Genetic variation in target-site resistance to pyrethroids and pirimicarb in Tunisian populations of the peach potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

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Abstract

BACKGROUND: We used molecular assays to diagnose resistance to pyrethroids and pirimicarb in samples of *Myzus persicae* from field crops or an insect suction trap in Tunisia. Genotypes for resistance loci were related to ones for polymorphic microsatellite loci in order to investigate breeding systems, patterns of genetic diversity and to inform resistance management tactics.

RESULTS: The *kdr* mutation L1014F conferring pyrethroid resistance was found in all samples. The M918T s-*kdr* mutation also occurred in most samples, but only in conjunction with *kdr*. We discovered a previously unreported genotype heterozygous for L1014F but homozygous for M918T. Samples with modified acetylcholinesterase (MACE) conferring resistance to pirimicarb were less common but widespread. 16% of samples contained both the *kdr* and MACE mutations. Many unique microsatellite genotypes were found, suggesting that *M. persicae* is holocyclic in Tunisia. There were no consistent associations between resistance and microsatellite markers.

CONCLUSION: This first study of insecticide resistance in *M. persicae* in North Africa showed genetic variation in insecticide resistance within microsatellite multilocus genotypes (MLG_Ms) and the same resistance mechanisms to be present in different MLG_Ms. This contrasts with variation in northern Europe where *M. persicae* is fully anholocyclic. Implications for selection and control strategies are discussed.

Keywords: *Myzus persicae*; insecticide resistance; knockdown resistance; acetylcholinesterase; microsatellite polymorphism; holocycly; resistance management

1 INTRODUCTION

Aphids (Hemiptera: Aphididae) are major agricultural pests that cause extensive damage to crops through both direct feeding and disease transmission.[1,2] Insecticides are the main means of control, but insecticide resistance has been reported in approximately 20 aphid species[3] including the peach-potato or green peach aphid, *Myzus persicae* (Sulzer). This species has a cosmopolitan distribution, is highly polyphagous, and can vector many plant viruses.[4] Insecticide resistance in *M. persicae* now extends to most classes of insecticide, including organophosphates, carbamates and pyrethroids.[5] The mechanisms conferring the most potent resistance in bioassays are those resulting from amino-acid substitutions leading to conformational changes in insecticide target sites. Target-site resistance to the carbamate insecticide pirimicarb involves a modification to the enzyme acetylcholinesterase (so-called MACE resistance) and results from a serine to phenylalanine substitution (S431F) within the enzyme's active site.[6,7,8] Knockdown resistance (kdr) conferring resistance to pyrethroids involves mutations in the voltage-gated sodium channel protein in nerve membranes. The best documented substitutions are leucine to phenylalanine (L1014F, '*kdr*') and methionine to threonine (M918T, 's-*kdr*').[9,10] Another substitution at the 918 site (M918L) has recently been described.[11] In addition, resistance of *M. persicae* to neonicotinoids is attributable to enhanced activity of a P450 monooxygenase enzyme,[12] and/or a mutation (R81T) in the nicotinic acetylcholine receptor.[13] These mechanisms frequently coexist, greatly limiting the availability of effective control agents.[14]

Although primarily a consequence of insecticide use, the evolution of resistance in *M. persicae* is also influenced by its life-cycle and other aspects of the agroecosystem.[15,16,17,18] Alternation between sexual and asexual reproduction (holocycly) can favour resistance, since sexual reproduction and recombination leads to new genetic combinations on which selection for resistance can act, while multiple asexual generations can promote the rapid build-up of

resistant individuals under exposure to insecticides. Both active flight and human transport of individuals can contribute to the dispersal of resistance genotypes to new regions.

In southern Europe, *M. persicae* is holocyclic with one generation of sexual reproduction in autumn on peach (*Prunus persica* L.), its primary host. Offspring from this sexual stage disperse from peach to secondary host plants (herbaceous crops and weeds) and reproduce by parthenogenesis during spring and summer.[4] However, in the absence of the primary host (as in the UK), asexual reproduction on secondary host plants (anholocycly) persists throughout the year.[15] In areas where holocycly predominates, *M. persicae* can be exposed to insecticides on both primary and secondary host plants, enhancing the selection pressure for resistance. However, fitness costs incurred by resistant aphids may lead to selection against resistance when insecticide use is relaxed.[19,20] Thus, the frequency of resistance can potentially follow cyclical dynamics corresponding to alternating periods of selection for resistance in spring and summer and selection against resistance in autumn and winter.[14,21,22] Spatial and/or temporal variation in host availability during different stages of the life-cycle can also affect clonal dynamics and genetic diversity.[23,24,25] However, many aspects of resistance dynamics and genetic diversity of *M. persicae* on its primary and secondary hosts remain poorly understood.[26]

In Tunisia, *M. persicae* is a major vector of plant viruses to crops such as potato, tomato, pepper and tobacco. Potatoes in particular are vulnerable to the non-persistent viruses *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV).[27,28,29] For several decades, insecticides including pyrethroids, organophosphates and carbamates have been used extensively against *M. persicae* and other aphid pests.[30] Repeated use of insecticides on potatoes and other crops containing aphids imposes a continual risk of resistance developing. To date, however, there has been no work on the status and dynamics of insecticide resistance in *M. persicae* in North Africa. We report here on the use of established molecular assays to diagnose the status of

resistance to pyrethroids and pirimicarb in samples of *M. persicae* collected from field crops and from an insect suction trap in northern Tunisia, where crops vulnerable to attack by this aphid are concentrated. Resistance profiles disclosed by these assays are then compared the diversity of genotypes at five polymorphic microsatellite loci in order to investigate the genetic composition and clonal diversity of *M. persicae* and inform attempts at resistance management.

2 MATERIALS AND METHODS

2.1 Aphid samples

Samples of *M. persicae* were collected from peach (*Prunus persica*) orchards and potatoes (*Solanum tuberosum*) at four sites in Tunisia (Fig. 1): (i) **Cap bon** (36°40'40"N 10°28'20"E) a coastal sub-humid zone with Kermes oak (*Quercus* cf. *coccifera* L.) forest, *Cystus* sp. and various crops (fruit trees, potato, tomato, pepper); (ii) **Manouba** (36°43'09"N 9°29'10"E) a semi-arid area with warm winter zone characterized by Oleo-lentisc forest with mixed farming; (iii) **Jendouba** (36°33'42"N 8°56'40"E) a continental sub-humid zone with Oleo-lentisc forest and cereal crops, sugar beet and vegetables; (iv) **Kairouan** (35°39'50"N 9°59'10"E), a continental zone with arid cold winter and steppe with a developing agricultural industry based on fruit trees and vegetables. Kairouan is a new site for producing Spunta and Nicola seed potatoes. Collections were made during five consecutive years. In the majority of cases, samples were taken twice per year in the spring and autumn, enabling a comparison of insects before and after the growing (=crop protection) season. At each site, aphids were collected from widely-spaced plants in order to limit the chance of sampling the same colony. In addition, aphids were obtained from a 12.2m suction trap at Cap bon and these were shipped in alcohol. Aphids confirmed as *M. persicae* were stored in microtubes filled with 70% ethanol and preserved at -80 C prior to genotypic testing. In total, 32 samples (26 from

123 field sites and 6 from suction traps), totalling 903 individuals were obtained. Sampling sites,
124 host plants and dates of collection are listed in table 1.

125 **2.2 DNA extraction**

126 Total genomic DNA was extracted from individual adult aphids using DNAzol (Invitrogen,
127 Carlsbad, California) at one fifth scale of the supplier's recommended protocol.
128 (http://www.invitrogen.com/content/sfs/manuals/10503.pdf). Each aphid was dried in a speed-
129 vac and then crushed using a Teflon pestle in a microcentrifuge tube in 200 µl of DNAzol
130 containing 1% (v/v) of polyacryl carrier (Invitrogen, Carlsbad, California). The homogenate
131 was centrifuged for 12 min at 10,000g after a 30 min incubation period at room temperature.
132 The supernatant was transferred to a new tube and a half volume of 100% ethanol was added.
133 The tube was cooled to - 20°C for 30 min and DNA pelleted by centrifuging at 10,000g for 15
134 min. The DNA pellet was dissolved in 50 µl of distilled, deionized water (ddH2O) after being
135 washed twice with 70% ethanol. The quality and quantity of DNA samples were assessed by
136 spectrophotometry (Nanodrop Technologies) and by running an aliquot on a 1% agarose gel.
137 All DNA samples were diluted to 40 ng/µl and stored at -20°C for future use.

138 **2.3 Insecticide resistance mechanisms**

139 Mutations conferring knockdown and MACE resistance were identified using TaqMan assays
140 to discriminate between wildtype and resistance alleles.[31,32] Reactions took place in a
141 STRATAGENE MX 3000 (Agilent Technologies, Santa Clara, CA) thermocycler. Diagnosis
142 of the presence or absence of the *kdr* (L1014F) and s-*kdr* (M918T) mutations in the sodium
143 channel gene, and of MACE (S431F) in the acetylcholinesterase gene, enabled each
144 individual to be classified as homozygous susceptible (SS), heterozygous (SR) or
145 homozygous resistant (RR) at each of the three loci. This in turn enabled each individual to be
146 allocated a multi-locus resistance genotype (MLG_R).

147 **2.4 Microsatellite genotyping**

148 A sub-sample of 153 individuals was subjected to microsatellite analysis to investigate the
149 genotypic variation of aphids from peach and potato at three of the collection sites. Each
150 aphid was genotyped using five microsatellite loci M40, M49, M63, M86 and myz9[33,34]
151 chosen on the basis of their level of pulymorphism (allele numbers of 7, 15, 11, 11 and 11,
152 respectively). These loci were amplified using fluorochrome primers labelled at the 5'end of
153 the reverse primer (M40 FAM, M49 HEX, M63 FAM, M86 TET, myz9 HEX; MWG
154 Biotech, Germany) and PCR ready-to-go beads (Amersham Biosciences, U.K.; for the
155 conditions used, see[35]). Products were then analysed on an ABI 377 (96) automated
156 sequencer with Genescan v3.4 and Genotyper v2.5 software (Applied Biosystems, Foster
157 City, California), for both visualization and analyses. Each individual was described by its
158 multilocus microsatellite genotype (MLG_M): the combination of alleles at all five
159 microsatellite loci.

160 **2.3 Statistical analysis**

161 *2.3.1 Genetic variability within samples*

162 Allele frequencies, mean number of alleles per locus and allelic richness were calculated
163 using FSTAT version 2.9.3.2.[36] Linkage disequilibrium (LD) between loci within each
164 population and departure from H-W equilibrium at each locus were tested using ARLEQUIN
165 version 3.11.[37]

166 *2.3.2 Genetic variation between samples*

167 Samples were pooled in three ways: (i) by geographical origin, (ii) by host plant, and (iii) by
168 year of collection. Population structure was assessed by calculating multilocus F_{ST} values[38]
169 for pairwise comparisons of samples using ARLEQUIN version 3.11.[37] The null distribution
170 of pairwise F_{ST} values under the hypothesis of no difference between the populations is
171 obtained by permuting diploid multilocus genotypes between populations. The *P* value of the
172 test is the proportion of 100 000 permutations leading to an F_{ST} value larger than or equal to

the observed one. The structure of the data was also investigated by analysis of molecular variance[39] (AMOVA) using Arlequin version 3.11. A permutation non-parametric approach was used for the significance of fixation indices described in Excoffier *et al.*[39]. Allelic differentiation between populations was examined using GENEPOP version 3.4. An unbiased estimate of the *P* value of the Fisher exact test was made using a Markov chain method described in Raymond and Rousset.[40] For microsatellite markers, analyses were performed without clonal copies, i.e., with the data reduced to a single representative of each multilocus genotype (MLG_M) per population, because the clonal amplification of genotypes inevitably leads to deviations from genetic equilibria.[41,42]

3 RESULTS

3.1 Frequency of insecticide resistance genes

3.1.1 Kdr resistance

The *kdr* mutation L1014F was present in heterozygous or homozygous form in 65% of individuals collected from the field crops (Table 1). The frequency of genotypes containing *kdr* varied between 3 and 100% for samples from both peach and potato across all years, with heterozygotes (RS) being the most common resistance genotype. This mutation was most frequent at Cap bon and Kairouan, and least frequent at Jendouba. The s-*kdr* mutation M918T was present in 21.7% of individuals collected from the field crops and was only ever found in conjunction with *kdr* (ie. no SSRS or SSRR genotypes; Table 2). This mutation was also widespread, being absent in only four of the 26 samples analysed (Table 1) and most frequent at the Kairouan locality. The resistance genotypes characterised included one heterozygous for L1014F but homozygous for M918T (SRRR) that has never been reported previously. The three most common knockdown resistance genotypes were SSSS (neither resistance mutation), SRSS (heterozygous for *kdr*, homozygous susceptible for s-*kdr*) and SRRR (heterozygous for *kdr*, homozygous resistant for s-*kdr*) (Table 2).With all data collated, *kdr*

198 and s-*kdr* mutations were in very strong linkage disequilibrium (P < 0.001), as expected
199 because of the close positioning of these two sites in the same gene.

200 *3.1.2 MACE resistance*

201 23.6% of the individuals collected from field crops were either heterozygous or homozygous
202 for the S431F mutation (Table 1), which was found at all four collection sites. It was most
203 common at Manouba, with heterozygotes being the most frequent genotype. Moreover, 16.4%
204 of individuals possessed both the *kdr* and MACE mutations, and 5.3% had all three resistance
205 mutations (*kdr*, s-*kdr* and MACE). There was no significant linkage disequilibrium between
206 MACE and *kdr* or MACE and s-*kdr* using data pooled across all collections (*P*=0.08 and 0.97,
207 respectively).

208 **3.2 Differences in resistance genotypes between crops and locations**

209 The *kdr* mutation was significantly more frequent (Fisher's exact test $P < 10^{-5}$) in aphids from
210 peach (73.3%) than in those from potato (31.7%). The F_{ST} values revealed significant
211 variation in MLG_R frequencies between locations (Table 3). There was no significant
212 difference between years in the same locality, thereby justifying the pooling of samples across
213 years for each locality. Pairwise comparisons between locations all yielded highly significant
214 levels of differentiation. A hierarchical AMOVA also revealed a significant differentiation
215 (F_{ST}=0.101; P < 0.001) over all mechanisms and locations, with most of the variance (89.8%)
216 being within location. The *kdr* mutation explained a high percentage of the between-locality
217 variance (12.17%) with an F_{ST} value of 0.121 (P < 0.001).

218 **3.3 Suction trap samples and their relationship to field samples**

219 Compared to collections from field crops, which are more likely to reflect localized events
220 including insecticide treatment regimes, collections from suction traps should be
221 representative of larger areas.[43] Our analysis was limited to comparisons between samples of
222 *M. persicae* collected from the field and a suction trap at a single site (Cap bon) (Table 1 and

4), due to the absence of suction traps at another sites. Each of the three resistance mutations (*kdr*, s-*kdr* and MACE) had a similar prevalence in samples collected from field crops and from the suction trap at Cap bon. For example, the *kdr* mutation L1014F was present in 65% of individuals collected from field crops and 61.5% of individuals collected from the suction trap. Similarly, the S431F mutation was detected in 23.6% of individuals collected from field crops and in 22.7% of individuals collected from the suction trap.

3.4 Microsatellite genotypes

The number of alleles identified for each microsatellite locus ranged from eight at M40 and M63 to 15 at M49. 49 alleles were detected across all loci. All 13 samples used for microsatellite analysis showed substantial genetic diversity, as expressed by the mean number of alleles per locus and the randomization test for population allelic richness (Table 5). Values of allelic richness ranged from 1.9 at locus M40 to 5.0 at locus M49.

120 different MLG_{M}s were found among 153 individuals genotyped. Estimates of clonal diversity (G) measured by dividing the numbers of genotypes by the number of individuals,[16] ranged from 0.62 to 1 across the 13 samples (Table 5). Only 16 of the 120 MLG_{M}s were found more than once (between two and six times in the samples). Most genotypes were unique to a particular sampling site although one was collected at multiple sites. When considering only one individual per MLG_{M}, significant linkage disequilibrium was detected only for a single pair of loci (M40 × M63, $P<0.001$). No linkage disequilibrium was detected between microsatellite loci and mutations conferring insecticide resistance with the exception of a significant association between s-*kdr* and M40.

3.5 Comparing MLG_{M}s between samples

Comparisons of genetic differentiation for microsatellite loci between locations or between host plants showed significant differentiation between the three locations (Table 6). A hierarchical AMOVA also revealed a significant F_{ST} (0.040; $P < 0.001$) variation over all loci

and locations, with most of the variance (94%) being within location. There was low but significant genetic differentiation between peach and potato samples ($P < 0.001$; $F_{ST} = 0.024$).

3.6 Comparison of resistance and microsatellite profiles

Among the 120 different MLG_Ms identified using microsatellite markers, only seven contained more than one MLG_R defined using the three resistance markers. Of the remaining MLG_Ms, 18 were susceptible for both resistance mechanisms, 95 contained the *kdr* mutation, 31 the s-*kdr* mutation and 28 the MACE mutation. Only 16 MLG_Ms contained both the *kdr* and MACE mutations. No strict associations could be established between microsatellite and resistance profiles since: (a) with one exception (see above) no significant linkage could be detected between microsatellites and insecticide resistance markers; (b) each resistance mechanism was found in different MLG_Ms; and (c) some resistance genes showed variation within MLG_Ms.

4 DISCUSSION

Heterozygotes and homozygotes for mutations conferring resistance to pyrethroids and pirimicarb were readily found using allelic discrimination PCR assays[31,32], showing that resistance to these functionally distinct compounds is well established in Tunisia. Differences between samples in the frequency of resistance mechanisms may reflect some spatial variation in selection pressure. The *kdr* (L1014F) mutation was present at all four collection sites and in samples from the single suction trap, in some cases in 100% of the insects tested. The frequencies of *kdr* were statistically higher in peach orchards than in potato fields, possibly a consequence of the selection pressure imposed by spring treatments in peach orchards against *M. persicae* and other pests including Mediterranean fruit fly *Ceratitis capitata*, Peach Twig Borer *Anarsia lineatella* and scale insects. Both the *kdr* and s-*kdr* mutations have now been identified in samples of *M. persicae* worldwide, and the status of

knockdown resistance in Tunisia mirrors its generally high frequency in Europe, USA and Japan[44,14,25] where it is a major constraint on the continuing use of pyrethroids for combating *M. persicae*.

Only some of the possible genotypic combinations of the *kdr* and s-*kdr* mutations were detected. Three alleles predominated: fully susceptible (SSSS), heterozygous at *kdr* but homozygous susceptible at s-*kdr* (SRSS), and heterozygous at *kdr* but homozygous resistant at s-*kdr* (SRRR). Prior to this study, M918T had never been observed in the absence of L1014F, leading to an assumption that both mutations are necessary for an enhanced resistance phenotype. The presence of the previously unreported SRRR genotype in our samples demonstrates the occurrence of an allele that is wild-type at the *kdr* locus but which contains the s-*kdr* mutation. The phenotype of insects with this genotype in terms of the expression and potency of resistance has not been investigated. The existence of the new allele implies that aphids with a SSRR genotype should be generated through outcrossing, although none were detected in the samples investigated.

No individuals were found that were homozygous resistant for both mutations (RRRR). Fenton *et al.*[15] also found a lack of the RRRR genotype in Scotland. The lack of such double homozygotes could implicate a fitness cost associated with such a genotype.[45,46] The absence of homozygous genotypes for *kdr* and s-*kdr* also matches observations in populations of *M. persicae* in mainland Europe, Zimbabwe and South East Australia, where there appears to be a strong selection pressure against homozygosity in *kdr* due to the high fitness costs associated with the trait.[46]

The frequency of MACE resistance was generally constant between years. Its relatively limited frequency in Tunisia could be due to a switch to insecticides other than pirimicarb, resulting in a situation where MACE is of no advantage. Although, as expected, there was strong linkage disequilibrium between *kdr* and s-*kdr*, there was no significant association

between MACE and either the *kdr* or s-*kdr* mutations, which could arise in areas treated with both pyrethroids and pirimicarb. In Tunisia, such an association is not apparent, presumably as a consequence of recombination during sexual reproduction.

The presence of 120 microsatellite genotypes in 153 individuals indicates a high level of genetic diversity similar to that found in *M. persicae* in France, where 100 genotypes were identified from 174 aphids collected from suction traps in 2000.[25] This level of variation contrasts markedly with that in Scotland (UK), where only 21 different genotypes were found in 1497 individuals collected from suction traps and secondary hosts.[18] This lack of variation was attributed to obligate anholocycly, since the primary host is absent. In Greece, the extent of variation in microsatellite markers was closely associated with the presence or absence of the primary host, with the number of unique MLG_Ms being much higher in peach-growing areas than in non-peach-growing areas[4]. Thus, the absence of genetic signatures of clonal reproduction (repeated genotypes, linkage disequilibrium) suggests that Tunisian samples are mostly constituted of cyclically parthenogenetic aphids. The variation observed was attributable to the fact that most of our collections were made from peach trees in spring, and were offspring of the founding females that emerged from sexually-produced eggs.

Significant pairwise F_{ST} values for pooled samples from different localities imply genetic differentiation even over small distances (Cap bon and Kairouan are less than 150 km apart, Fig. 1). For *Sitobion avenae*, Simon *et al.*[47] obtained an average F_{ST} value of 0.032 in France, and Llewellyn *et al.*[48] reported most values lower than 0.05 in the UK. For *Rhopalosiphum padi*, Delmotte *et al.*[49] reported values of 0.022 and 0.032 for anholocyclic and cyclically parthenogenetic genotypes, respectively. The authors suggested that genetic homogeneity over a large geographical scale results from the high migratory habits of two aphid pests of cereal crops. However, *M. persicae* seems to differ in this respect, as shown also by previous studies in Australia[24] (F_{ST} = 0.058–0.202, with an average 0.087); France[25] (F_{ST} up to 0.17–

0.21 in some cases) and Greece[50] (F_{ST} = 0.05–0.174, with an average 0.062). It should be noted that local differentiation does not mean that intense migration and long-distance flights do not occur in *M. persicae*, but rather that long-distance migration may be rare or that the success rate of migration may be low.[51] Tunisian samples showed variation in insecticide resistance genotypes (MLG_{R}s) was present within MLG_{M}s and that the same insecticide resistance mutations were present in different MLG_{M}s. This again points to the existence of sexual recombination. Recombination resulting from sexual reproduction can lead to a polymorphism at resistance genes within MLGs, and this conclusion is supported by the presence of peach, the primary host, and by the high level of genetic diversity revealed by microsatellite analysis.

In conclusion, molecular analyses of the diversity of insecticide resistance mutations in *M. persicae* can assist in determining the levels and types of resistance mechanism present. This information can strengthen strategies for preserving the effectiveness and increasing the performance of insecticides currently used for managing *M. persicae*, and for deploying insecticides alongside other control strategies. Work in cropping systems in Tunisia has helped to reveal how patterns of variation in insecticide resistance genes relate to those in microsatellite markers and compliments information from other continents to provide a global perspective on the evolution of resistance in one of the world's most economically-significant agricultural pests.

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525 **Figure caption**

526 Figure 1. Map of Tunisia showing the location of sampling sites.

527

528 **Table 1.** Percentage of *M. persicae* individuals with different resistance genotypes, collected
529 from field crops in Tunisia.
530

Location	Crop	Date	Abbreviation*	n	*Kdr* genotype			super-*kdr* genotype			MACE genotype		
					SS	*SR*	*RR*	*SS*	SR	RR	SS	SR	RR
Cap bon	Peach	20/05/2005	Cpe5	30	16.6	63.3	20.0	76.7	0.0	23.3	76.7	16.7	6.66
	Peach	24/03/2006	Cpe6	77	14.3	55.8	29.9	77.9	1.29	20.8	85.7	7.79	6.49
	Peach	01/12/2006		20	20.0	75.0	5.0	90.0	5.0	5.0	70.0	30.0	0.0
	Peach	27/04/2007		21	38.1	19.0	42.9	81.0	0	19.0	33.3	952	57.1
	Peach	18/04/2008	CpeL8	43	23.3	62.8	13.9	76.7	9.30	13.9	79.1	18.6	2.32
	Peach	26/10/2008	CpeE8	34	2.92	91.2	5.88	73.5	2.84	23.6	61.8	35.3	2.94
	Peach	23/04/2009	Cpe9	56	8.92	83.9	7.14	66.1	3.57	30.4	69.6	21.4	8.92
	Potato	21/04/2006	Cpt6	20	85.0	15.0	0.0	90.0	0.0	10.0	50.0	30.0	20.0
	Potato	08/12/2006		20	80.0	20.0	0.0	85.0	10.0	5.0	65.0	30.0	5.0
	Potato	11/05/2007		12	91.7	8.33	0.0	100	0.0	0.0	75.0	25.0	0.0
	Potato	18/04/2008	CptL8	32	96.9	0.0	3.12	100	0.0	0.0	62.5	21.9	15.6
	Potato	28/09/2008	CptE8	32	62.5	37.1	0.0	84.4	0.0	15.6	78.1	18.8	3.12
Total				397	35.0	51.9	13.1	80.4	2.77	16.9	71.5	18.9	9.57
Manouba	Peach	16/06/2005		12	16.7	50.0	33.3	83.3	0.0	16.7	66.7	25	8.33
	Peach	28/03/2006		24	0.0	16.7	83.3	95.8	4.16	0.0	20.8	79.2	0.0
	Potato	17/04/2006		12	0.0	83.3	16.7	75.0	0.0	15.0	41.7	41.7	16.7
Total				48	4.16	41.7	54.2	87.5	2.08	10.4	37.5	56.3	6.25
Jendouba	Peach	06/03/2005	Jpe5	20	25.0	75.0	0.0	85.0	0.0	15.0	55.0	45.0	0.0
	Peach	17/04/2006		24	91.7	8.33	0.0	100	0.0	0.0	100	0.0	0.0
	Peach	22/03/2007	Jpe7	68	86.8	13.2	0.0	95.6	2.94	1.47	91.2	7.35	1.7
	Potato	22/05/2007		12	83.3	16.7	0.0	100	0.0	0.0	100	0.0	0.0
Total				124	77.4	22.6	0.0	95.2	1.61	3.22	87.9	11.3	0.8
Kairouan	Peach	30/04/2005		12	8.33	83.3	8.33	75.0	8.33	16.7	58.3	33.3	8.33
	Peach	10/04/2006	Kpe6	61	6.55	80.3	13.1	55.8	0.0	44.3	93.4	6.55	0.0
	Peach	06/04/2008	Kpe8	65	12.3	86.2	1.53	49.2	1.53	49.2	84.6	12.3	3.07
	Peach	03/12/2008		24	20.8	75.0	4.16	83.3	0.0	16.7	87.5	8.33	4.16
	Peach	02/06/2009		26	38.5	53.8	7.69	92.3	7.69	0.0	100	0.0	0.0
	Potato	06/05/2008	Kpt8	15	13.3	73.3	13.3	46.7	0.0	53.3	86.7	13.3	0.0
	Potato	03/12/2008		12	50.0	50.0	0.0	75.0	0.0	15.0	91.7	8.33	0.0
Total				215	16.7	76.3	6.97	62.8	1.86	35.3	88.4	9.76	1.86

Total	784	34.8	53.3	11.9	78.3	2.29	19.4	76.3	18.0	5.74

n is the number of individuals tested. An asterisk identifies samples analysed for microsatellite variation

535 **Table 2**. Proportion of knockdown resistance genotypes in Tunisian samples of *Myzus*

536 *persicae*

Kdr resistance genotype	Location				Total	Percentage
	Capbon n=516	Manouba n=48	Jendouba n=124	Kairouan n=215		
SSSS	0.35	0.04	0.77	0.16	319	35.3
SRSS	0.33	0.31	0.17	0.4	298	33.0
RRSS	0.10	0.52	0	0.05	91	9.96
RRSR	0.01	0.02	0	0.01	11	1.21
SSSR	0	0	0	0	0	0
SSRR	0	0	0	0	0	0
SRSR	0.01	0.004	0.01	0.004	11	1.10
SRRR	0.17	0.1	0.03	0.35	173	19.2
RRRR	0	0	0	0	0	0

537 *n* is the number of aphids sampled from each site

538

539 **Table 2**. Proportion of knockdown resistance genotypes in Tunisian samples of *Myzus*

540 *persicae*

Kdr resistance genotype	Location				Total	Percentage
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SRSS	0.33	0.31	0.17	0.4	298	33.0
RRSS	0.10	0.52	0	0.05	91	9.96
RRSR	0.01	0.02	0	0.01	11	1.21
SSSR	0	0	0	0	0	0
SSRR	0	0	0	0	0	0
SRSR	0.01	0.004	0.01	0.004	11	1.10
SRRR	0.17	0.1	0.03	0.35	173	19.2
RRRR	0	0	0	0	0	0

541 *n* is the number of aphids sampled from each site

542

543

544 **Table 4.** Percentage of *M. persicae* individuals for suction trap samples with different
545 resistance mechanisms.
546

Date	n	*Kdr* genotype			super-*kdr* genotype			MACE genotype		
		SS	SR	RR	SS	SR	RR	SS	SR	RR
2006	23	65.2	34.8	0.0	74.0	4.34	21.7	78.3	21.7	0.0
2006	24	29.2	62.5	8.33	70.8	4.16	25.0	58.3	41.7	0.0
2007	12	75.0	25.0	0.0	91.7	0.0	8.33	100	0.0	0.0
2007	12	50.0	41.7	8.33	91.7	0.0	8.33	75.0	25.0	0.0
2008	24	20.8	75.0	4.16	75.0	8.33	16.7	87.5	12.5	0.0
2008	24	16.7	70.8	12.5	83.3	4.16	12.5	75.0	20.8	4.16
Total	119	38.7	55.5	5.88	79.0	4.20	16.8	77.3	21.8	0.84

547 *n* is the number of individuals tested
548

549 **Table 5.** Number of genotypes, clonal diversity (G), mean number of alleles per locus and
550 allelic richness per locus for each population. Values of allelic richness were calculated based
551 on a minimal sample size of 3 individuals

	Populations												
	Cpe5	Cpe6	CpeL8	CpeE8	Cpe9	Cpt6	CptL8	CptE8	Kpe6	Kpe8	Kpt8	Jpe5	Jpe7
	N=(10)	N=(15)	N=(7)	N=(3)	N=(18)	N=(6)	N=(3)	N=(8)	N=(21)	N=(24)	N=(4)	N=(18)	N=(16)
No. of multilocus genotypes	9	13	7	2	18	5	3	8	14	23	4	15	10
G	0.9	0.86	1	0.66	1	0.83	1	1	0.66	0.95	1	0.83	0.62
Mean no. alleles per locus	4.6	4.2	3.4	2.8	5.2	4.4	4.0	6.2	6.2	6.4	4.4	5.4	4.4
Allelic Richness per locus													
M49	4.49	4.19	3.50	3.00	3.60	4.15	5.00	3.85	4.11	4.40	4.39	3.19	3.23
M63	1.98	2.13	1.99	2.00	1.93	3.78	4.00	3.34	2.97	2.84	3.25	3.39	3.23
M86	2.52	3.45	2.81	3.00	3.53	3.23	4.00	4.67	3.55	2.70	4.21	3.87	3.32
M40	3.29	1.90	2.77	2.00	3.20	1.99	4.00	3.62	2.16	2.83	3.50	2.36	2.96
Myz9	2.69	1.86	2.83	4.00	2.62	3.71	3.00	3.74	2.74	2.60	3.46	2.13	1.82
Mean	2.99	2.70	2.78	2.8	2.97	3.27	3.8	3.84	2.96	3.07	3.76	2.98	2.91

552

553

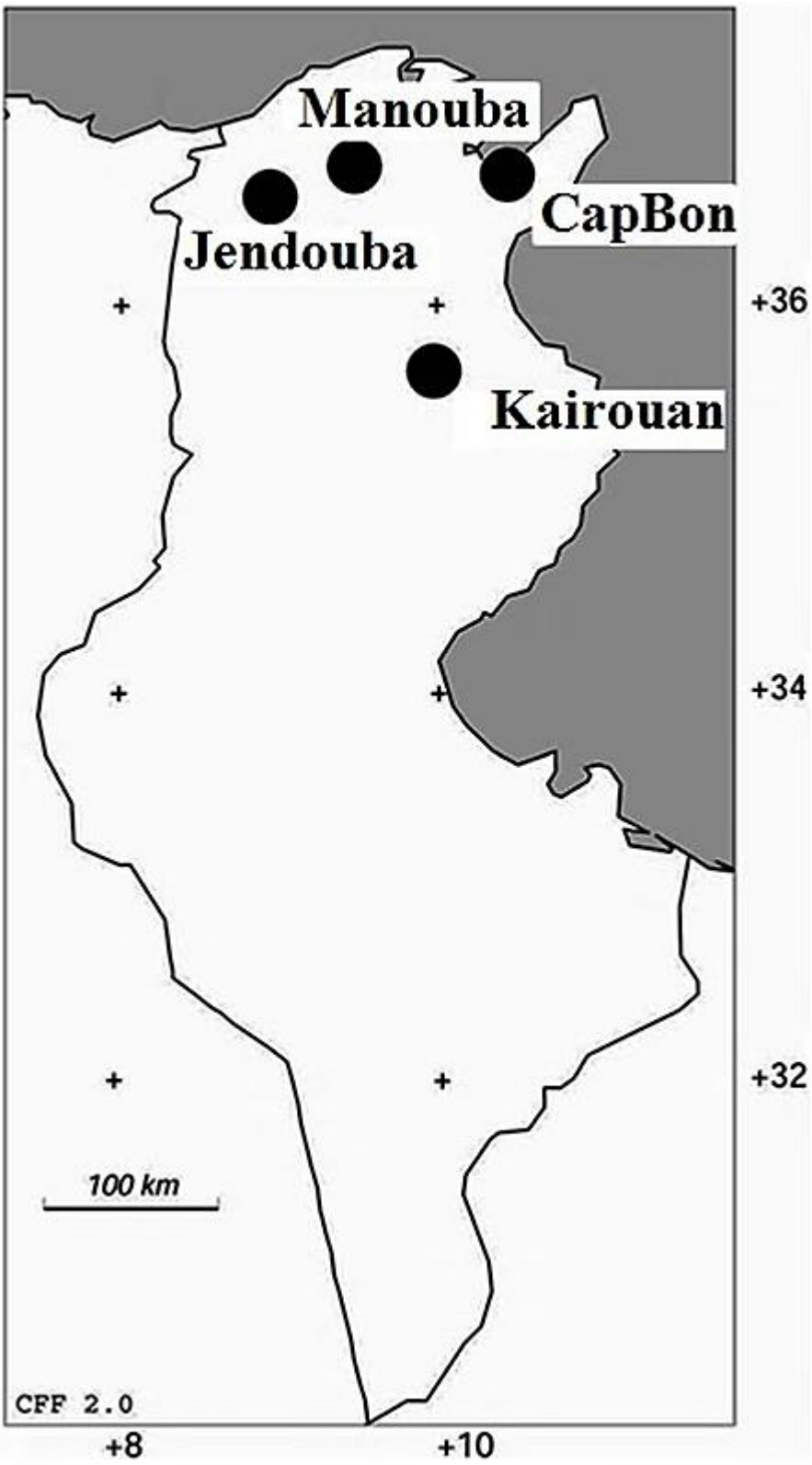
554 **Table 6.** Genetic differentiation in microsatellite loci expressed as F_{ST} values for pairs of

555 samples pooled by geographical origin

	Cap bon	Jendouba	kairouan
Cap bon	-		
Jendouba	0.040*	-	
kairouan	0.023*	0.071*	-

556 Above F_{ST} value * $P < 0.001$

557


Manouba
CapBon
Jendouba
Kairouan
+36
+34
+32
100 km
CFF 2.0
+8
+10