Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of AgriculturalFaculty of GaziosmanpasaUniversity
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*ResearchArticle*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(3) 136-142
doi:10.55507/gopzfd.1144369

Acetamiprid Resistance in the Green Peach Aphid *Myzuspersicae*(Sulzer) (Hemiptera: Aphididae): Selection, Cross-Resistance, Biochemical and Molecular Resistance Mechanisms

Gizem BERBER[1] Berke DEMİRCİ[2] Umut TOPRAK[2] Emre İNAK[2] Sibel YORULMAZ[1*]

[1]Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta
[2]Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara
***Corresponding author's email:** sibelyorulmaz@isparta.edu.tr

Alındığıtarih (Received): 16.07.2022 Kabul tarihi (Accepted): 27.09.2022

Abstract: *Myzus persicae*(Sulzer) (Hemiptera: Aphididae) is a polyphagous pest that causes significant losses in many crops. In the present study, the biochemical and molecular mechanism of acetamiprid resistance in a laboratory-selected *M.persicae* population of which the resistance ratios reached 57.5-fold were investigated. This study was conducted in the Isparta University of Applied Sciences, Agriculture Faculty, Department of Plant Protection in 2018 and 2020. Synergism, biochemical and molecular assays showed the absence of increased P450 activity in selected population. In addition, no point mutation in nicotinic acetylcholine receptor (nAChR), the target-site of neonicotinoids including acetamiprid, was detected in the selected population. These results suggests that high level of acetamiprid resistance might be developed via the mechanisms other than well-known mechanisms, such as increased P450 activity and target-site mutations. The population selected with acetamiprid showed decreased susceptibility to imidacloprid, sulfoxaflor, beta-cyfluthrin, and tau-fluvanite ranging from 1.54 to 4.76. Nonetheless, more studies are needed to support cross-resistance by *M. persicae* populations having different genetic backgrounds.

Keywords:acetamiprid, *CYP6CY3*, monooxygenase, *Myzus persicae*, resistance

Yeşil Şeftali Yaprakbiti *Myzus persicae*'de (Sulzer) (Hemiptera: Aphididae) Acetamiprid Direnci: Seleksiyon, Çapraz Direnç, Biyokimyasal ve Moleküler Direnç Mekanizmaları

Öz : *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), birçok üründe önemli kayıplara neden olan polifag bir zararlıdır.Bu çalışmada, laboratuarda seleksiyon baskısı sonucunda direnç oranı 57.5 kata ulaşan bir *M. persicae* popülasyonunda acetamipriddirencinin biyokimyasal ve moleküler mekanizması araştırılmıştır. Çalışma 2018-2020 yılları arasında Isparta Uygulamalı Bilimler Üniversitesi, Ziraat Fakültesi, Bitki Koruma bölümünde yürütülmüştür. Sinerjistik, biyokimyasal ve moleküler çalışmalar, seleksiyon popülasyonunda artan P450 aktivitesinin olmadığını göstermiştir. Buna ilaveten, seleksiyon popülasyonunda, acetamiprid dahil neonikotinoidlerin hedef bölgesi olan nikotinik asetilkolin reseptöründe (nAChR) herhangi bir nokta mutasyonu belirlenmemiştir. Bu sonuçlar, seleksiyon popülasyonunda artan P450 aktivitesi ve hedef bölge mutasyonları dışındaki mekanizmalar yoluyla yüksek düzeyde asetamiprid direncinin geliştirilebileceğini düşündürmektedir. Acetamipridile selekte edilen popülasyonda, imidacloprid, sulfoxaflor, beta-cyfluthrin ve tau-fluvanite karşı 1.54 ila 4.76 kat arasında duyarlılık azalması belirlenmiştir. Bununla birlikte, farklı genetik geçmişlere sahip *M. persicae* popülasyonlarında çapraz direncin belirlemek için daha fazla çalışmaya ihtiyaç bulunmaktadır.

Anahtar sözcükler: acetamiprid,*CYP6CY3*, monooksijenaz, *Myzus persicae*, direnç

1. Introduction

The green peach aphid, *Myzus persicae*(Sulzer) (Hemiptera: Aphididae) is phytophage pest in the world due to its highly polyphagous nature, short life cycle, and ability to spread rapidly (Emden &Harrington, 2017). In addition, they can transmit nearly 30% of all plant-infecting viruses (Harris & Maramorosch, 1977; Ng &Perry, 2004; Brault et al., 2010). The main control of aphids relies heavily on chemical insecticides (Dedryver et al., 2010; Bass et al., 2014). However, due to their excellent ability to evolve mechanisms that cope with xenobiotics, failure in chemical control has often been reported (Bass et al., 2014; Simon & Peccoud, 2018).To date, a total of 477 resistance cases against 81 active ingredients, including traditional (such as organophosphates and carbamates);

as well as those from relatively newer insecticide classes (such as neonicotinoids), have been documented (Mota-Sanchez &Wise, 2022).

Neonicotinoids, synthetic derivatives of nicotine, are one of the most important insecticide classes, accounting roughly for 25% of the global insecticide sale (Sparks et al., 2020). Acetamiprid is a neonicotinoid insecticide that is commonly used to control sucking pests, such as aphids and whiteflies (Yamada et al., 1999). However, its chemical structure differs from that of many other members of this group, hence it is categorized as part of the N-cyanoamidines subgroup (Jeschke et al., 2011). Acetamiprid belongs to subclass 4A, which is known as a nicotinic acetylcholine receptor (nAChR) agonist, according to the Insecticide Resistance Action Committee (IRAC) classification (Yamada et al., 1999; Nauen and Denholm, 2005; Sparks et al., 2020) .In insects, nAChRs (a member of cys-loop ligand-gated ion channel superfamily) is widely and densely distributed in the neuropil regions of the central nervous system (Tomizawa & Casida, 2003). Neonicotinoids bind to these receptors, inhibiting the excitatory neurotransmitter acetylcholine from binding to these receptors, leading to disruption of the neuronal transmission and eventually death of the insects (Abbink,1991; Stenersen, 2004).

Insecticide resistance in *M. persicae* develops mostly through metabolic pathways or target-site mutations, similar to other insects (Bass et al., 2014, Crossthwaite et al., 2017; Troczka et al., 2021).One of such mechanisms underlying resistance has been previously suggested to be the increased cytochrome P450 monoxygenase (P450s) enzyme activity (Philippou et al., 2009).More specifically, Puinean et al. (2010) reported that overexpression of a P450 gene, *CYP6CY3*, is associated with neonicotinoid resistance. In addition, the R81T mutation in β1 subunit of the nicotinic acetylcholine receptor, the target site of neonicotinoids, has been linked to resistance development (Bass et al., 2011) and is further supported by molecular modeling (Wang et al., 2016). Recently, another mutation in the same subunit,V101I, has been uncovered in an imidacloprid-resistant *M. persicae* population (Xu et al., 2022).

So far, the knowledge on resistance mechanisms to neoniconitoids mainly comes from the studies on imidacloprid. However, together with the limitation of imidacloprid usage, more attention needs to be given to the resistance mechanisms to other neonicotinoids, such as acetamiprid. In this study, the biochemical and molecular mechanisms of acetamiprid resistance were investigated using a laboratory population of *M. persicae*. In addition, cross-resistance between acetamiprid and other insecticides were examined to contribute better use of insecticides in rotation.

2.Materialmethod

2.1.Origin and production of *Myzus persicae*

The population used in acetamiprid selection was collected from pepper plants in Adana/Turkey in 2004. Since then, the population has been reared on radish plants (*Raphanus sativus* L.) (Brassicales: Brassicaceae) without any pesticide exposure in climate cabinets with 26±1°C temperature, 60-65% humidity, and 16:8 photoperiod conditions.This study was conducted in the Isparta University of Applied Sciences, Department of Plant Protection in 2018 and 2020.

2.2.Bioassays, selection and cross-resistance

In toxicity assays, the method recommended by IRAC (No. 19) was followed using a commercial formulation of acetamiprid (GOLDPLAN 20 SP). Before choosing the appropriate concentrations, preliminary tests were performed to determine the various mortality rates between 10-90%. Bioassays were performed using at least 5 gradually (25 mg/100 ml water, 12,5 mg/100 ml water, 6,25 mg/100 ml water, 3,125 mg/100 ml water and 1,5625 mg/100 ml water) diluted concentration and a control group that was sprayed only with distilled water. Three replications including 15 adult aphids for each replicate were used at each insecticide concentration.

After clean radish leaves were dipped into insecticide solution for 10 seconds and placed on petri dishes with agar, aphids were gently transferred onto the leaves. The petri dishes were then placed in the climate cabinets. Mortality was assessed after 72h and aphids that could not move when touched were considered dead. Acetamiprid-related mortality, slope values, and Lethal Concentration (LC50,60) values were determined bythe POLO computer package program (Software, 2002).

LC_{60} values determined via bioassays were used in each selection step. Thirty adult aphids with 15 replicates were used in assays. Bioassays were performed as mentioned above, and survived aphids were transferred to radish plants. Resistance ratios (RR) were determined by dividing the LC values.

Next, we determined the susceptibility of the *M. persicae* population before and after acetamiprid

selection against four insecticides registered for aphid control to investigate potential cross-resistance. The tested insecticides were tau-fluvanite (Mavrik® 2F), beta-cyfluthrin (Hawk® 25 EC), imidacloprid (Confidor® SC 350) and sulfoxaflor (Breaker™ 240 SC).

2.3.Synergism studies

In order to investigate the role of P450 in acetamiprid resistance, piperonyl butoxide (PBO) inhibiting P450 activity was used. PBO (1000 mg L^{-1}) was prepared and applied as previously described by Carletto et al., (2010).Two hours after synergist treatment, acetamiprid solutions were applied. The rest of the experiment was the same as described in the bioassay section. Synergist ratios (SR) were determined by dividing the LC values.

2.4.Specific activity of P450 monooxygenases

To determine P450 enzyme activity in selected and initial aphid populations, the method suggested by Li et al., (2016) was used. Briefly, a pool of 60 adult aphids were homogenized in 2 mL of PBS buffer (0.1 mol L^{-1}, pH 7.8). Nitroanisole (0.05 mol L-1) dissolved in acetone was used as a substrate. Supernatant and NADPH were added to microplate cells and incubated at 37 °C for 30 minutes. Then, 1 mol of L^{-1} hydrochloric acid was added to the microplate cells. Kinetic readings were made at 400 nm using SpectraMax® M2 (Molecular devices, USA) after the addition of chloroform and 0.5 mol L^{-1} NaOH to the reaction cells.

Kinetic measurement of P450 enzymes in microplate cells was performed with four replications. Bovine Serum Albumin (B.S.A) was used as the Bradford (1976) standard in the total protein method (Doumas, 1975).Duncan multiple comparison test was performed by applying the one-way analysis of variance technique (One-Way ANOVA) to compare MFO enzyme activities between populations.

2.5.Target-site amplification and relative expression of *CYP6CY3*

Following the manufacturer's instructions, total RNA was extracted from two pools of ten adults for each population using the GeneMATRIX Universal RNA/miRNA Purification Kit (EURX, Poland). Gel electrophoresis and spectrophotometry were used to evaluate the quality and quantity of extracted RNA (Thermo Scientific, NanoDrop™ 2000). The iScript cDNA Synthesis Kit (Biorad, USA) was used to make 2000) of cDNA for the PCR and qPCR steps.

The primers used for nAChR1 amplification and sequencing, and also the PCR conditions, were as described by Puinean et al., (2010). Based on visual inspection of sequencing chromatographs, target-site sequences of susceptible and selected F5 populations were compared using BioEdit 7.0.5 software (Hall 1999) (İnak et al., 2019).

The primer pair suggested by Puinean et al., (2010) was used to determine the expression level of *CYP6CY3*. As previously suggested, the reference genes actin, voltage-gated sodium channel, and acetylcholinesterase were used (Bass et al., 2011).

The qPCR was performed in a total volume of 20 µl, including 10 µl of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA), 1 µl of forward and reverse primers (10µM stock), 1 µl of cDNA (100 ng/µl), and 7 µl of nuclease-free water using a BioRad CFX96 TouchTM. The qPCR conditions were as follows: 98 degrees Celsius for 30 seconds, followed by 39 cycles of 95 degrees Celsius for 10 seconds and 57 degrees Celsius for 30 seconds. To confirm the lack of non-specific bindings, the melting curve was examined at temperatures ranging from 60°C to 95°C. The ΔΔCt method was used to compute relative expression levels (Livak and Schmittgen, 2001). An unpaired t-test was used to calculate P-values (CFX MaestroTM Software, Bio-Rad). For each analysis, three biological replicates were used.

3.Results and Discussion

3.1.Selection of laboratory *Myzus persicae* population with acetamiprid

$LC_{50,60}$ values and resistance ratio of *M. persicae* populations obtained after acetamiprid selection are given in Table 1. According to the LC_{50} value of the initial population, the resistance ratios obtained after consecutive selections were 11-, 13-, 18.5- and, 47-fold, respectively.In the last selection, the population was achieved 57.5-fold acetamiprid resistance (here after called F5).

Resistance development to insecticides is a growing global issue for agricultural pests (Sparks et al., 2021). In addition, increasing legal pressure on insecticides resulting in restrictions or bans on several widely-used insecticides together with increasing costs for developing novel active compounds limit the design of insecticide rotation programs (Sparks, 2013; Sparks & Lorsbach, 2017). Therefore, the mechanisms underlying the resistance should be elucidated in detail in order to manage or delay the insecticide resistance

development. In the present study, we artificially selected a *M. persicae* population with acetamiprid to investigate the biochemical and molecular mechanisms of resistance.

Table 1. $LC_{50,60}$ values and resistance ratios of each generations of *Myzus persicae* selected with acetamiprid
Çizelge 1. *Asetamiprid ile selekte edilen Myzus persicae popülasyonlarında $LC_{50,60}$ değerleri ve direnç oranları*

Populations	n*	Slope±SE	LC_{50} (mg a.i L^{-1}) (0.95% Cl[a])	LC_{60} (mg a.i L^{-1}) (0.95% Cl)	χ^2	df	RR[b]
Initial population	270	1.268±0.161	8.0 (4.0-12.0)	12.0 (8.0-16.0)	13.8	13	-
Selection -1	270	0.799±0.160	88.0 (56.0-136.0)	102.0 (68.0-164.0)	11.5	13	11.0
Selection -2	270	1.318±0.241	104.0 (60.0-148.0)	160.0 (108.0-236.0)	8.5	13	13.0
Selection -3	270	1.380±0.238	148.0 (92.0-208.0)	228.0 (156.0-324.0)	12.6	13	18.5
Selection -4	270	1.735±0.262	376.0 (252.0508.0)	528.0 (380.0-704.0)	6.8	13	47.0
Selection -5 (F5)	270	1.594±0.267	460.0 (280.0-648.0)	664.0 (448.0-916.0)	11.7	13	57.5

n*: the number of individuals used in the experiment
[a]:Cl = Confidence limit
[b]: RR = Resistance ratio

Five consecutive selections with acetamiprid resulted in 57.5-fold resistance. Compared to selection studies in other aphid species, *Aphis gossypii* Glover (Hemiptera: Aphididae), (Ullah et al., 2020), the LC_{50} value of our initial population was high, indicating the presence of resistance alleles conferring low-level acetamiprid resistance. The fact that the LC_{50} value of a population grown in a pesticide-free environment for many years exceeds the registered field rate of acetamiprid (50 mg a.i. L^{-1}) with a single selection emphasizes the need to be careful in the rotation of acetamiprid in field conditions.

3.2.Cross-resistance between acetamiprid and other insecticides

The susceptibility of acetamiprid-selected F5 population against imidacloprid, sulfoxaflor, beta-cyfluthrin, and tau-fluvanite is given in Table 2. Cross-resistance results of the F5 population against imidacloprid, sulfoxaflor, beta-cyfluthrin, and tau-fluvanite were determined as 2.62, 4.76, 1.54 and 3.13 fold, respectively.

Next, we evaluated whether acetamiprid selection would cause decreasing susceptibility to other insecticides registered for *M. persicae* even if they had not been exposed to them. The lack of cross-resistance assumption is of vital importance since it contributes to forming the basis of insecticide resistance management programs in field conditions. All tested insecticides (imidacloprid, sulfoxaflor, beta-cyfluthrin, and tau-fluvanite) showed more than 1.5-fold resistance ratio after acetamiprid selection.

Table 2. LC_{50} values and cross-resistance ratios determined against other insecticides in the F5 population
Çizelge 2. *F5 popülasyonunda diğer insektisitlerekarşı belirlenen LC_{50} değerleri ve çoklu direnç oranları*

Insecticide	Population	n*	Slope±SE	LC_{50} (mg a.i L−1)(95% Cl[a])	χ^2	df	RR[b]
Imidacloprid	F5	270	1.729±0.260	47.638 (33.557-63.492)	5.8	13	2.62
	Initial	270	1.500±0.248	18.129 (11.227-25.284)	6.5	13	-
Sulfoxaflor	F5	270	1.380±0.229	21.354 (14.855-29.364)	12.5	13	4.76
	Initial	270	1.754±0.259	4.484 (3.260-5.914)	11.7	13	-
Beta-cyflutrin	F5	270	1.977±0.281	93.792 (67.868-122.048)	18.5	13	1.54
	Initial	270	2.196±0.295	60.566 (45.755-76.458)	18.7	13	-
Tau-fluvanite	F5	270	2.103±0.417	84.084 (55.035-112.964)	12.4	13	3.13
	Initial	270	1.632±0.260	26.831 (17.535-36.549)	12.9	13	-

n*: the number of individuals used in the experiment
[a]:Cl = Confidence limit
[b]: RR = Resistance ratio

Cross-resistance among neonicotinoids has been known due to *CYP6CY3* and target site mutations (Bass et al., 2011; Bass et al., 2013; Koo et al., 2014).However, in the present study, the cross-resistance arises via mechanisms other than those mentioned. Therefore, possible mechanisms underlying the neonicotinoid cross-resistance should be elucidated. Similarly, acetamiprid selection resulted in increased imidacloprid resistance in whiteflies (Basit et al., 2011). On the other hand, the debate about cross-resistance between neonicotinoids and sulfoxaflor seems to be more complicated since there are some contradictory results (Cutler et al., 2013; Watson et al., 2021).Although sulfoxaflor has a similar target site with neonicotinoids, its mode of action and chemical structure is different, and it belongs to the group of sulfoxamines (Sparks et al., 2013). However, Cutler et al., (2013) reported that the mechanism of action of

sulfoxaflor is same to that of neonicotinoids, and therefore there is a possibility of cross-resistance. In the present study, acetamiprid selection led to approximately 5-fold sulfoxaflor resistance. Although they are both pyrethroid, beta-cyfluthrin and tau-fluvanite showed different cross-resistance outcomes, which have 1.54 and 3.13-fold resistance, respectively, indicating the putative role of non-selective enzymes in cross-resistance. Overall, beta-cyfluthrin should be considered for insecticide rotation programs for the control of acetamiprid-resistant *M.persicae* populations. Notably, the resistance mechanisms conferring cross-resistance between insecticides belonging to different modes of action groups (such as neonicotinoids and pyrethroids) are often caused by unselective detoxification enzymes, which should be further investigated.

3.3.Synergistic effect of PBO on acetamiprid toxicity

The synergistic effect of P450 inhibiting PBO in acetamiprid toxicity was determined in the initial and selected *M. persicae* populations (Table 3). The results showed that fold the synergism ratios were less than 1.1.

Table 3. LC_{50} values of acetamiprid with and without PBO treatment for *Myzus persicae* populations
Çizelge 3. *Myzus persicae popülasyonlarında PBO+asetamiprid LC_{50} değerleri*

Population	Insecticide	n*	Slope±SE	LC_{50} (mg a.i L^{-1})(0.95% Cl[a])	χ^2	df	SR[b]
F5 population	Acetamiprid	270	1.594±0.267	664.0 (448.0-916.0)	11.7	13	-
	Acetamiprid+ PBO	270	1.516±0.262	609.1 (402.8-875.9)	9.8	13	1.09
Initial population	Acetamiprid	270	1.268±0.161	8.0 (4.0-12.0)	13.8	13	-
	Acetamiprid+ PBO	270	1.742±0.267	8.1 (4.1-12.6)	15.8	13	1.00

n*: the number of individuals used in the experiment
[a]:CL = Confidence limit
[b]: SR = Synergism ratio

3.4.Specific activity of P450 monoxygenases

The P450 enzyme activities for the initial and selected F5 populations of *M. persicae* were found to be 0.2387 and 0.2357 mOD/min/mg protein, respectively (Table 4). Statistical analysis did not show any significant differences.

Table 4. Specific activity of P450 enzymes in selected and pre-selected *Myzus persicae* populations
Çizelge 4. *Myzus persicae popülasyonlarında P450 enzim seviyeleri*

Populations	N*	mOD/min/mg protein(±SE)
F5	4	0,2357(±0.002) a
Initial	4	0,2387 (±0.004) a

*: Number of repetitions

3.5.Screening of target-site mutations

The sequences of the beta subunit of the nicotinic acetylcholine receptor of both the initial susceptible and acetamiprid-selected populations were compared to investigate the presence of target-site mutations. In addition, these sequences were also compared with reference sequences in GenBank (AJ251838.1, XM_022309582.1). However, no target-site mutation was detected in the populations.

3.6.Relative expression of *CYP6CY3* gene

The relative expression of a resistance-associated P450, *CYP6CY3*, was investigated. Although population F5 had a higher expression level, this difference was not statistically significant (Figure 1).

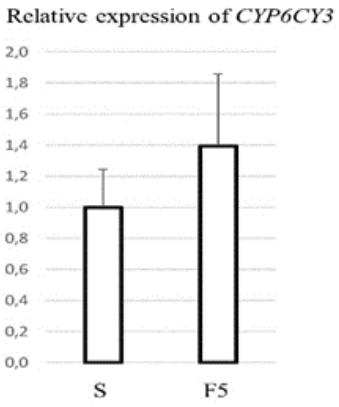

Figure 1. Relative expression of *CYP6CY3* in the initial (S) and selection populations (F5) in *Myzus persicae*
Şekil 1. *Myzus persicae'nin başlangıç (S) ve seleksiyon (F5) popülasyonlarında CYP6CY3 ifadesi*

P450-mediatedacetamiprid resistance has been documented in different aphid species (Ullah et al., 2020; Sial et al., 2022).In the present study, synergism and biochemical assays were performed to determine the role of P450s in decreased susceptibility to acetamiprid. PBO did not synergize the acetamiprid toxicity in both initial and selected F5 population. Thus, the specific activity of P450s in both populations was similar ($p>0.05$), indicating the absence of

elevated P450 activity in acetamiprid resistance. More specifically, the*CYP6CY3* gene, a well-known, globally distributed gene responsible for neonicotinoid resistance (Puinean et al., 2010; Singh et al., 2021; Sial et al., 2022), was measured at mRNA level in the initial and acetamiprid-selected F5 population. Although the F5 population had a higher *CYP6CY3* expression compared to control, this difference was statistically insignificant ($p<0.05$). These results indicate that the acetamiprid resistance is likely to be associated with the mechanisms other than increased P450 activity.

In addition to elevated P450 activity, a point mutation (R81T) in the loop D region of the β1 subunit of the nicotinic acetylcholine receptor has been associated with neonicotinoid resistance (Bass et al., 2011) and common in worldwide populations of *M. persicae* (Singh et al., 2021). Recently, another amino acid substitution, V101I, in the same subunit of an acetamiprid-resistant *M. persicae* population has been reported (Xu et al., 2022). In our study, the initial population did not harb our any mutations in β1 subunit of the nicotinic acetylcholine receptor; therefore, no mutation was expected in the selected F5 population. However, since only limited number of individuals were used in mutation screening, the presence of mutation might be missed. Thus, we still screened for the presence of target site mutations in the F5 population. However, no mutation was detected in the selected F5 population. Examination of more populations with different genetic backgrounds would be helpful to make a conclusive statement.

4. Conclusion

This study showed that mechanisms other than the well-known neonicotinoid resistance mechanisms might lead to high acetamiprid resistance.It should be noted that investigations on resistance mechanisms using laboratory selections do not always reflect the resistance in field conditions. Therefore, studies other than artificial selection in laboratory conditions are necessary. Nonetheless, mechanisms underlying the cross-resistance should be examined to implement robust resistance management programs.

Acknowledgements

We greatly acknowledge Isparta University of Applied Sciences, Scientific Research Projects Coordination Unit for the financial support of this project (project no: 2019-MYL-0041).

References

Abbink, J.(1991). The biochemistry of imidacloprid. *Pflanzenschutz-Nachrichten Bayer*,(44), 198-195.

Basit, M., Sayyed, A.H., Saleem, M.A. & Saeed, S. (2011). Cross-resistance, inheritance and stability of resistance to acetamiprid in cotton whitefly, *Bemisia tabaci* Genn (Hemiptera: Aleyrodidae). *Crop Protection*, 30(6), 705-712.

Bass, C., Puinean, A.M., Andrews, M., Cutler, P., Daniels, M., Elias, J.,Paul, V.L., Crosswaite, A.J., Denholm, I., Field, L.M., Foster, S.P., Lind,R., Williamson M.S. & Slater, R. (2011). Mutation of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *BMC Neuroscience*, 12(1), 1-11.

Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P. Williamson, M.S. (2014). The evolutionof insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochemistry and Molecular Biology*, 51, 41-51.

Bass, C., Zimmer, C.T., Riveron, J.M., Wilding, C.S., Wondji, C.S., Kaussmann, M. & Nauen, R. (2013). Gene amplification and microsatellite polymorphism underlie a recent insect host shift. *The Proceedings of the National Academy of Sciences*, 110(48), 19460-19465.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72 (1-2),248-254.

Brault, V., Uzest, M., Monsion, B., Jacquot, E. & Blanc,S. (2010). Aphids as transport devices for plant viruses. *Comptes Rendus Biologies*, 333(6-7), 524-538.

Carletto, J., Martin, T., Vanlerberghe-Masuttia, F. & Brevault, T. (2010). Insecticide resistance traits differ among and within host races in *Aphis gossypii*. *Pest Management Science*, 66, 301–307.

Crossthwaite, A.J., Bigot, A., Camblin, P., Goodchild, J., Lind, R.J., Slater, R. & Maienfisch, P. (2017). The invertebrate pharmacology of insecticides acting at nicotinic acetylcholine receptors. *Journal of Pest Science*, 7, 17-19.

Cutler, P., Slater, R., Edmunds, A.J., Maienfisch, P., Hall, R.G., Earley, F.G. & Crossthwaite, A.J. (2013). Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid. *Pest Management Science*, 69(5), 607-619.

Dedryver, C.A., Le Ralec, A. & Fabre, F. (2010). The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *Comptes Rendus Biologies*, 333(6-7), 539-553.

Doumas, B.T. (1975). Standards for total serum protein assays—a collaborative study. *Clinical Chemistry*, 21 (8), 1159-1166.

Emden, H.F. & Harrington, R. (2017). Aphids as crop pests. CABI, London, UK, Nosworthy Way, Wallingford, Oxfordshire OX10 8DE, UK.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41,95–98.

Harris, K.F. & Maramorosch, K. (Eds.) (1977). Aphids as virus vectors. Elsevier.

Jeschke, P., Nauen, R., Schindler, M. & Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry*, 59(7), 2897-2908.

Koo, H.N., An, J.J., Park, S.E., Kim,J.I. & Kim, G.H. (2014). Regional susceptibilities to 12 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point mutation associated with imidacloprid resistance. *Crop Protection*, 55, 91-97.

Li, Y., Xu, Z., Shi, L., Shen, G. & He,L. (2016). Insecticide

resistance monitoring and metabolic mechanism study of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), in Chongqing, China. *Pesticide Biochemistry and Physiology*, 132, 21–28.

Livak, K.J. & Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, 25(4),402-408.

Mota-Sanchez, D. & Wise, J.C. (2022). The Arthropod Pesticide Resistance Database. Michigan State University. https://www.pesticideresistance.org/.

Nauen, R. & Denholm, I. (2005). Resistance of insect peststo neonicotinoid insecticides: current status and futureprospects. *Archives of Insect Biochemistry and Physiology*, 58 (4), 200-215.

James, C. & Perry, K.L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, 5(5): 505-511.

Philippou, D., Field, L.M. & Moores, G.D. (2009). Metabolic enzyme(s) confer imidacloprid resistance in a clone of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) from Greece. *Pest Management Science*, 66, 390–395.

Puinean, A.M., Foster, S.P., Oliphant, L., Denholm, I., Field, L.M., Millar, N.S., Williamson, M.S. & Bass, C. (2010). Amplification of a cytochrome P450 gene is sssociated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *PLoS Genetics*, 6(6). e1000999. doi:10.1371/journal.pgen.1000999.

Sial, M.U., Mehmood, K.,Saeed,S.,Husain,M.,Rasool,K.G. & Aldawood, A.S. (2022). Neonicotinoid's resistance monitoring, diagnostic mechanisms and cytochrome P450 expression in green peach aphid [*Myzus persicae* (Sulzer) (Hemiptera: Aphididae)]. *Plos One*, 17(1), e0261090. https://doi.org/10.1371/journal.pone.0261090.

Simon, J.C. & Peccoud, J. (2018). Rapid evolution of aphid pests in agricultural environments. *Current Opinion in Insect Science*, 26, 17-24.

Singh, K.S., Cordeiro, E.M., Troczka, B.J., Pym, A., Mackisack, J., Mathers, T.C., Bass, C. (2021). Global patterns in genomic diversity underpinning the evolution of insecticide resistance in the aphid crop pest *Myzus persicae*. *Communications Biology*, 4(1),1-16.

Software, L. (2002). POLO-Plus, a user's guide to probit or logic analysis.LeOra Software, Berkeley, CA

Sparks, T.C. (2013). Insecticide discovery: an evaluation and analysis. *Pesticide Biochemistry and Physiology*, 107(1),8-17.

Sparks, T.C. & Lorsbach, B.A. (2017). Perspectives on the agrochemical industry and agrochemical discovery. *Pest Management Science*, 73(4), 672-677.

Sparks, T.C., Crossthwaite, A. J., Nauen, R., Banba, S., Cordova, D., Earley, F. & Wessels, F.J. (2020). Insecticides, biologics and nematicides: Updates to IRAC's mode of action classification-a tool for resistance management. *Pesticide Biochemistry and Physiology*,167,104587.

Sparks, T.C., Storer, N., Porter, A., Slater, R. & Nauen,R. (2021). Insecticide resistance management and industr y: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. *Pest Management Science*, 77(6), 2609-2619.

Sparks, T.C., Watson, G.B., Loso, M.R., Geng, C., Babcock, J.M. & Thomas, J.D. (2013). Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects. *Pesticide Biochemistry and Physiology*, 107(1), 1-7.

Stenersen, J. (2004). Nicotinoids and neonicotinoids chemical pesticides. Mode of Action and Toxicology, CRC Press, USA.

Tomizawa, M. & Casida, J.E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinik receptors. *Annual Review of Entomology*, 48,339–364.

Troczka, B.J.,Singh, K.S., Zimmer, C.T., Vontas, J., Nauen, R., Hayward, A. & Bass, C. (2021). Molecular innovations underlying resistance to nicotine and neonicotinoids in the aphid Myzus persicae. *Pest Management Science*, 77(12), 5311-5320.

Ullah, F., Gula, H., Tariq, K., Desneuxe, N., Gaoa, X. & Songa, D. (2020). Functional analysis of cytochrome P450 genes linked with acetamiprid resistance in melon aphid, *Aphis gossypii*. *Pesticide Biochemistry and Physiology*, https://doi.org/10.1016/j.pestbp.2020.104687.

Wang, N.X., Watson, G.B., Loso, M.R. & Sparks, T.C. (2016). Molecular modeling of sulfoxaflor and neonicotinoid binding in insect nicotinic acetylcholine receptors: Impact of the Myzus β 1 R81T mutation. *Pest Management Science*, 72(8), 1467-1474.

Watson, G.B., Siebert, M.W., Wang, N.X., Loso, M.R. & Sparks, T.C. (2021). Sulfoxaflor–A sulfoximine insecticide: Review and analysis of mode of action, resistance and cross-resistance. *Pesticide Biochemistry and Physiology*,178,104924.

Xu, X., Ding, Q., Wang, X., Wang, R.,Ullah, F., Gao, X. & Song, D. (2022). V101I and R81T mutations in the nicotinic acetylcholine receptor β1 subunit are associated with neonicotinoid resistance in *Myzus persicae*. *Pest Management Science*, 78,1500-1507.

Yamada, T., Takahashi, H. & Hatano, R. (1999). A novel insecticide, acetamiprid. In: Yamamoto I., Casida J.E. (eds) Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Springer, Tokyo. https://doi.org/10.1007/978-4-431-67933-2_7.