

POPULATION STRUCTURE IN MYZUS PERSICAE AND DISPERSAL OF RESISTANCE GENES



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M. persicae. Picture L. Roy

INTRODUCTION

Myzus persicae (Sulzer, 1776) is a generalist aphid that parasitizes a diversity of crop plants. While populations developing on oilseed rape in France in 2001 widely exhibited the kdr mutation on the voltage dependent sodium channel, but lacked the Mace mutation on acetylcholinesterase (Zamoum *et al.* 2005), recurrent failures of carbamates to control these aphid populations in oilseed rape fields were reported from 2005. Kdr and Mace mutations are known to be associated with the highest resistance level against pyrethroids and carbamates, respectively (target resistance). Additionally, most of individuals sampled in 2001 were also overproducing either E4 or FE4 carboxylesterases, which were shown to be associated with efficient detoxication of both pyrethroids and carbamates (metabolic resistance). Therefore, and because target resistance is known to be associated with higher resistance levels than metabolic resistance, one may wonder whether the prevalence of target mutations would not have been altered during the last decade. If so, what is the scenario of changes? We explored the distribution of the above target mutations in similar aphid populations and started a preliminary study based on microsatellite genotyping. Given

the above rationale, this study is partly based on the comparison between results obtained in a present study (sampling dates 2009-2010) and those obtained in Zamoum et al. (2005; sampling date 2001). Isolates in Zamoum et al. (2005) and isolates in the present study were sampled in France on the same crop during the same season and in partially overlapping regions during two periods separated by a decade (2001, 2009-2010).

MATERIAL AND METHODS



BIOLOGICAL MATERIALS. One isolate = a group of individuals sampled in a single oilseed rape field. Sampling was performed during autumn of 2009 & 2010 in North-Eastern France. **GENOTYPING - MARKERS UNDER THE SELECTIVE PRESSURE OF PESTICIDES.** Each individual was described by the presence or absence of two non synonymous mutations: kdr on the *para* gene (RFLP-PCR; coded protein: voltage sensitive sodium channel; amino-acid substitution L1014F) and Mace on the *ace2* gene (Allele-Specific PCR; coded protein: acetylcholinesterase 2; amino-acid substitution S431F). **GENOTYPING - NEUTRAL MARKERS.** Each individual was described by its neutral multilocus genotype (MLG), the allelic combination at the six microsatellite loci M40, M55, myz25, S16b, S17b, S23, following

Zamoum *et al.* (2005). **ANALYSIS OF NEUTRAL MLGs.** Some statistical tests were performed on the obtained 6-loci MLGs dataset in order to estimate the clonality rate (G:N), linkage disequilibrium between pairs of loci, and F_{IS} (the last two using Genepop 4.1 and Arlequin 3.11). The relationships between the 6-loci MLGs were assessed by building a neighbour-joining tree based on Shared Allele Distance and the mean of Factorial Correspondance Analyses (FCA) of a dataset composed of single copies of each individual locus (Genetix and Matlab). Additionally, Bayesian clustering and assignment were processed on the whole MLG dataset (Structure 2.3.3, allelic frequency; number of subpopulations estimated using Evanno et al. 2005's ΔK method).



Distribution of the frequencies of the Mace/kdr genotypes. *Left:* distribution from Zamoum et al. (2005, 247 individuals). *Right*, distribution obtained in present study (997 individuals).



Neighbor-joining tree of the 16 MLGs detected in isolates 2009-2010 based on Shared Allele Distance. With six loci, a 'one allele' distance is 1/12=0.0833. No = bootstrap support values. Percentages = frequencies of the two majority clones Gpp1 and Gpp2.

Colored areas represent the clustering assignment (K=2, Ln threshold >0.7).

Genetic diversity

 Mean G:N per isolate

 present study
 0.37 (0.13 - 0.75)

 Zamoum et al. (2005)
 0.11 (0.03 - 0.32)

Global G:N

present study 0.10 Zamoum et al. (2005) 0.06 (0,065 8-loci MLG; 0,057 6-loci MLG)



The analyses resulted in very high linkage disequilibrium between pairs of loci (consistent with a high clonality rate) and important variations among F_{IS} values per locus (consistent with the rare occurrence of sexual recombination).

Global distribution of 6-loci MLGs in Zamoum *et al.* (2005, 247 individuals)(*left*) and in the present study (153 individuals)(*right*).



Graphic representation of the first two factors for individual MLGs (FCA). Percentage of factor values are represented in the axes. 1 dot = 1 MLG; dot color refers to the clustering assignments and dot shape refers to the resistant genotype found in association with each MLG (top).

CONCLUSION

Resistance genotype frequencies suggest that an inversion of the proportion of Mace/kdr⁺ individuals has occurred and that a Mace⁺/kdr⁻ clone, while very rare in 2001, strongly amplified during the last decade. No inversion of population structure is revealed as estimated based on neutral markers, but instead a diversification of the majority genotypes is present (1 in 90% of population in 2001, 2 sharing 70% of population in 2009-10). Convergent results generated by a DAS tree, FCA and clustering revealed that 2 neutral genotypes (green cluster) were distant from the 14 others in the 2009-10 isolates. Moreover, the two majority genotypes are each assigned to one of the two distant clusters and a strong increase in G:N per isolate has occurred without any marked increase in global G:N. That suggests that some recent secondary contact has occurred between two sub-populations of *M. persicae* on oilseed rape in France.