Assessment of the Dominance Level of the R81T Target Resistance to Two Neonicotinoid Insecticides in Myzus persicae (Hemiptera: Aphididae)

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Abstract

Myzus persicae (Sulzer, 1776), a major crop pest worldwide, displays insecticide resistance to most molecules. The R81T substitution on the β1 subunit of nicotinic receptors of acetylcholine (nAChR) confers target site resistance to neonicotinoids and is widespread in aphid populations colonizing peach tree orchards in Southern Europe. But the impact of this resistance in the field, as well as ways to optimize its management, depends largely on the dominance level of the R81T mutation. In this study, we measured by in vitro assays the response of R81T mutation to two neonicotinoids (imidacloprid and thiacloprid) in 23 M. persicae clones with different resistance genotypes in order to assess the dominance status of this allele. In this study, all homozygous clones for the R81T mutation (genotype 81TT) showed a much higher level of resistance to both active substances than other clones. The heterozygous clones 81RT displayed a slightly higher level of resistance than wild homozygous, though resistance phenotypes against both neonicotinoids in these two genotypes were overlapping. A great variation of resistance level was found within these two latter clones’ categories. The dominance level of insecticide resistance (DLC) strongly suggested that the mutant allele 81T is semirecessive (the wild 81R allele being rather dominant) for both insecticide molecules under test. Mean DLC values were 0.316 for imidacloprid and 0.351 for thiacloprid. Cross-resistance was shown between imidacloprid and thiacloprid. This partial recessivity is valuable information to broaden the knowledge on neonicotinoid resistance, a prerequisite for devising adapted management strategies against insecticide-resistant populations of M. persicae.

Key words: Myzus persicae, resistance, neonicotinoid, R81T mutation, dominance level
signal (hyperpolarization and inhibition of excitation; Jones and Sattelle 2010, Dederer 2013). Sustained activation of the receptor results in the inability of acetylcholinesterases to break down the pesticide (Matsuda and Sattelle 2004). Both metabolic (Paincean et al. 2010) and target site (Bass et al. 2011) resistances were found in this aphid species against neonicotinoids. The R81T substitution in the β1 subunit of nAChR in M. persicae was the first target site mutation selected by neonicotinoids in the field, while the first one was found in a laboratory-selected strain of the plant hopper, Nilapavarta lugens (Liu et al. 2005). In M. persicae, 81T is the only currently known mutation in this target gene, so that only two different cDNA alleles are known to date (wild 81R and mutant 81T). Bass et al. (2011) characterized the resistance phenotype of a clone of M. persicae possessing the mutant 81T allele at the homozygous status. Based on spray bioassays, they showed that this homozygous mutant had a 1,679-fold and 225-fold resistance level against imidacloprid and thiamethoxam, respectively. Slater et al. (2012) and Panini et al. (2014) found that the 81T allele was widespread in M. persicae populations sampled in peach tree orchards over Southern France, Spain, and in Italy.

Dominance of one allele over another one is the result of interactions between alternative alleles at a specific locus. In the case of complete dominance, the heterozygote phenotype is indistinguishable from that of one of the two homozygous parents, while intermediate levels of dominance result in heterozygote phenotype being more or less close to it (incomplete dominance). Codominance results in the two phenotypes of both homozygote parents being simultaneously expressed in the same offspring organism. In the case of target resistances, Bourguet and Raymond (1998) highlighted that the physiological impacts of target proteins and the insecticide mode of action underpin the relationships between alleles in the phenotypic expression. Especially, they have shown that when an insecticide acts by opening an ion channel, the mutant allele associated with a decreased affinity of the insecticide molecule with the target protein is mostly recessive to semirecessive, the wild allele being dominant. They explained this as follows: the permanent opening of only a few channels is usually sufficient to cause death (e.g. sodium channels). Heterozygous insects which possess 50% of sensitive channels are therefore phenotypically similar to susceptible insects in the presence of insecticide. The impact of target site resistances in the field largely depends on their dominance level: in case of recessive resistant allele, some recommendations may be drawn. Indeed, management strategies for controlling the development of pest resistance, when assuming monogenic resistance, may be mainly based on the fitness of the heterozygotes to that of the two homozygotes at a given insecticide dose in the presence (DWT) or in the absence (DWNT) of insecticide. Assessing these three traits of resistance dominance requires important and time-consuming bioassays. Yet, stating the first dominance trait (DLC) may represent a useful first step in order to estimate whether further research toward the development of recessive-based resistance management strategies might be feasible. Indeed, in case of full dominance level in the lab, it is likely that no such strategy might be developed to delay insecticide resistance. In order to evaluate whether estimation of DML and DWT could be helpful for field recommendation, we assessed the dominance level of the mutant allele 81T relative to the wild allele 81R (nAChR beta 1 subunit [Bass et al. 2011]) in multiple clones of M. persicae.

It has already been established that R81T substitution can be an effective target resistance and that it is widespread in Southern Europe (Bass et al. 2014). However, to get a complete picture of R81T target resistance, the phenotype(s) associated with each genotype had to be characterized. For that purpose, the resistance could not be explored at the population level, but had to be assessed at the individual level. Our study provides extensive bioassays on synchronized clonal lineages, establishing a direct link between individual genotypes on the 81 position and associated phenotype with regard to neonicotinoid resistance. We measured by in vitro assays the impact of R81T mutation on sensitivity to different neonicotinoids in different homozygous and heterozygous clones. Connecting phenotypes with their associated genotype in numerous clones allowed us to investigate the dominance level associated with this allele, which, to date, no study has done. It also gives insights into the range of levels of resistance to neonicotinoids observed in the three genotypes 81RR, 81RT, and 81TT borne by different clones of M. persicae.

Materials and Methods

Biological Material

Parthenogenetic female individuals of M. persicae were sampled during spring 2011, 2012, and 2013 in France in peach orchards (Table 1). One aphid individual was collected per leaf and was maintained alive and individually isolated on Chinese cabbage in a controlled environment room at 20–22°C with a photoperiod of 16:8 (L:D) h. Such a rearing method allowed the reproduction of each aphid as a clonal colony. In addition, three clones were also included in the bioassays as nAChR sensitive references: 4106A, a fully drug-sensitive reference clone; 4916A, a clone overproducing carboxylesterases (R3 level), but devoid of any of the mutations cited above; and 384C, a clone sampled on French oilseed rape in 2010 and carrying both Mace (Modified Acetylcholinesterase, a mutation associated with high-level resistance against carbamates) and M918L (a mutation associated to high-level resistance against pyrethroids), but not R81T (homozygous for the wild-type allele).

In order to check that clones under study had distinct genotypes, one adult of each clone was genotyped using published microsatellite loci following Wilson et al. (2004) and Zamoum et al. (2005).

Artificial Feeding Bioassays

Three weeks before the bioassays, aphids reared on Chinese cabbage plants were transferred to cabbage leaf discs disposed on 12% agar in 4-cm-diameter pillboxes. Each clone was multiplied in pillboxes until enough individuals were produced for the tests.
Table 1. Sampling information and in vitro assay results on 23 field clones and three reference clones treated with imidacloprid or thiacloprid

<table>
<thead>
<tr>
<th>Clone ID</th>
<th>Field sampling information</th>
<th>nAChR 81</th>
<th>n</th>
<th>Slope (±SE) Imidacloprid LC₅₀ (µg liter⁻¹) [95% CL]</th>
<th>Chi-square df RL</th>
<th>n</th>
<th>Slope (±SE) Thiacloprid LC₅₀ (µg liter⁻¹) [95% CL]</th>
<th>Chi-square df RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4106A (lab clone)</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>3,743</td>
<td>2.75 (0.20)</td>
<td>40.97 [34.62–47.07]</td>
<td>0.0024125</td>
<td>7</td>
</tr>
<tr>
<td>384C (lab clone)</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>244</td>
<td>2.34 (0.04)</td>
<td>42.2 [29.7–54.3]</td>
<td>0.3079251</td>
<td>5</td>
</tr>
<tr>
<td>4916A (lab clone)</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>1,088</td>
<td>2.34 (0.34)</td>
<td>42.2 [29.7–54.3]</td>
<td>0.3079251</td>
<td>5</td>
</tr>
<tr>
<td>12-069-004 Peach 2012 Roche de Glun, RA</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>715</td>
<td>2.68 (0.24)</td>
<td>67.0 [57.1–76.7]</td>
<td>0.9159961</td>
<td>5</td>
</tr>
<tr>
<td>11-037-001 Peach 2011 Fossur-Mer, PACA</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>466</td>
<td>3.08 (0.29)</td>
<td>199.8 [173.3–228.3]</td>
<td>0.9924539</td>
<td>7</td>
</tr>
<tr>
<td>13-001-050 Peach 2013 Etoile sur Rhône, RA</td>
<td>– –</td>
<td>81</td>
<td>RR</td>
<td>815</td>
<td>3.53 (0.29)</td>
<td>75.7 [68.1–83.9]</td>
<td>0.0672275</td>
<td>5</td>
</tr>
<tr>
<td>11-062-016 Peach 2011 Beaucoisin, LR</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>463</td>
<td>2.81 (0.27)</td>
<td>177.2 [152.7–208.6]</td>
<td>0.2908136</td>
<td>8</td>
</tr>
<tr>
<td>11-037-018 Peach 2011 Fos-sur-Mer, PACA</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>313</td>
<td>2.45 (0.32)</td>
<td>70.0 [55.8–84.8]</td>
<td>0.8190045</td>
<td>7</td>
</tr>
<tr>
<td>11-037-001 Peach 2011 Fos-sur-Mer, PACA</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>367</td>
<td>2.81 (0.27)</td>
<td>177.2 [152.7–208.6]</td>
<td>0.2908136</td>
<td>8</td>
</tr>
<tr>
<td>11-037-018 Peach 2011 Fos-sur-Mer, PACA</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>313</td>
<td>2.45 (0.32)</td>
<td>70.0 [55.8–84.8]</td>
<td>0.8190045</td>
<td>7</td>
</tr>
<tr>
<td>12-067-023 Peach 2012 Loriol, RA</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>744</td>
<td>2.51 (0.36)</td>
<td>237.4 [160.3–326.1]</td>
<td>0.0010954</td>
<td>5</td>
</tr>
<tr>
<td>12-068-004 Peach 2012 Roche de Glun, RA</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>735</td>
<td>2.68 (0.22)</td>
<td>267.0 [234.0–299.9]</td>
<td>0.3071127</td>
<td>3</td>
</tr>
<tr>
<td>11-039-103 Peach 2011 St Félin d’Avall, LR</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>268</td>
<td>2.68 (0.24)</td>
<td>8,623.3 [7,193.4–10,358]</td>
<td>0.6624780</td>
<td>4</td>
</tr>
<tr>
<td>11-039-003 Peach 2011 St Félin d’Avall, LR</td>
<td>– –</td>
<td>81</td>
<td>RT</td>
<td>265</td>
<td>3.26 (0.32)</td>
<td>497.5 [448.7–550.9]</td>
<td>0.9739978</td>
<td>7</td>
</tr>
<tr>
<td>13-001-048 Peach 2013 Etoile sur Rhône, RA</td>
<td>– –</td>
<td>81</td>
<td>TT</td>
<td>901</td>
<td>4.36 (0.36)</td>
<td>680.5 [622.44–741.91]</td>
<td>0.0448992</td>
<td>3</td>
</tr>
<tr>
<td>11-039-001 Peach 2011 St Félin d’Avall, LR</td>
<td>– –</td>
<td>81</td>
<td>TT</td>
<td>286</td>
<td>2.64 (0.52)</td>
<td>10,212 [6,448–18,441]</td>
<td>0.0336702</td>
<td>4</td>
</tr>
<tr>
<td>11-039-001 Peach 2011 St Félin d’Avall, LR</td>
<td>– –</td>
<td>81</td>
<td>TT</td>
<td>286</td>
<td>2.64 (0.52)</td>
<td>10,212 [6,448–18,441]</td>
<td>0.0336702</td>
<td>4</td>
</tr>
</tbody>
</table>

nAChR 81: Genotype of 81 amino-acid residue in the nAChR β1 subunit.

n: Number of tested individuals per genotype.

LC₅₀: Lethal concentration resulting in 50% dead or poorly co-ordinated aphids. 95% CL: confidence limits at 95%.

RL: Resistant level = LC₅₀ for clone/LC₅₀ for 4106A.

Different protocols have been used for determining the sensitivity of *M. persicae* against neonicotinoids (Foster et al. 2003, Beckingham et al. 2013, Panini et al. 2014). Because of the systemic mode of action of neonicotinoids (Buchholz and Nauen 2002), we selected a bioassay based on controlled insecticide ingestion, which seems more relevant to specifically evaluate the differences in sensitivity in the studied clones. As we used a different method as the aforementioned authors, we could not compare directly our measures with those obtained from earlier studies. We decided to work using only newborn L1 instar larvae, because 1) besides being easier to discriminate from other instars, it permits to work on a more homogenous batch of individuals with same age (responses are thus expected to be more repeatable), and 2) they had no time to feed on plants prior transfer to artificial medium, so we were not allowed to get used to feed on plant, thus avoiding any repellent effect of the artificial device. Prior research has shown that some aphids experience poor performance on a given host plant when having previously experienced other food source (e.g., Ramirez and Niemeyer 2000).

The artificial feeding bioassays were adapted from the satchets method developed by Rahbé and Febvay (1993). Between 25 to 35 adult females were placed for 24 h in a PVC ring (4 cm in diameter) covered with two membrane layers of parafilm containing the artificial diet AP3 (provided by G. Febvay and Y. Rahbé). Newborn L1 nymphs (0–24 h old) produced by the females aforementioned were then used to determine the oral toxicity of imidacloprid and thiacloprid. They were transferred to a fresh PVC ring (3 cm in diameter) covered with two membrane layers of parafilm containing the artificial medium amended with insecticide. Two commercial products were tested separately: Confidor (imidacloprid) [Bayer CropScience, Lyon, France] and Calypso (thiacloprid) [Bayer CropScience, Lyon, France]. Commercial products were used instead of technical product (as commonly used in resistance bioassays), because the latter has to be diluted in alcohol, and thus cannot be incorporated into the artificial medium. Artificial diet AP3 was supplied with 5% of a solution of the commercial product with different concentrations of the active substance (imidacloprid or thiacloprid, respectively) so as to obtain final insecticide concentrations ranging from 10 µg liter⁻¹ to 40,960 µg liter⁻¹ (with an increment of 2 in each dose). For each concentration, two replicates or more, involving at least 15 L1 per replicate, were tested.

To assess Abbott mortality (Abbott 1925), control tests with insecticide-free conditions were carried out in parallel with each tested insecticide. The absolute mortality in both insecticide and control tests was scored 48 h after the transfer to the AP3 medium. Aphids were turned on to their back; those unable to turn back and move after 15 s were scored as dead. Tests were repeated until ca 100 aphids per dose were assayed.

**Molecular Analyses**

DNA from a single individual of *M. persicae* for each studied clone was extracted and purified using Nucleospin Tissue DNA extraction kit (Macherey Nagel, Hoerd, Germany) according to the manufacturer’s recommendations.

The R81T mutation in the β1 subunit of nAChR was detected by dCAPS (derived cleaved amplified polymorphic sequence) PCR. A restriction site is created when the allele is nonmutated with a forward primer placed close to the codon 81, one of which bases is not complementary to the sequence. The PCR is followed by an enzyme digestion in order to discriminate the wild-type allele (two fragments of 116 pb and 37 pb) from the mutant allele lacking any restriction site (a single fragment of 153 pb).

PCR were performed in a final volume of 25 µl with 200 µM of dNTPs, 300 µM of each primer (forward primer MpB1F-SmlII: AAAAGAGTCAAATAATGAAATCAAACGTTTG—reverse primer MpB1TMIR from Slater et al. 2012), 2 mM of MgCl₂, 0.1 µg/µl of BSA, 1.5 units of Taq polymerase in the appropriate buffer (Fermentas, Villebon sur Yvette, France), and 1 µl of DNA. The PCR cycle consisted of an initial denaturation phase of 5 min at 94°C and then 35 cycles including denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and then extension at 72°C for 30 s. PCR ended with a final extension of 5 min at 72°C. Digestion of 10 µl of PCR products was performed with 5 units of SmII enzyme (NEB) at 55°C overnight. PCR products from a wild individual, a mutant heterozygote, and a mutant homozygote were systematically included as references. Restriction fragments were separated and visualized using QIAxcel (an automated capillary electrophoresis system by Qiagen, Hilden, Germany) with the QIAxcel DNA Screening Kit (alignment marker: 15pb–1 kb, molecular weight marker: 50–800 pb) and method AM420. The sizes of fragments were automatically calculated and exported using the bioCalculator software, which provided a gel view and an electrophoregram of the fragments separation.

**Statistics**

Aphid mortality was subjected to dose-probit analysis using the Finney equivalent option of PriProbit software (Finney 1971, Finney 1978, Sakuma 1998). This yielded LC₅₀ values (concentrations estimated to be lethal to 50% of the clonal population under test). Their 95% confidence limits and the goodness-of-fit χ² statistics were also calculated. When the P-value associated with the χ² was <0.05, the heterogeneity factor H was calculated by the PriProbit software and applied for the calculation of LC₅₀ values and their associated confidence limits (as recommended by Raymond [1985]). Resistance levels (RL) were calculated by dividing the LC₅₀ of a clonal test population for a particular chemical by the corresponding value for the susceptible reference clone 4106A.

The level of significance of differences among LC₅₀ between clones having different nAChR genotypes was measured using nonparametric tests (Kruskal–Wallis analysis of variance by ranks and post-hoc Wilcoxon Mann–Whitney). The dominance level (Dₜₐₜ) was calculated following equation [2] in Bourguet et al. (2000):

$$ D_{L_{C}} = (\log_{L_{C_3}} - \log_{L_{C_1}})/(\log_{L_{C_2}} - \log_{L_{C_1}}) $$

In order to state whether there is cross resistance associated with the 81T allele for both imidacloprid and thiacloprid, the Spearman correlation coefficient was calculated to assess association between the LC₅₀ of imidacloprid and the LC₅₀ of thiacloprid in genotypes carrying the R81T mutation. A P value of ≤0.05 was considered indicative of a statistically significant correlation.

**Results**

Twenty-two of the 24 sampled clones (all coming from peach fields) had unique multilocus microsatellite genotypes. Only two (clones 11-060-006 and 11-062-013) had the same multilocus genotype, so likely belonged to the same clonal lineage. To avoid a bias due to the inclusion of the same genotype twice in the study, we kept only one (11-060-006) of these two clones in the bioassays.

The artificial feeding bioassays were carried out on these 23 clonal populations (i.e., possessing various β1 nAChR genotypes) and on three laboratory strains with the wild 81RR nAChR genotype (Table 1). This collection of 23 clones included the three nAChR genotypes, with 6 clones with 81RR, 12 with 81RT, and 5 with 81TT.

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Mortality in the untreated control ranged from 0 to 7%. The three laboratory strains had similar estimated LC$_{50}$ with imidacloprid (ca. 40 µg liter$^{-1}$/C0). In contrast, the 95% confidence interval of the estimated thiacloprid LC$_{50}$ for the laboratory clone 4106A was not overlapping the 95% confidence intervals of the two other laboratory clones (384C and 4916A), suggesting that this clone was a little more sensitive than the two other laboratory clones to this molecule (LC$_{50}$: 85.9 µg liter$^{-1}$/C0 [75.17–97.44] vs. ca. 154.9 µg liter$^{-1}$/C0 for 384C and 151.1 µg liter$^{-1}$/C0 [135.38–168.39] for 4916A).

The five homozygous clones for the R81T mutation (genotype 81TT) showed a much higher level of resistance to both active substances under test than other clones (LC$_{50}$ > 6,400 µg liter$^{-1}$/C0 and RL > 100 for both active substances, with a maximum of 16,908 µg liter$^{-1}$/C0 and RL = 412). For both insecticide molecules, a conspicuous gap was visible between mortality curves of the resistant homozygous clones and those of both heterozygous and sensitive homozygous clones (Fig. 1), as well as between 95% CL of estimated LC$_{50}$ (4,648.7–19,026 µg liter$^{-1}$/C0 for 81RR and 29.6–816.6 µg liter$^{-1}$/C0 for both 81RR and 81RT genotypes).

Heterozygous and sensitive homozygous clones both displayed a great range of responses, observable in mortality curves obtained with either imidacloprid (Fig. 1A) or thiacloprid (Fig. 1B): for imidacloprid, sensitive homozygous LC$_{50}$ ranged from 67.0 to 199.8 µg liter$^{-1}$/C0 and heterozygous from 158.0 to 727.4 µg liter$^{-1}$/C0; for thiacloprid, sensitive homozygous LC$_{50}$ ranged from 183.6 to 876.0 µg liter$^{-1}$/C0 and heterozygous from 494.8 to 2,246.5 µg liter$^{-1}$/C0.

Mean D$_{LC}$ values were 0.316 for imidacloprid and 0.351 for thiacloprid, as calculated based on the most commonly considered median lethal concentration (LC$_{50}$). These values did not vary much when the calculation was processed on different % lethality (e.g., with LC$_{10}$ and LC$_{90}$, D$_{LC}$ were 0.344 and 0.323 resp. for imidacloprid and 0.344 and 0.367 resp. for thiacloprid). Although sensitive homozygous and heterozygous clones provided overlapping 95% CL of estimated LC$_{50}$ and no gap between their mortality curves, the LC$_{50}$ of all three groups of clones (genotypes 81RR, 81RT, and 81TT) appeared to be significantly different according to the Kruskal–Wallis analysis of variance by ranks ($P$ < 0.0001 for both imidacloprid and thiacloprid bioassays) and post hoc Wilcoxon Mann–Whitney pairwise analyses ($P$ < 0.05 for every pair in both series of bioassays after Bonferroni correction). All the heterozygous clones 81RT were less sensitive than laboratory sensitive reference clones to both active substances. 95% CL for estimated LC$_{50}$ of a few sensitive homozygote field clones (only wild type allele—nAChR genotype 81RR) and heterozygous clones (nAChR genotype 81RT) were overlapping. Indeed, for imidacloprid, 95% CL extended from 57.1 to 84.8 µg liter$^{-1}$/C0 for all 81RR clones except two (11-037-001 and 11-062-016, 95% CL from 152.7 to 228.3 µg liter$^{-1}$/C0), whose 95% CL were overlapping.

Fig. 1. Imidacloprid (A) and thiacloprid (B) dose response curves for the 23 clones tested and the three reference clones (genotype [RR] is shown in light grey, [RT] in medium grey, and [TT] in dark grey).
with two 81\textsuperscript{RT} clones' (13-001-032 and 12-067-023, 95% CL from 126.53 to 326.1 \mu g liter\textsuperscript{-1}).

The ratio of thiacloprid LC\textsubscript{50} over imidacloprid LC\textsubscript{50} ratio was close to 1 in each of the five 81\textsuperscript{TT} clones tested (mean 1.16 ± 0.12), which was expected in regards to the similar molar weights of imidacloprid and thiacloprid (253.66 g mol\textsuperscript{-1} and 252.72 g mol\textsuperscript{-1}, respectively). In contrast, great differences were observed between LC\textsubscript{50} for the two chemicals per clone in both 81\textsuperscript{RR} and 81\textsuperscript{RT} genotypes (thiacloprid LC\textsubscript{50}/imidacloprid LC\textsubscript{50} ratio = 4.43 ± 1.05 for sensitive homozygous clones 81\textsuperscript{RR}, 3.62 ± 1.59 for heterozygous clones 81\textsuperscript{RT}).

Cross-resistance between imidacloprid and thiacloprid in \textit{M. persicae} clones carrying the R81T mutation was also noticed. LC\textsubscript{50} values for imidacloprid and thiacloprid for these clones showed a high significant positive correlation (Spearman coefficient rs = 0.90—P < 0.00001; see linear regression on Fig. 2). This is consistent with other neonicotinoid cross-resistances (mainly imidacloprid–thiamethoxam cross-resistances) previously reported in \textit{M. persicae} clones possessing the R81T mutation (Bass et al. 2011, Slater et al. 2012, Cutler et al. 2013).

**Discussion**

Here we provide an interesting overview of the range of resistance phenotypes against neonicotinoids found in \textit{M. persicae} from French peach orchards according to codon genotype of position 81 in the \(\beta\)1 nAChR subunit.

First, we observed highly resistant phenotypes (118 ≤ RL ≤ 412) associated with the homozygous mutant genotype 81\textsuperscript{TT} for the codon 81 of the \(\beta\)1 nAChR subunit as previously established. It was shown to be implicated in a drastic loss of sensitivity to different active substances belonging to neonicotinoids family (Bass et al. 2011, Slater et al. 2012, Panini et al. 2014).

Second, the \(D\textsubscript{LC}\) values as calculated following Bourguet et al. (2000) allow to assign the mutant allele 81T to the semirecessive category (ca. 0.3 for both insecticide molecules tested). Note that the equation used to calculate the dominance level is based on log values of lethal concentrations, which makes interval between 0.3 and 0.5 a considerable one. Completely to Bourguet and Raymond (1998)'s categorization, this value falls within the range of the semirecessive category but is excluded from that of the codominant category. This allele appears to be at least partly recessive over the wild 81R allele, which is consistent with the known mode of action of neonicotinoid molecules (their binding induce the opening of the cation channel nAChR): as previously reported by Bourguet and Raymond (1998), mutant alleles preventing an insecticide to open a channel are commonly found recessive to semirecessive, as only a few open channels may be lethal. In the present case, the ca. 0.30–0.35 \(D\textsubscript{LC}\) values obtained using different lethal concentrations (10, 50, and 95) for both molecules are within the range of values obtained by these authors with other ion channels.

Slater et al. (2012) observed that the expression of the mutant allele in heterozygous individuals was effective. The fact that, despite above evoked overlapping of 95% CL, the 12 heterozygous clones tested provided significantly different LC\textsubscript{50} values as compared to the 81\textsuperscript{RR} genotypes raises the question of the influence of the mutant allele in heterozygous status in the resistance phenotype. As expressed by the continuous values of \(D\textsubscript{LC}\) (from 0 to 1 with 0 for complete recessivity and 1 for complete dominance), the recessivity is not a binary trait, so that a limited role of the 81T allele in the presence of 81R is not excluded. Nicotinic acetylcholine receptors (nAChRs) are ion channels that open transiently after binding two agonist molecules (Auerbach and Akk 1998). Channel closure is allowed when the agonist acetylcholine (ACH) molecule is hydrolyzed by acetylcholinesterase (which is the most common case in the absence of neonicotinoid with any of the 81 alleles or in the presence of neonicotinoid with the mutant allele 81T allele). The involvement of two agonist molecules in the channel position may result in more or less unpredictable effect of mixed mutant and wild amino-acid sequences. All the more that the nAChR are pentameric receptors (five subunits are arranged around the central ion channel), which are formed by identical (homomeric) or different (heteromeric) subunits (Jones and Sattelle 2010). Although the specific composition of insect receptors is not yet well known, several different subunits are known to be present in insect nAChR. Recently, using the full genome of \textit{Acyrtosiphon pisum}, Dale et al. (2010) isolated 11 genes encoding putative nAChR subunits in this aphid, suggesting the presence of several different nAChR subtypes in single individuals, as in vertebrates. As a result, insecticide-nAChR protein interactions may be highly complex. This makes possible that inclusion of both mutant and wild alleles in different nAChR subtypes does not exactly result in the strict abolition of advantage conferred by the mutant allele.

Other important modifiers such as metabolic resistance related with P450 cytochrome are likely to play a role in the phenotypic variations observed in both wild homozygotes and in heterozygotes. Such an involvement was shown in \textit{M. persicae} resistance to neonicotinoid insecticides (Puinean et al. 2010). In previous studies carried out with homozygous resistant genotypes, a significant decrease of resistance level was observed when bioassays were realized after a piperonyl butoxide (PBO) treatment, which inhibits P450 enzyme activity (Bass et al. 2011, Panini et al. 2014). It is likely that such modifiers, as well as others not yet identified, are also responsible for mortality curve differences between sensitive homozygous and heterozygous clones. Both sensitive homozygous 81\textsuperscript{RR}, and, to a lesser extent, heterozygous 81\textsuperscript{RT}, display a great range of phenotypes, for both imidacloprid, and even more so, for thiacloprid. This is also consistent with the marked differences between thiacloprid and imidacloprid LC\textsubscript{50} which were observed in 81\textsuperscript{RR} and 81\textsuperscript{RT} genotypes, but not in 81\textsuperscript{TT} genotype. According to the semirecessive profile of 81T, only clones with the 81\textsuperscript{TT} genotype possess fully effective target resistance, and therefore likely have reached a maximum threshold of resistance. In contrast, the less sensitive clones

![Fig. 2. Correlation between LC\textsubscript{50} values for imidacloprid and thiacloprid showing cross-resistance between these two molecules.](image-url)
among both 81RR and 81RT genotypes most likely have developed other resistance mechanisms, such as overproduction of detoxifying enzymes, the efficiency of which may differ among molecules.

In conclusion, the partial recessivity of the mutant 81T allele over the wild 81R allele, established for two neonicotinoids, by bioassays on 23 clonal populations, adds a new facet to the understanding of neonicotinoid resistance in *M. persicae*. Given the ca. 0.3 DL$_{50}$ values and the large gap between heterozygous and sensitive homozygous clones on one hand and resistant homozygous clones on the other hand, it is not unlikely that some field-applicable doses may kill all or almost all heterozygous individuals. Assessing these doses could be of great help for managing such resistance on peach trees in the presence of refuge zones, although such a management method should be considered with caution, considering that cyclical parthenogenetic reproduction is likely to allow rapid amplification of homozygous genotypes. Since peach tree is the primary host of *M. persicae*, all aphids developing on this plant result from recent sexual reproduction events. As a consequence, the advantage of maintained homozygosity during asexual generation could be lost in these populations by the regular occurrence of sexual reproduction, with associated recombination events breaking the association of the two resistant alleles (unless this locus is seated in a region of low recombination). However, exerting some such selective pressure on primary host may result in increased resistance on secondary hosts under the same insecticide selective pressure (selected homozygous resistant genotypes being able to swarm to secondary hosts where only parthenogenetic reproduction occurs). With this risk in mind, measuring the $D_{50}$$_{50}$ by assessing the relative mortality level for insects with associated recombination events would provide a helpful recommendation for resistance management against neonicotinoids on the primary host. Bioassays involving comparisons in the presence and in the absence of synergist PBO would allow stating to which extent known metabolic resistance mechanism against neonicotinoids contribute as modifiers to the observed high sensitivity variations amongst both wild and heterozygous genotypes, and would complete the picture of neonicotinoid resistance in *M. persicae*.

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**References**


