (24 x 24 x 3 cm) covered with a fine screen and maintained in Fitotron Chambers at 65% relative humidity. A temperature of 18°C was used for *S. graminum* and 20°C for *S. avenae* and *R. padi*. We analyzed nymphs, apterous and alate adults. Apterous and alate individuals are considered different forms of adults. They change from apterous to alate depending on environmental conditions. In this work these were considered to be two ontogenetic stages. Twenty-eight specimens of each ontogenetic stage (nymphs, apterous and alate adults) from each species from Passo Fundo and the same number of *S. graminum* from Jaguariúna and Dourados were analyzed for three enzymatic systems: glutamate oxalacetate transaminase (GOT) (EC 2.6.1.1), malate dehydrogenase (MDH) (EC 1.1.1.37) and esterase (EST) (EC 3.1.1) (IUPAC-IUB 1979). We used *Metopholophium dirhodum* as a control for the relative migration of each electromorph.

The isoenzyme patterns were obtained by electrophoresis on 7% polyacrylamide gel for GOT and MDH and on 6% gel for EST, using the buffer system of Brown (1983) for GOT, that of Roose and Gottlieb (1976) for MDH and that of Scandalios (1969) for EST. A potential difference of 10 v/cm was applied and 9-cm migration was allowed to occur.

The staining procedures were performed according to the methods used by Vallejos (1983) for GOT, Brewer and Sing (1970) for MDH and Scandalios (1969) for EST. Nei distance (Nei et al., 1983) was used in the generation of neighbor-joining dendrograms (Saitou and Nei, 1987) in order to evaluate the genetic relationships among localities and species.

## RESULTS

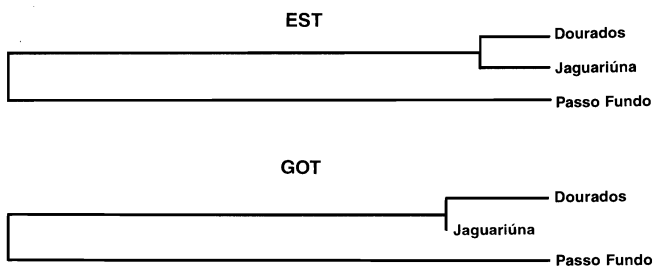
### Intraspecific variation

Three electromorphs of the GOT system (Got) were found in Passo Fundo and Jaguariúna while Dourados showed four electromorphs (Table I). Got 0.93 was found in apterous and alate adults of the three geographic localities, and was the most frequent electromorph for these stages. Got 0.92 was found in nymphs of the three localities but was the only electromorph found in Passo Fundo, whereas in Dourados and Jaguariúna it was the least frequent. This electromorph was exchanged for Got 0.87 in the two latter localities where it was the most frequent. Got 0.94

**Table I** - Frequency of EST and GOT electromorphs in nymphs, apterous and alate adults of *Schizaphis graminum* collected from Passo Fundo, Dourados and Jaguariúna, Brazil.

Enzymatic system	Localities	Passo Fundo			Dourados			Jaguariúna		
		Stages	1	2	3	1	2	3	1	2
<b>EST</b>										
0.25		-	-	-	-	-	-	0.04	-	-
0.42		0.05	0.10	0.13	-	-	-	-	-	-
0.75		-	-	-	0.05	-	-	-	-	-
0.77		-	-	-	-	-	-	0.03	0.07	-
0.92		0.95	0.90	0.87	-	-	-	-	-	-
0.94		-	-	-	0.01	0.25	0.18	-	-	-
0.95		-	-	-	-	-	-	0.03	0.03	0.05
1.00		-	-	-	0.94	0.75	0.82	0.90	0.90	0.95
<b>GOT</b>										
0.87		-	-	-	0.98	-	-	0.91	-	-
0.92		1.00	-	-	0.02	-	-	0.09	-	-
0.93		-	1.00	0.97	-	1.00	0.99	-	1.00	1.00
0.94		-	-	0.03	-	-	0.01	-	-	-

1 - Nymphs; 2 - apterous adults; 3 - alate adults.



**Figure 1** - Dendrograms obtained for three Brazilian collecting localities of nymphs, apterous and alate adults of *Schizaphis graminum*, considering EST and GOT data.

was found only in alate adults of Passo Fundo and Dourados at a low frequency.

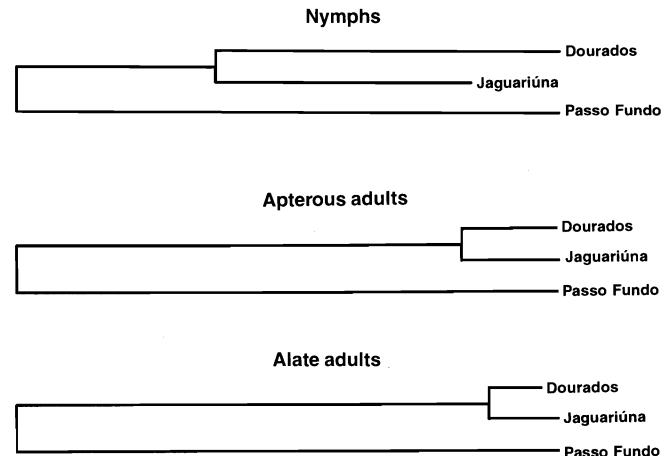
In the EST system two electromorphs (Est) were found in Passo Fundo, three in Dourados and four in Jaguariúna. While Dourados and Jaguariúna shared electromorph 1.0, which was the most frequent in the three stages, the population of Passo Fundo did not share any electromorphs with these two populations and Est 0.92 was the most frequent electromorph.

Using the two isoenzymatic systems at all stages, Dourados and Jaguariúna were more similar to each other than Passo Fundo, as can be seen in Figure 1. When the three ontogenetic periods were clustered and analyzed separately by isoenzymatic system we found that both GOT and EST distinguish Passo Fundo from Dourados and Jaguariúna, as can be seen in the dendrogram in Figure 2.

## Interspecific variation

The frequencies of the different isoenzymatic electromorphs for the three enzymatic systems and three ontogenetic periods of *S. graminum*, *S. avenae*, and *R. padi* are shown in Table II. A total of 26 different electromorphs were observed for all enzymatic systems, 12 for GOT, five for MDH and nine for EST. *S. avenae* and *R. padi* always shared some electromorphs, as is the case for Got 0.27 in nymphs and apterous adults and Got 0.58 in apterous adults. Mdh 0.50 and Est 1.0 were shared in the three ontogenetic periods in both these aphid species. *S. graminum* did not share any electromorph with these species.

In general, nymphs presented a higher or equal number of electromorphs when compared to the other ontogenetic stages. However, while the same MDH pattern was observed for all three ontogenetic stages, only *S. graminum* shared the same GOT electromorphs in the apterous and alate adults. Many EST electromorphs were shared by different ontogenetic periods (Table II).



**Figure 2** - Dendrograms of nymphs, apterous and alate adults lumped together of *Schizaphis graminum* obtained from three Brazilian collecting localities.

It was observed that for some isoenzymatic systems the same electromorph was not always the most frequent in all ontogenetic stages or species. For instance, Got 0.85 was more frequent in nymphs whereas Got 0.93 was more frequent in apterous and alate adults in *S. graminum* (Table II).

The similarity among species were analyzed separately by ontogenetic period: nymphs, apterous and alate adults. At all stages *S. avenae* and *R. padi* were more similar to each other than to *S. graminum* which presented a different isoenzymatic pattern (Figure 3).

However, when we clustered the three ontogenetic periods and analyzed by isoenzymatic system separately, we found that, whereas GOT differentiates *S. avenae* from *R. padi* and *S. graminum*, EST and MDH separated *S. graminum* from *S. avenae* and *R. padi* (Figure 4). However, the MDH system also differentiated the latter two species because while Mdh 0.80 was more frequent for all stages of *S. avenae*, Mdh 0.50 was more frequent in *R. padi* (Table II).

## DISCUSSION

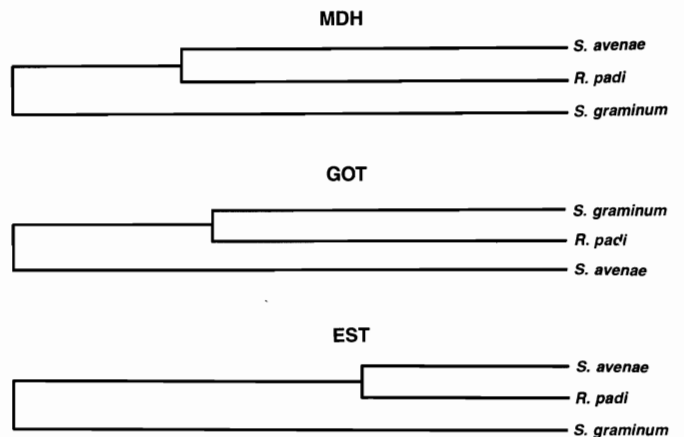
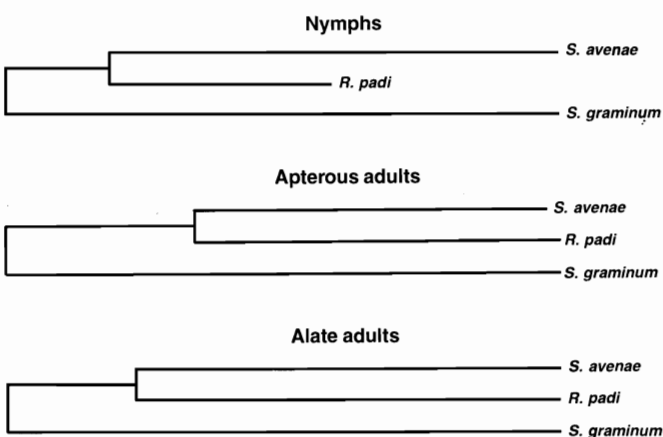
### Intraspecific variation

Electromorph variation within *S. graminum* localities was small, as expected for parthenogenetic organisms, but sufficient to discriminate among them. While for apterous and alate adults the most frequent Got electromorph was the same in the three localities, this did not occur for the nymphal stage. The most

**Table II** - Frequencies of the electromorphs for EST, GOT and MDH in nymphs, apterous and alate adults of *Schizaphis graminum*, *Sitobion avenae* and *Rhopalosiphum padi*.

Electromorph	Species Stages	<i>S. graminum</i>			<i>S. avenae</i>			<i>R. padi</i>		
		1	2	3	1	2	3	1	2	3
<b>MDH</b>										
0.50		-	-	-	0.18	0.10	0.05	0.90	0.98	0.95
0.66		0.37	0.22	0.23	-	-	-	-	-	-
0.73		-	-	-	-	-	-	0.10	0.02	0.05
0.80		-	-	-	0.82	0.90	0.95	-	-	-
0.86		0.63	0.78	0.77	-	-	-	-	-	-
<b>GOT</b>										
0.27		-	-	-	0.82	-	-	-	0.78	-
0.28		-	-	-	0.18	-	-	-	-	-
0.58		-	-	-	-	0.09	-	-	0.22	-
0.85		0.73	-	-	-	-	-	0.50	-	-
0.87		-	-	-	-	-	-	0.50	-	-
0.90		-	-	-	-	-	-	-	-	1.00
0.91		-	-	-	-	-	1.00	-	-	-
0.92		0.18	-	-	-	-	-	-	-	-
0.93		-	1.00	0.97	-	-	-	-	-	-
0.94		-	-	0.03	-	-	-	-	-	-
0.95		0.09	-	-	-	-	-	-	-	-
0.96		-	-	-	-	0.91	-	-	-	-
<b>EST</b>										
0.21		-	-	-	0.03	-	0.06	-	-	-
0.58		-	-	-	0.14	0.19	0.10	-	-	-
0.60		-	-	-	0.14	0.15	0.25	-	-	-
0.69		0.03	-	0.03	-	-	-	-	-	-
0.71		-	-	-	0.01	0.06	-	-	-	-
0.73		0.03	0.01	-	-	-	-	-	-	-
0.87		-	-	-	-	-	-	0.10	0.35	0.58
0.90		0.94	0.99	0.97	-	-	-	-	-	-
1.00		-	-	-	0.68	0.60	0.59	0.90	0.65	0.42

1 - Nymphs; 2 - apterous adults; 3 - alate adults.



**Figure 3** - Dendrograms obtained for nymphs, apterous and alate adults of *Schizaphis graminum*, *Sitobion avenae* and *Rhopalosiphum padi*, considering EST, GOT and MDH data.

**Figure 4** - Dendrograms obtained for MDH, GOT and EST of *Schizaphis graminum*, *Sitobion avenae* and *Rhopalosiphum padi* using nymphs, apterous and alate adults lumped together.

frequent EST electromorph was the same for all three developmental stages.

The EST and GOT systems could differentiate the Passo Fundo sample (Figures 1 and 2).

Voegtlin *et al.* (1987) found that in *Rhopalosiphum maidis* only hexokinase (Hk-1) showed geographic variation. Loxdale *et al.* (1983) also observed that phosphoglucosmutase showed both intra- and interspecific variation in staining intensity. Most of the enzymes which presented variation within *R. maidis* (Lázzari, 1992) were also mentioned by Steiner *et al.* (1985b), who showed that intraspecific variation in *R. padi* occurred in nine out of the 22 loci examined. Mdh-1 and Mdh-2 were consistently polymorphic for all populations, with two alternative homozygous forms. Hk-1 was clearly heterozygous for all populations and polymorphism was also observed in Hk-2, Got-1 and  $\alpha$ -Gpdh for some clones. PEP was heterozygous for at least half of the populations. Steiner *et al.* (1985b) found fixed genetic differences in Got-F, Hk-1 and Idh-F and suggest that these findings clearly demonstrate that genetic differentiation may arise in a widespread aphid population and may be a function of reproduction coupled with the clonal nature of their parthenogenetic mode of population founding.

Our results showed that isoenzymatic patterns of the various ontogenetic stages may also be used for intraspecific differentiation.

### Interspecific variation

The isoenzymatic pattern for GOT, MDH and EST, found in each ontogenetic period, showed that all stages (nymphs, apterous and alate adults) *S. avenae* and *R. padi* could be grouped by the neighbor-joining method. Nevertheless, when the three species were analyzed using the isoenzymatic system, taking into account all three ontogenetic periods, it was found that MDH discriminated *R. padi*, whereas GOT distinguished *S. avenae* and EST separated *S. graminum*.

Significant polymorphism of the EST gene occurs, but it appears to be related to insecticide resistance (Beranek, 1974a,b; Sudderuddin, 1973). Nevertheless, fixed protein differences have been reported between species (Tomiuk and Wöhrmann, 1983; Tomiuk *et al.*, 1979; Odermatt, 1981; Singh and Winningham, 1981).

Lázzari (1992) observed that Est-1, Lap-2, Pgm, Got-1 and  $\alpha$ -Gpdh separate *R. padi* and *R. insertum*. The enzymes Est-2, G-3Pdh, Sdh and Got-2 were useful to separate *R. maidis* from *R. padi*, while Est-1, Lap-2, Got-1 and  $\alpha$ -Gpdh, in addition to the first three mentioned above, separated *R. maidis* from *R. insertum*. Singh and

Winningham (1981) also suggest that EST is useful to distinguish species. This enzyme, added to leucine-alanine peptidase, produces "diagnostic" patterns specific to individual species. Loxdale *et al.* (1983) also show that, among other enzymes, EST is the most useful enzyme for inter-generic and interspecific separation. These same authors found that *R. maidis* and *R. padi* showed a different pattern for Mdh-1 (which apparently was lacking in *R. padi*), peroxidase and especially sorbitol dehydrogenase.

Steiner *et al.* (1985b) reported specific electromorph markers to assay variation in *R. padi* and *R. maidis*. He also observed that *S. graminum* shares an electromorph with the two other species, *R. padi* and *R. maidis*, only at Mdh-s and additionally with *R. maidis* at Got-F and with *R. padi* at  $\alpha$ -Gpdh. Thus, 14.3% of loci present some genetic similarity for *S. graminum* to the *Rhopalosiphum* species. Among the three species, 3.1% of electromorphs are shared, with 5.9% shared between *S. graminum* and *R. maidis* and 8% shared between *S. graminum* and *R. padi*.

Thus, electrophoretic patterns of enzymes constitute a powerful tool to access both interspecific and intraspecific genetic variability. Its use has already been well disseminated to facilitate the recognition of several plant and animal species (Steiner, 1988). In the case of the aphids *S. graminum*, *R. padi* and *S. avenae* studied in this work, which are morphologically very similar, this robust method, despite being old, proved to be valuable not only for taxonomical but also for agricultural purposes, since it makes it easier to identify the species, which is important for their control, because they cause great damage to wheat cultures.

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### RESUMO

Os padrões eletroforéticos de esterase (EST), glutamato oxalacetato transaminase (GOT) e malato desidrogenase (MDH) foram estudados por eletroforese em três diferentes momentos ontogenéticos (ninfa, adulto áptero e adulto alado) de *Sitobion avenae*, *Rhopalosiphum padi* e

*Schizaphis graminum* coletadas em Passo Fundo, Rio Grande do Sul. Esta última espécie foi também estudada em duas localidades brasileiras: Dourados e Jaguariúna. Nove eletromorfos foram encontrados para EST, 12 para GOT e cinco para MDH e geralmente as ninfas mostraram número mais alto de eletromorfos. Alguns eletromorfos foram restritos a uma espécie, assim a eletroforese pode ser usada para distinguir espécies de afídeos apesar de sua característica partenogênese. Populações da mesma espécie podem também ser diferenciadas por esta técnica. As amostras das localidades de Dourados e Jaguariúna foram mais similares do que as de Passo Fundo em todos os estágios. A amostra de Passo Fundo foi mais dissimilar para GOT e EST quando comparada com Dourados e Jaguariúna. *S. avenae* e *R. padi* foram mais similares que *S. graminum*, que apresentou diferente padrão isoenzimático quando cada estágio ontogenético foi analisado para os três sistemas isoenzimáticos. *S. avenae*, *R. padi* e *S. graminum* podem ser diferenciadas pelo padrão ontogenético agrupado observado para GOT, MDH e EST, respectivamente. Assim, PAGE é uma técnica poderosa não somente para o taxonomista mas também para fins agrícolas, porque estas espécies são responsáveis por considerável dano às plantas de trigo e precisam ser bem identificadas para facilitar o seu controle.

## REFERENCES

- Beranek, A.P.** (1974a). Esterase variation and organophosphate resistance in populations of *Aphis fabae* and *Myzus persicae*. *Exp. Appl. Ent.* 17: 129-142.
- Beranek, A.P.** (1974b). Stable and non-stable resistance to demethoate in the peach-potato aphid (*Myzus persicae*). *Exp. Appl. Ent.* 17: 381-390.
- Berlorcher, S.H.** (1980). An electrophoretic key for distinguishing species of the genus *Rhagoletis* (Diptera: Tephritidae) as larvae, pupae, or adults. *Ann. Entom. Soc. Am.* 73: 131-137.
- Brewer, G.J. and Sing, C.F.** (1970). *An Introduction to Isozyme Techniques*. Academic Press, New York, pp. 186.
- Brown, A.H.G.** (1983). Barley. In: *Isozymes in Plant Genetics and Breeding*. Part B. (Tansley, S.D. and Orton, T.J., eds.). Elsevier Science Publishers, Amsterdam, pp. 219-239.
- Lázzari, S.M.N.** (1992). Characterization and discrimination of three *Rhopalosiphum* species (Homoptera: Aphididae) based on isozymes. *Rev. Bras. Zool.* 9(1/2): 139-151.
- Loxdale, H.D., Castanera, P. and Brookes, C.P.** (1983). Electrophoretic study of enzymes from cereal aphid populations. I. Electrophoretic - techniques and staining systems for characterizing isoenzymes from six species of cereal aphids (Homoptera: Aphididae). *Bull. Entom. Res.* 73: 645-657.
- Nei, M., Tajima, F. and Tateno, Y.** (1983). Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* 19: 153-170.
- Odermatt, von Frieda** (1981). Die starke-gel Electrophorese als Methode zur Artidifferenzierung der "Schwarzen Blatlaus" *Aphis fabae* Scop. und verwandte. Arten (Homoptera - Aphididae). *Z. ang. Ent.* 92: 398-408.
- Roose, M.L. and Gottlieb, L.D.** (1976). Genetic and biochemical consequences of polyploidy in tragopogon. *Evolution* 30: 818-830.
- Saitou, N. and Nei, M.** (1987). The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol. Biol. Evol.* 4: 406-425.
- Scandalios, J.G.** (1969). Genetic control of multiple forms of enzymes in plants: a review. *Biochem. Gen.* 3: 37-79.
- Service, P.M. and Lenski, R.E.** (1982). Aphid genotypes, plant phenotypes and genetic diversity: a demographic analysis of experimental data. *Evolution* 36: 1276-1282.
- Singh, S.M. and Wainwright, T.K.** (1981). Morphological and genetic differentiation in aphids (Aphididae). *Can. Ent.* 113: 539-549.
- Steiner, W.W.M.** (1988). Electrophoretic techniques for the genetic study of aphids. In: *Aphids, their Biology, Natural Enemies and Control* (Minks, A.K. and Harrewijn, P., eds.). Elsevier Science Publishers, Amsterdam, pp. 135-143.
- Steiner, W.W.M., Voegtlin, D.J. and Irwin, M.E.** (1985a). Genetic differentiation and its bearing on migration in North American populations of the corn leaf aphid; *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae). *Ann. Entomol. Soc. Am.* 78: 518-525.
- Steiner, W.W.M., Voegtlin, D.J., Irwin, M.E. and Kampmeier, G.** (1985b). Electrophoretic comparison of aphid species: detecting differences based on taxonomic status and host plant. *Comp. Biochem. Physiol.* 81B: 205-299.
- Sudderuddin, K.J.** (1973). An electrophoretic study of some hydrolases from an OP-susceptible and OP-resistant-strain of the green peach aphid, *Myzus persicae* (Sulz.). *Comp. Biochem. Physiol.* 44B: 923-929.
- Tomiuk, J. and Wöhrmann, K.** (1983). Enzyme polymorphism and taxonomy of aphid species. *Z. Zool. Syst. Evolution Sforsch.* 21: 266-274.
- Tomiuk, J., Wöhrmann, K. and Eggers Schumacher, H.A.** (1979). Enzyme patterns as a characteristic for the identification of aphids. *Z. ang. Ent.* 88: 440-446.
- Vallejos, C.E.** (1983). Enzyme activity staining. In: *Isozymes in Plant Genetics and Breeding*. Part A. (Tansley, S.D. and Orton, T.J., eds.). Elsevier Science Publishers, Amsterdam, pp. 469-515.
- Voegtlin, D.J., Steiner, W.W.M. and Irwin, M.E.** (1987). Searching for the source of the annual spring migrants of *Rhopalosiphum maidis* (Homoptera: Aphididae) in North America: In: *Populations Structure, Genetics and Taxonomy of Aphids and Thysanoptera* (Holman, J., Pelikán, J., Dixon, A.F.G. and Weismann, L., eds.). SPB Academic Publishing, The Hague, pp. 120-133.

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