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Mutations in the voltage-gated sodium channel gene associated with deltamethrin resistance in commercially sourced *Phytoseiulus persimilis*

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Abstract

The implementation of Integrated Pest Management (IPM) in current agricultural practice is a convenient and very effective strategy to maintain pest populations under control. The use of biological control agents, like *Phytoseiulus persimilis*, is key for the success of such an approach. This predatory mite is widely used since it is very effective for controlling *Tetranychus urticae*, one of the most devastating crop pests.

Here we identify several mutations located in the Voltage Gated Sodium Channel (VGSC) of commercially sourced *P. persimilis* that correlate with a reduced susceptibility to the pyrethroid deltamethrin. We found that the mites sourced from two different biocontrol product companies have intrinsic genotypic differences that correlate with their phenotype when tested with different concentrations of deltamethrin. Mites from Syngenta Bioline, carrying the mutations M918L and A1536T, were able to survive deltamethrin concentrations of up to 10 ppm, while the mites from Koppert Biological Systems, with the combination M918L, L925V and S1539T, survived treatment with 40 ppm. All of the point mutations identified in the predatory mite samples are located in a particular region of the VGSC, previously proposed as the binding site for this family of pesticides and identified as a 'hot spot' for resistance.

Introduction

Integrated Pest Management (IPM) is considered the most effective and environmentally sensitive approach to combating arthropod pests, assimilating cultural and physical practices and the use of chemical and biological control measures. Indeed, the overall objective of the EU is to establish "a framework to achieve the sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of Integrated Pest Management and of alternative approaches or techniques such as non-chemical alternatives to pesticides" (Directive 2009/128/EC). This is a science-based, decision-making strategy intended to maintain pests or diseases below Economic Injury Levels (EILs). The use of Biological Control Agents (BCAs) to reduce pest populations is key for the success of such a strategy and it is considered the most convenient approach to reduce the negative side-effects currently associated with the use of chemical pesticides. In Almeria (Spain), a highly intensive region for European agriculture, farmers have embraced this approach with enthusiasm. The crop growing area dedicated to biological control has increased approximately 300% since 2005-2006, encompassing 25,700 ha in 2016 (Alarcón-Roldán, 2016).

Phytoseiulus persimilis Athias-Henriot (Acari: Phytoseiidae) is a fast-moving predatory mite that feeds exclusively on *Tetranychus* species (McMurtry and Croft, 1997) and is one of the most valuable and widely used BCAs (van Lenteren et al., 2018). It is particularly effective against *Tetranychus urticae* Koch (Acari: Tetranychidae), the two-spotted spider mite, which is one of the most damaging pests in agriculture since it is capable of feeding on more than 1,100 host plant species belonging to more than 140 different plant families (Helle and Sabelis, 1985) and shows a great capacity to adapt to different environmental conditions and

60 food sources (Grbic et al., 2011). *Phytoseiulus persimilis* has been used successfully in
61 augmentative biological control programs to reduce *T. urticae* infestations in greenhouses
62 since 1968 (Fathipour and Maleknia, 2016). In augmentative biological control, BCA's are
63 mass-reared either to be released in large numbers to obtain rapid pest control (inundative
64 biological control) or to be released in low numbers to control the pest for several generations
65 (seasonal inoculative biological control) (van Lenteren et al., 2018). To guarantee the success
66 of this approach, the release of BCA's must be integrated with other crop protection practices,
67 like the use of conventional pesticides targeting other pests on which the released natural
68 enemies do not act (Jacas and Urbaneja, 2008). It is therefore important to use selective
69 pesticides that maintain and preserve BCAs and are not disruptive to the biological control
70 programme (Argolo et al., 2014; Desneux et al., 2007; Urbaneja et al., 2008).

71 Synthetic pyrethroids are frequently used to control pests in agricultural, veterinary and
72 domestic settings and still command a significant share of the pesticides market (Davies and
73 Williamson, 2009; Sparks and Nauen, 2015). This family of compounds exhibit high toxicity
74 against a wide range of arthropod pests through their specific interactions with the voltage-
75 gated sodium channel (VGSC), a large protein located in the membrane of axons and other
76 excitable cells that is essential for the initiation and transmission of action potentials in nerve
77 signalling (Catterall, 2000). As with many other pesticides, intensive use of pyrethroids has
78 led to the development of resistance in many pest populations. Although some of these cases
79 have been attributed to alterations in the expression of certain detoxification enzymes
80 (Feyereisen et al., 2015; Van Leeuwen and Dermauw, 2016), the most common mechanism
81 of resistance is the substitution of key residues within the VGSC (Davies et al., 2007). These
82 residues are located mainly in the linker between transmembrane segments four and five of

domain II (IIS4-S5), in segments five (IIS5) and six (IIS6) of the same domain and in segment six of domain III (IIIS6) (Dong et al., 2014). Protein structure modelling studies suggest that these regions form a binding site for pyrethroids, comprising a hydrophobic pocket close to the intracellular mouth of the activated (open) channel into which the pyrethroids bind tightly (O'Reilly et al., 2006). Several of the substitutions in this region are known to confer high levels of resistance to pyrethroids in arthropods, often referred to as kdr (knockdown resistance) or super-kdr mutations, and usually include methionine 918 (M918T,L,V,I), leucine 925 (L925I, V), threonine 929 (T929I,C,V,N), leucine 932 (L932F), leucine 1014 (L1014F,S,H,W,C) phenylalanine 1534 (F1534C) and phenylalanine 1538 (F1538I) (reviewed by Dong et al., 2014. All numbering matches that of the *Musca domestica* L. VGSC (GenBank Accession X96668)). In mites, resistance to pyrethroids has already been associated with these target-site modifications in *T. urticae*, *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae), *Panonychus citri* (McGregor) (Acari: Tetranychidae), *Varroa destructor* Anderson and Trueman (Acari: Varroidae) and *Sarcoptes scabiei* L. (Acari: Sarcoptidae) (reviewed by Van Leeuwen and Dermauw 2016).

Here we report the identification of analogous mutations in the VGSC of commercially available *P. persimilis* strains. The mutations are present in a significant proportion of the populations and show a strong correlation with the reduction of susceptibility to deltamethrin. The potential implications for optimised integration of *P. persimilis* within an IPM strategy are discussed.

Results

VGSC sequence variability

105 Comparison of the relevant genomic sequence of domains II and III, obtained after sequencing
106 two separate batches of insects per commercial source (Syngenta Bioline or Koppert
107 Biological Systems), revealed high similarity for the *P. persimilis* VGSC sequence between the
108 two suppliers, except for certain non-synonymous single nucleotide polymorphisms (SNPs)
109 resulting in amino acids substitutions at positions 918 and 925 of domain II or 1536 and 1539
110 of domain III (Fig. 1A and Supplementary material Fig. S1). The *P. persimilis* samples analysed
111 in this study have either a Leucine at (the super-kdr) position 918 or they are heterozygous
112 for Leucine and Methionine at this position (Fig 1A). We did not identify mites that were
113 homozygous for Methionine at this position, although this is normally a highly conserved
114 residue among arthropod species (Fig. 1B). The other residues showing variation in *P.*
115 *persimilis* samples are also well conserved among arthropods (Fig. 1B). We sequenced mite
116 samples having Leucine at position 925, Alanine at 1536 or Threonine at 1539, which are the
117 'normal' wild-type residues at these positions in arthropods, but also identified individual
118 mites with Valine at 925, Threonine at 1536 and Serine at 1539. For these three positions
119 (925, 1536 and 1539), we found samples with each of the residues as homozygotes or as
120 heterozygotes, and in multiple combinations (data not shown).

121 *Susceptibility to pyrethroid insecticide*

122 Mites from the commercial colonies, obtained from Syngenta Bioline or from Koppert
123 Biological Systems, were bioassayed with deltamethrin to test for susceptibility. The mites
124 from Syngenta Bioline showed 74 % mortality at 5 ppm, 97 % mortality at 10 ppm and 100 %
125 mortality when tested at higher concentrations (Fig. 2). Mortalities of treated mites were
126 significantly different from that of the untreated controls (Kruskal-Wallis test, $P < 0.0001$).
127 Mites from Koppert showed a mean mortality of only 12.9 % at 40 ppm, with a variability

among batches ranging from 2 % to 36 % mortality, which makes it non-statistically different from the untreated controls (Mann-Whitney U = 171.5, *P* = 0.0703) (Fig. 2).

Molecular characterization following bioassay

For both commercial sources, mites surviving the treatment with deltamethrin were separated from those that were susceptible in each case. For Syngenta Bioline samples, we sequenced DNA from single mites and found that those surviving treatments of up to 10 ppm deltamethrin had the Leucine substitution at position 918 and a Threonine at position 1536. Susceptible mites were heterozygous for Methionine and Leucine at position 918, for Leucine and Valine at position 925 and for Threonine and Alanine at position 1536 (Table 2). The mites obtained from Koppert that survived the treatment with 40 ppm deltamethrin were pooled and were found to be homozygous for Leucine at position 918, Valine at position 925 and Threonine at position 1539. Susceptible mites were also homozygous but for Leucine at 918 and 925 and for Serine at position 1539 (Table 2 and Supplementary material Fig. S2).

To test for the presence of other alleles in the population from Koppert Biological Systems, ten mites were selected randomly for DNA extraction. The sequence of DNA from these single mites showed that all of them were homozygous for Leucine at position 918 and for Alanine at position 1536. For the other positions, we found different combinations as homozygotes and heterozygotes, but in all cases when there was a Valine at position 925 there was a Threonine at position 1539, and when there was a Leucine at position 925 there was a Serine at position 1539 (examples of the profiles detected are shown in Fig. 3).

Discussion

Resistance to synthetic pyrethroids is usually associated with amino acid substitutions in

150 certain key regions of the VGSC, the primary target site for pyrethroid insecticides (Dong et
151 al., 2014). Here we evidenced that a significant percentage of the mites from commercial
152 populations of *P. persimilis* showed reduced susceptibility to deltamethrin, and that there are
153 indeed mutations in the VGSC associated with these phenotypes. Our data suggest that there
154 is a direct correlation between the reduction in susceptibility recorded and the presence of
155 certain mutations. For example, mites with the substitutions M918L (IIS4-S5 linker) and
156 A1536T (IIIS6) were able to survive when treated with 10 ppm of deltamethrin. On the other
157 hand, mites with a combination of M918L, L925V (IIS5) and S1539T (IIIS6) survived a
158 treatment with 40 ppm of deltamethrin.

159 The aforementioned substitutions are all located in known 'hot-spots' for resistance.
160 Mutations located in these regions, either alone or in combination with others, have been
161 associated with high (super-kdr) levels of resistance to pyrethroids in many arthropod species
162 (Dong et al., 2014). The M918L and L925V mutations identified in this study, that map to
163 domain II, have been described previously in *Aphis gossypii* (Glover) (Hemiptera: Aphididae)
164 (Carletto et al., 2010), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Panini et al., 2015),
165 *Hyalella azteca* (Saussure) (Amphipoda: Hyalellidae) (Weston et al., 2013), *Trialeurodes*
166 *vaporariorum* Westwood (Hemiptera: Aleyrodidae) (Karatolos et al., 2012) and *Thrips tabaci*
167 Lindeman (Thysanoptera: Thripidae) (Wu et al., 2014) and *V. destructor* (González-Cabrera et
168 al., 2013). However, those mapping to domain IIIS6 (A1536T and S1539T) have not previously
169 been identified as associated with resistance. Nonetheless, other authors have described
170 amino acid substitutions mapping at IIIS6 as associated with resistance to pyrethroids in two
171 mosquito species (Kasai et al., 2007; Kawada et al., 2009), in cattle ticks (He et al., 1999) and
172 spider mites (Feng et al., 2011; Tsagkarakou et al., 2009).

It is not surprising that particular amino acid combinations mapping to these regions of the channel protein might be conferring resistance to pyrethroids. A previous modelling study has proposed that the IIS4-S5 linker, IIS5 and IIIS6 regions of the channel form a hydrophobic pocket that can accommodate pyrethroids, forming a high affinity binding site for these compounds (O'Reilly et al., 2006). This model was further refined to show in detail the interaction of pyrethroids with amino acid residues within the hydrophobic pocket in ticks and mites (O'Reilly et al. 2014). According to this model, amino acid residues that line the pocket stabilize pyrethroid binding, so any modification of this 'spatial configuration' would impair binding and confer resistance. Based on this assumption, we can hypothesize that the destabilization caused by the combination of M918L and A1536T is lower than that caused by M918L, L925V and S1539T, given the different levels of resistance recorded with each of these combinations. However, further testing is needed to measure the pharmacological properties of the mite channel and to determine how each of these amino acid substitutions and their combinations are affecting its normal function and response to pyrethroid insecticides.

Resistance to various pesticides in BCA populations has been reported to evolve in response to selection pressure in the field or after selection in the laboratory (Bonafos et al., 2007; Bonafos et al., 2008; Sato et al., 2000; Tirello et al., 2012). For example, in *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae), resistance to methidathion, an organophosphate insecticide, increased by 311-fold after four rounds of selection in the laboratory (Sato et al., 2000) and in a *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) population selection with mancozeb resulted in a 73-fold increase of resistance after ten cycles of selection (Auger et al., 2004). However, there is no information available regarding the mechanisms underlying these reports of resistance.

196 Biological control can be very effective, but there are times when relying on natural enemies
197 alone is not enough. There are occasions where outbreaks of additional pest species (that are
198 not killed by the natural enemy of the target pest) occur and alternative means of control are
199 required. The use of BCA colonies having resistance to certain pesticides can be a timely
200 solution to maintain control of outbreaks in these situations, effectively reducing the input of
201 conventional pesticides to the crop. It was previously reported that, in the UK,
202 organophosphate and carbaryl resistant strains of the predator *T. pyri* were used to control
203 the European red mite, *Panonychus ulmi* Kock (Acari: Tetranychidae) and the apple rust mite,
204 *Aculus schlechtendali* (Nalepa) (Acari: Eriophyidae). The trial lasted four years, and the
205 combination of biological and conventional approaches were sufficient to control the pests
206 to such an extent that no acaricides were needed during the fourth year (Solomon et al.,
207 1993).

208 The significant reduction in the susceptibility to deltamethrin of commercial colonies of *P.*
209 *persimilis*, described here, might provide an opportunity to design similar IPM strategies to
210 better control *T. urticae* along with other pest species affecting protected crops. These
211 strategies will be based on the high efficiency of the predatory mite for controlling *T. urticae*
212 and the judicious application of pyrethroids when necessary to control other pests. This would
213 reduce to the minimum the level of chemical residues in fresh fruits and vegetables, a long-
214 standing demand from supermarkets and consumers.

215 ## Experimental procedures

216 *Phytoseiulus persimilis populations*

Mites used for bioassays were purchased from Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands (Spidex®, 2000 mites per bottle) and from Syngenta Bioline Ltd, Little Clacton, UK (PhytoLine p, 2000 mites per bottle). The variability of relevant regions in the VGSC was assessed sequencing two separate batches of each of these two commercial colonies.

Bioassays

For mites purchased from Syngenta Bioline, groups of 10 mites per replicate (10 replicates), were treated with 5, 10, 20, 40 and 100 ppm of deltamethrin (Sigma-Aldrich, 45423) dissolved in 20 % acetone (Labkem, ACET-G0P-1K0). The field rate was estimated as 12.5 ppm according to the datasheet of Decis® Protech 10 mL (Bayer CropScience, 80269285). Control mites were treated with 20 % acetone. Briefly, the mites were collected directly from the shipped bottle using a fine paintbrush and placed on moist Whatman No 1 filter paper (100 µl of distilled water) in a 5 cm Petri dish. No distinction between males and females was made. Each mite was then treated with one microliter of the relevant concentration of deltamethrin using an automatic micropipette. Given the size of the mite, with this methodology we guarantee that the mite is completely soaked by the deltamethrin solution (literally submerged inside the drop). After application, the mite either walked away from the drop totally wet or it remained motionless until the applied product was absorbed by the filter paper. Approximately 50 *T. urticae* individuals from a stock colony, originally maintained at Instituto Valenciano de Investigaciones Agrarias (Moncada, Valencia, Spain), were added to the plate as a food source and the plate was sealed with Parafilm®. The plates were left undisturbed at 25 ± 2 ºC, 16:8 h L:D, for 24 hours. After this period, the mortality was assessed, and the live and dead mites were placed in separate vials and stored at -20 ºC. Mites from Koppert Biological Systems

were bioassayed following essentially the same procedure but testing only with 40 ppm of deltamethrin.

Analysis of sequences encoding Domains II and III of P. persimilis VGSC.

Genomic DNA was extracted from individual mites or pools of at least 10 mites using DNAzol® (Thermo Fisher Scientific) following the manufacturer's protocol. The relevant regions of Domains II (residues 884-1013) and III (residues 1492-1801) (*M. domestica* numbering) were PCR amplified with the following primer combinations: for Domain II, primers were 1F_IIS5-6 and 1R_IIS5-6 (Table 1) and for Domain III, 1F_IIIS6 and 1R_IIIS6 (Table 1). For each amplification, the reaction mixture contained 0.4 µM of each primer, 12.5 µl of DreamTaq Green PCR Master Mix (2×) (ThermoFisher Scientific) and 1 µl of genomic DNA, in a final volume of 25 µl. Cycling conditions were: 95 °C for 1 min, followed by 35 cycles of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR fragments were purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel GmbH & Co. KG) and direct sequenced (STAB VIDA, Caparica, Portugal) with primers 2F_IIS5-6 for Domain II and 2F_IIIS6 for Domain III (Table 1). All primers were designed, and the sequences analysed, using Geneious software (Version 10.1.3, http://www.geneious.com/).

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References

Alarcón-Roldán, R. (2016) Control Biológico, apuesta de futuro, in: Gutiérrez-Escobar, J.A. (Ed.), Anuario Agrícola 2016: Horticultura intensiva de Almería. fhalmeria, p. 88.

Argolo, P.S., Jacas, J.A., Urbaneja, A. (2014) Comparative toxicity of pesticides in three phytoseiid mites with different life-style occurring in citrus: *Euseius stipulatus*, *Neoseiulus californicus* and *Phytoseiulus persimilis*. Exp Appl Acarol, **62**, 33-46.

Auger, P., Bonafos, R., Kreiter, S. (2004) Mancozeb resistance patterns among *Kampimodromus aberrans* and *Typhlodromus pyri* (Acari : Phytoseiidae) strains from French vineyards. Canadian Entomologis, **136**, 663-673.

Bonafos, R., Serrano, E., Auger, P., Kreiter, S. (2007) Resistance to deltamethrin, lambda-cyhalothrin and chlorpyriphos-ethyl in some populations of *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari : Phytoseiidae) from vineyards in the south-west of France. Crop Protection, **26**, 169-172.

Bonafos, R., Vigues, V., Serrano, E., Auger, P. (2008) Resistance monitoring to deltamethrin and chlorpyriphos-ethyl in 13 populations of *Typhlodromus pyri* Scheuten (Acari : Phytoseiidae) from vineyards in the southwest of France. Crop Protection, **27**, 855-858.

Carletto, J., Martin, T., Vanlerberghe-Masutti, F., Brevault, T. (2010) Insecticide resistance traits differ among and within host races in *Aphis gossypii*. Pest Manag Sci, **66**, 301-307.

Catterall, W.A. (2000) From ionic currents to molecular mechanisms: The structure and function of voltage-gated sodium channels. Neuron, **26**, 13-25.

290 Davies, T.G.E., Field, L.M., Usherwood, P.N.R., Williamson, M.S. (2007) DDT, pyrethrins,
291 pyrethroids and insect sodium channels. IUBMB life, **59**, 151-162.

292 Davies, T.G.E., Williamson, M.S. (2009) Interactions of pyrethroids with the voltage-gated
293 sodium channel. Bayer CropScience Journal, **62**, 159-177.

294 Desneux, N., Decourtye, A., Delpuech, J.M. (2007) The sublethal effects of pesticides on
295 beneficial arthropods. Annual Review of Entomology, **52**, 81-106.

296 Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009, OJ
297 L 309, 24.11.2009, p. 71–86 http://data.europa.eu/eli/dir/2009/128/oj

298 Dong, K., Du, Y., Rinkevich, F., Nomura, Y., Xu, P., Wang, L., Silver, K., Zhorov, B.S. (2014)
299 Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochem Mol
300 Biol, **50C**, 1-17.

301 Fathipour, Y., Maleknia, B. (2016) Mite predators, in: Omkar (Ed.), Ecofriendly Pest
302 Management for Food Security. Academic Press, San Diego, pp. 329-366.

303 Feng, Y.N., Zhao, S., Sun, W., Li, M., Lu, W.C., He, L. (2011) The sodium channel gene in
304 *Tetranychus cinnabarinus* (Boisduval): identification and expression analysis of a mutation
305 associated with pyrethroid resistance. Pest Manag Sci, **67**, 904-912.

306 Feyereisen, R., Dermauw, W., Van Leeuwen, T. (2015) Genotype to phenotype, the molecular
307 and physiological dimensions of resistance in arthropods. Pestic Biochem Physiol, **121**, 61-
308 77.

309 González-Cabrera, J., Davies, T.G.E., Field, L.M., Kennedy, P.J., Williamson, M.S. (2013) An
310 amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa*
311 *destructor*. PLoS ONE, **8**, e82941.

312 Grbic, M., Van Leeuwen, T., Clark, R.M., Rombauts, S., Rouze, P., Grbic, V., Osborne, E.J.,
313 Dermauw, W., Ngoc, P.C., Ortego, F., Hernandez-Crespo, P., Diaz, I., Martinez, M., Navajas,
314 M., Sucena, E., Magalhaes, S., Nagy, L., Pace, R.M., Djuranovic, S., Smagghe, G., Iga, M.,
315 Christiaens, O., Veenstra, J.A., Ewer, J., Villalobos, R.M., Hutter, J.L., Hudson, S.D., Velez, M.,
316 Yi, S.V., Zeng, J., Pires-daSilva, A., Roch, F., Cazaux, M., Navarro, M., Zhurov, V., Acevedo, G.,
317 Bjelica, A., Fawcett, J.A., Bonnet, E., Martens, C., Baele, G., Wissler, L., Sanchez-Rodriguez,
318 A., Tirry, L., Blais, C., Demeestere, K., Henz, S.R., Gregory, T.R., Mathieu, J., Verdon, L.,
319 Farinelli, L., Schmutz, J., Lindquist, E., Feyereisen, R., Van de Peer, Y. (2011) The genome of
320 *Tetranychus urticae* reveals herbivorous pest adaptations. Nature, **479**, 487-492.

321 He, H., Chen, A.C., Davey, R.B., Ivie, G.W., George, J.E. (1999) Identification of a point mutation
322 in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. Biochem
323 Biophys Res Commun, **261**, 558-561.

Helle, W., Sabelis, M.W. (1985) Spider mites: their biology, natural enemies and control: vol. **1B**. Elsevier, Amsterdam.

Jacas, J., Urbaneja, A. (2008) Control biológico de plagas agrícolas. Phytoma-España, Valencia, Spain.

Karatolos, N., Gorman, K., Williamson, M.S., Denholm, I. (2012) Mutations in the sodium channel associated with pyrethroid resistance in the greenhouse whitefly, *Trialeurodes vaporariorum*. Pest Manag Sci, **68**, 834-838.

Kasai, S., Shono, T., Komagata, O., Tsuda, Y., Kobayashi, M., Motoki, M., Kashima, I., Tanikanna, T., Yoshida, M., Tanaka, I., Shinjo, G., Hashimoto, T., Ishikanaa, T., Takahashi, T., Higa, Y., Tomita, T. (2007) Insecticide resistance in potential vector mosquitoes for west nile virus in Japan. Journal of Medical Entomology, **44**, 822-829.

Kawada, H., Higa, Y., Komagata, O., Kasai, S., Tomita, T., Thi Yen, N., Loan, L.L., Sanchez, R.A., Takagi, M. (2009) Widespread distribution of a newly found point mutation in voltage-gated sodium channel in pyrethroid-resistant *Aedes aegypti* populations in Vietnam. PLoS Negl Trop Dis, **3**, e527.

McMurtry, J.A., Croft, B.A. (1997) Life-styles of Phytoseiid mites and their roles in biological control. Annu Rev Entomol, **42**, 291-321.

O'Reilly, A.O., Khambay, B.P.S., Williamson, M.S., Field, L.M., Wallace, B.A., Davies, T.G.E. (2006) Modelling insecticide-binding sites in the voltage-gated sodium channel. Biochemical Journal, **396**, 255-263.

O'Reilly, AO, Williamson, MS, González-Cabrera, J, Turberg, A, Field, LM, Wallace, BA and Davies, TG (2014) Predictive 3D modelling of the interactions of pyrethroids with the voltage-gated sodium channels of ticks and mites. *Pest Manag Sci* **70**: 369-77.

Panini, M., Anaclerio, M., Puggioni, V., Stagnati, L., Nauen, R., Mazzoni, E. (2015) Presence and impact of allelic variations of two alternative s-kdr mutations, M918T and M918L, in the voltage-gated sodium channel of the green peach aphid *Myzus persicae*. Pest Manag Sci, **71**, 878-884.

Sato, M.E., Miyata, T., Kawai, A., Nakano, O. (2000) Selection for resistance and susceptibility to methidathion and cross resistance in *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae). Applied Entomology and Zoology, **35**, 393-399.

Solomon, M.G., Easterbrook, M.A., Fitz Gerald, J.D. (1993) Mite-management programs based on organophosphate-resistant *Typhlodromus pyri* in UK apple orchards. Crop Protection, **12**, 249-254.

Sparks,T. C., Nauen, R. (2015) IRAC: mode of action classification and insecticide resistance management. Pestic Biochem Physiol, **121**, 122-128.

Tirello, P., Pozzebon, A., Duso, C. (2012) Resistance to chlorpyriphos in the predatory mite *Kampimodromus aberrans*. Exp Appl Acarol, **56**, 1-8.

Tsagkarakou, A., Van Leeuwen, T., Khajehali, J., Ilias, A., Grispou, M., Williamson, M.S., Tirry, L., Vontas, J. (2009) Identification of pyrethroid resistance associated mutations in the para sodium channel of the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Insect molecular biology, **18**, 583-593.

Urbaneja, A., Pascual-Ruiz, S., Pina, T., Abad-Moyano, R., Vanaclocha, P., Montón, H., Dembilio, O., Castañera, P., Jacas, J.A. (2008) Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. Pest Manag Sci, **64**, 834-842.

Van Leeuwen, T., Dermauw, W., (2016) The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. Annu Rev Entomol, **61**, 475-498.

Van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A. (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl, **63**, 39-59.

Weston, D.P., Poynton, H.C., Wellborn, G.A., Lydy, M.J., Blalock, B.J., Sepulveda, M.S., Colbourne, J.K. (2013) Multiple origins of pyrethroid insecticide resistance across the species complex of a nontarget aquatic crustacean, *Hyalella azteca*. Proc Natl Acad Sci U S A, **110**, 16532-16537.

Wu, M., Gotoh, H., Waters, T., Walsh, D.B., Lavine, L.C. (2014) Identification of an alternative knockdown resistance (kdr)-like mutation, M918L, and a novel mutation, V1010A, in the *Thrips tabaci* voltage-gated sodium channel gene. Pest Manag Sci, **70**, 977-981.

382 **Tables and Figure legends**

383 **Table 1:** Oligonucleotides used to amplify and sequence *P. persimilis* VGSC PCR fragments.

Primer	Sequence 5' - 3'
1F_IIS5-6	GCTTAGAAGGCGTCCAAGGA
2F_IIS5-6	AAGGCGTCCAAGGATTGTCG
1R_IIS5-6	AGGTTGCCAATAACGACGGT
1F_IIIS6	CAAGTGGCCACGTTCAAAGG
2F_IIIS6	GCCACGTTCAAAGGTTGGAC
1R_IIIS6	GTTTCGTCCATGATCGCAGC

384

385

386 **Table 2:** Genetic differences in key residues of the *P. persimilis* VGSC between mites

387 surviving or dying when bioassayed with 5 or 10 ppm (Syngenta Bioline) or 40 ppm (Koppert

388 Biological Systems) of deltamethrin.

Source	Treatment	Phenotype[1]	Position at VGSC			
			918	925	1536	1539
Syngenta	5, 10 ppm	Alive (10)	L	L	T	T
		Dead (37)	M/L	L/V	T/A	T
Koppert	40 ppm	Alive (>30)	L	V	A	T
		Dead (>30)	L	L	A	S

389

390 [1]Numbers in brackets refer to the numbers of mites sequenced in each case. For Syngenta

391 Bioline individual mites were sequenced and for Koppert Biological Systems the sequencing

392 was carried out with 3 pools of at least 10 mites each.

393

Figure 1. Sequence alignments of a VGSC region containing fragments of domain II, IIS4-S5 linker and IIS5 helix and domain III, IIIS6. Boxed numbers (*Musca domestica* numbering) indicate the residues where SNPs were found in this study. **A:** Sequences from two different batches of *P. persimilis* as supplied by Koppert Biological Systems and Syngenta Bioline. **B:** Sequences from acarine and insect species. The sequences of *Phytoseiulus persimilis* were obtained from this work, the rest of sequences was obtained from NCBI. *Varroa destructor* (honeybee mite; AAP13992), *Ixodes scapularis* (black-legged tick; XP_002407119.1), *Rhipicephalus (Boophilus) microplus* (cattle tick; AAD23600.2), *Metaseiulus occidentalis* (predatory mite; XP_003741737.1) , *Tetranychus urticae* (two-spotted spider mite; ADB92110.1), *Apis mellifera* (Western honeybee; NP_001159377.1), *Periplaneta americana* (common cockroach; GQ132119) *Tribolium castaneum* (red flour beetle; XM_015981899), *Musca domestica* (common housefly; CAA65448) and *Spodoptera exigua* (beet armyworm; KU739058).

Figure 2. Mortality of mites from **A:** Syngenta Bioline and **B:** Koppert Biological Systems when treated with different concentration of deltamethrin. Error bars represent the Standard Error of Mean (SEM)

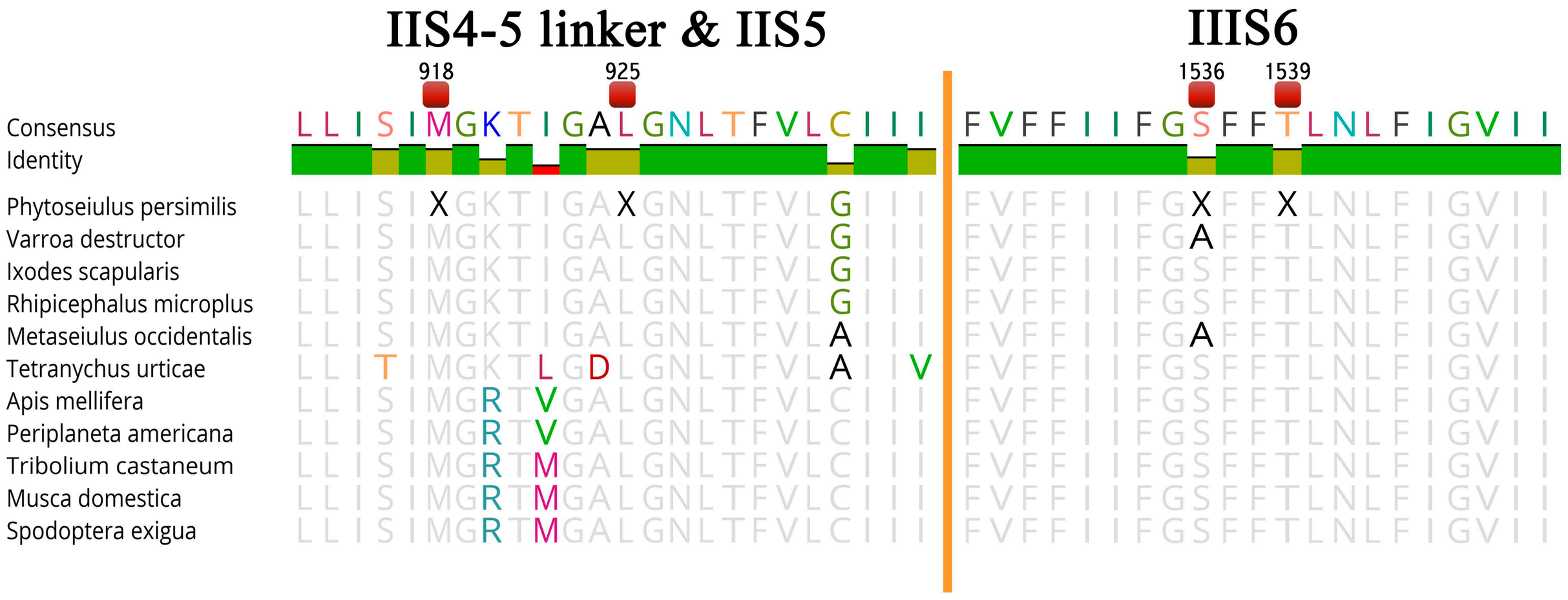
Figure 3: Sequence alignments of a VGSC region from single mites supplied by Koppert Biological Systems. The region comprises fragments of domain II, IIS4-S5 linker and IIS5 helix and domain III, IIIS6. Boxed numbers indicate the residues where SNPs were found in this study. These are an example of the 3 different profiles present in the population.

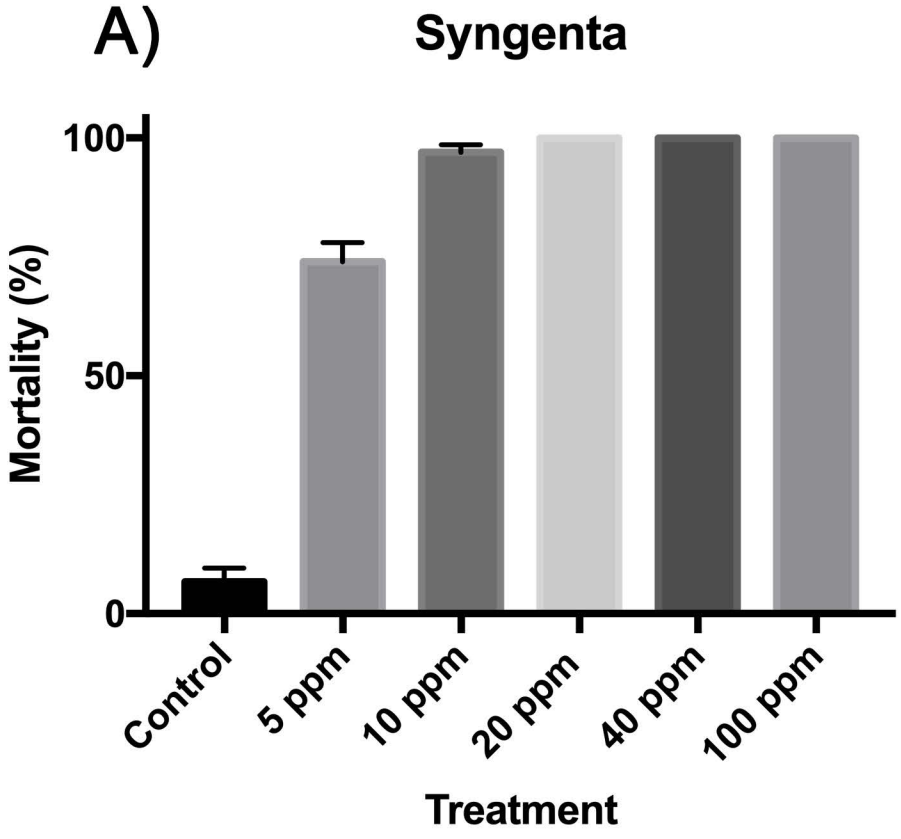
Supplementary material

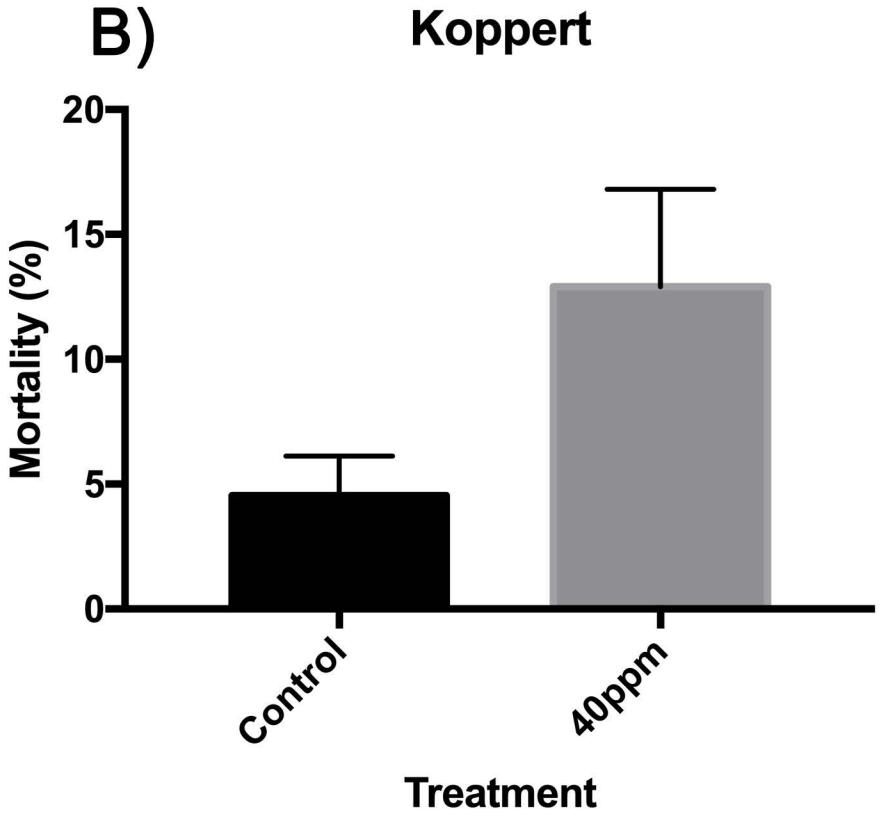
Figure S1: Electropherograms of a fragment from domains II (IIS4-S5 linker and IIS5 helix) and III (IIIS6 helix) of single mites showing the different allele combinations found at positions 925 and 1539 of the VGSC. These are an example of the 3 different profiles present in the population.

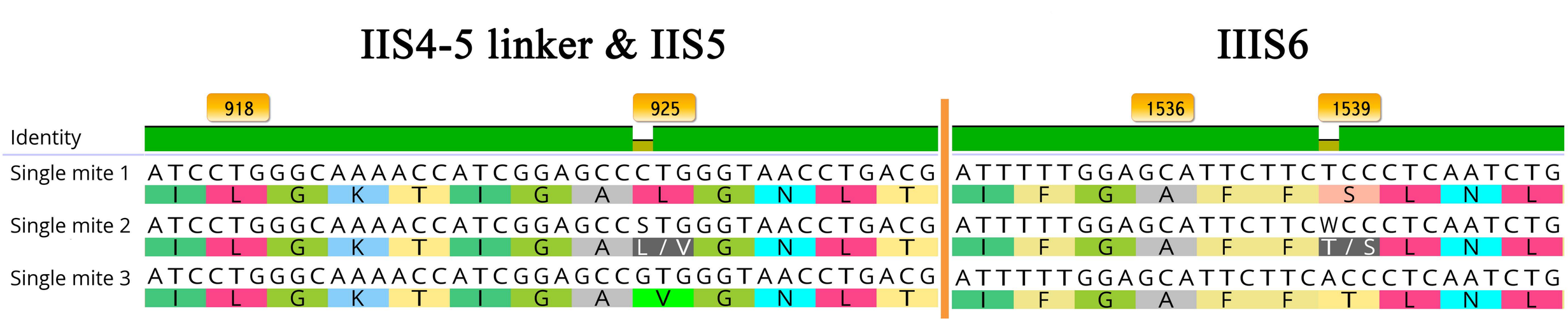
Figure S2: Electropherograms of a fragment from domains II (IIS4-S5 linker and IIS5 helix) and III (IIIS6 helix) from mites (Koppert Biological Systems) surviving or dying after treatment with 40 ppm deltamethrin.


IIS4-5 linker & IIS5
IIIS6
918
925
1536
1539
Identity
Koppert
ATCCTGGGCAAAACCATCGGAGCCSTGGGTAACCTGACG
ATTTTTGGAGCATTCTTCWCCCTCAATCTG
I L G K T I G A L/V G N L T
I F G A F F T/S L N L
Syngenta
ATCMTGGGCAAAACCATCGGAGCCSTGGGTAACCTGACG
ATTTTTGGARCATTCTTCACCCTCAATCTG
I M/L G K T I G A L/V G N L T
I F G T/A F F T L N L


IIS4-5 linker & IIS5
IIIS6
918
925
1536
1539
Consensus
Identity
Phytoseiulus persimilis
Varroa destructor
Ixodes scapularis
Rhipicephalus microplus
Metaseiulus occidentalis
Tetranychus urticae
Apis mellifera
Periplaneta americana
Tribolium castaneum
Musca domestica
Spodoptera exigua

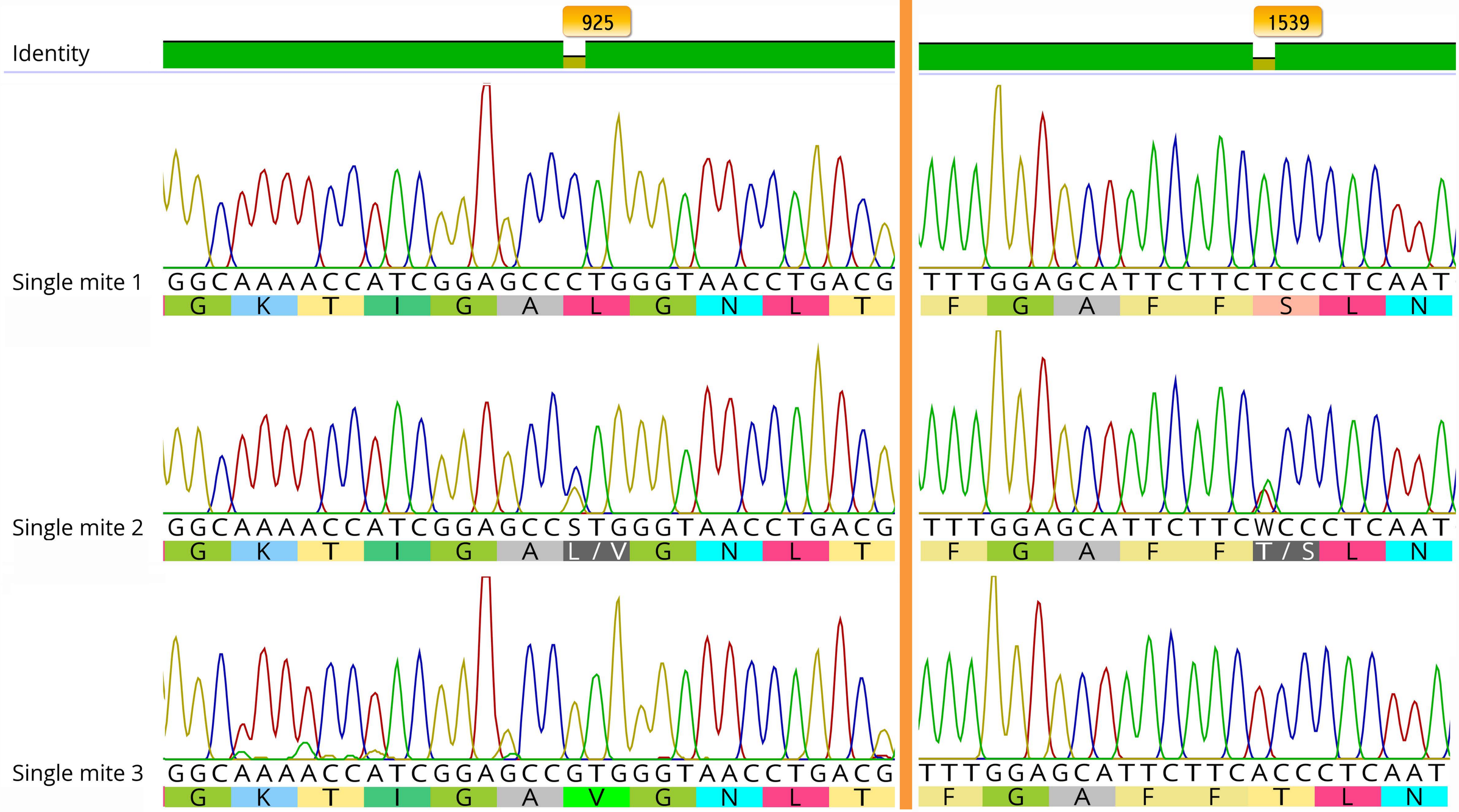

A)
Syngenta
100
50
0
Mortality (%)
Control
5 ppm
10 ppm
20 ppm
40 ppm
100 ppm
Treatment


B)
Koppert
20
15
10
5
0
Mortality (%)
Control
40ppm
Treatment

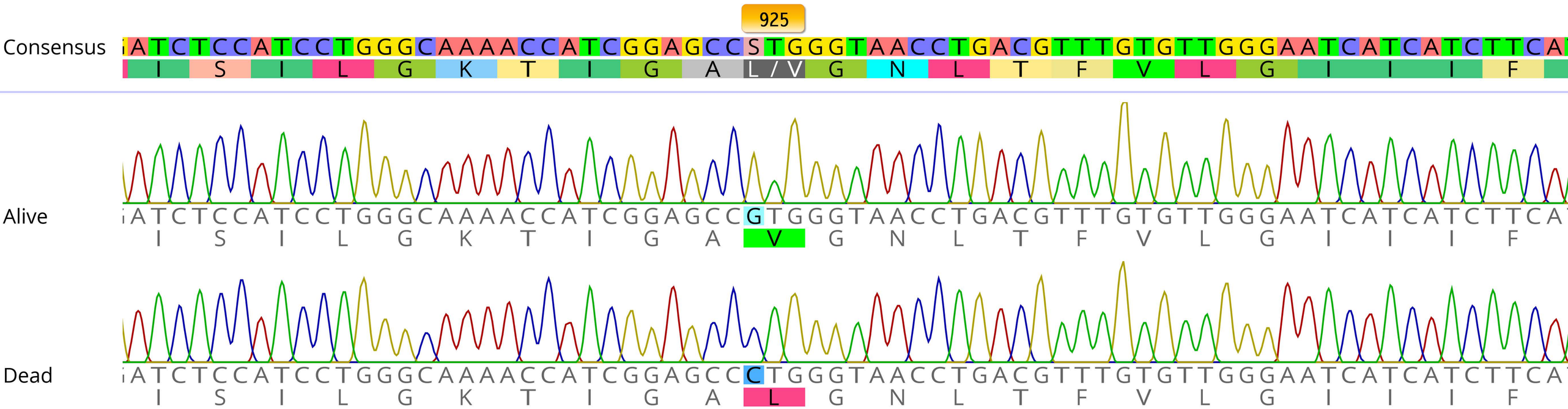

IIS4-5 linker & IIS5
IIIS6
918
925
1536
1539
Identity
Single mite 1
ATCCTGGGCAAAACCATCGGAGCCCTGGGTAACCTGACG
ILGKTIGALGNLT
ATTTTTGGAGCATTCTTCTCCCTCAATCTG
IFGAFFSLNL
Single mite 2
ATCCTGGGCAAAACCATCGGAGCCSTGGGTAACCTGACG
ILGKTIGAL/VGNLT
ATTTTTGGAGCATTCTTCWCCCTCAATCTG
IFGAFFT/SLNL
Single mite 3
ATCCTGGGCAAAACCATCGGAGCCGTGGGTAACCTGACG
ILGKTIGAVGNLT
ATTTTTGGAGCATTCTTCACCCTCAATCTG
IFGAFFTLNL

IIS4-5 linker & IIS5

IIIS6



IIS4-5 linker & IIS5



IIIS6