Selectivity and molecular stress responses to classical and botanical acaricides in the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae).

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32 ## Abstract

33 BACKGROUND: Acaricide application remains an integral component of IPM for the two-spotted
34 spider mite *Tetranychus urticae*. Species and strains of phytoseiid predatory mites can vary
35 significantly in their response to acaricides. For the success of IPM, it is imperative to identify the
36 determinants of selectivity and molecular stress responses of acaricides in predatory mites.

37 RESULTS: The three classical acaricides bifenazate, cyflumetofen, and fenbutatin oxide did not
38 affect survival and fecundity of *Phytoseiulus persimilis* regardless of the route of exposure.
39 Selectivity of the orange oil- and terpenoid blend- based botanical acaricides was low via a
40 combination of direct exposure, acaricide-laced diet, and residual exposure but improved when
41 limiting exposure only to the diet. To gain insights into the molecular stress responses, the
42 transcriptome of *P. persimilis* was assembled. Subsequent gene expression analysis of predatory
43 mites orally exposed to fenbutatin oxide and orange oil yielded only a limited xenobiotic stress
44 response. In contrast, *P. persimilis* exhibited target-site resistance mutations, including I260M in
45 SdhB, I1017M in CHS1, and kdr and super-kdr in VGSC. Extending the screen using available
46 Phytoseiidae sequences uncovered I136T, S141F in cytb, G119S in AChE, and A2083V in ACC, well-
47 known target sites of acaricides.

48 CONCLUSION: Selectivity of the tested botanical acaricides to *P. persimilis* was low but could be
49 enhanced by restricting exposure to a single route. Differential gene expression analysis did not
50 show a robust induced stress response after sub-lethal exposure. In contrast, this study uncovered
51 target-site mutations that may help explain the physiological selectivity of several classical
52 acaricides to phytoseiid predators.

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Keywords: acaricide selectivity, Phytoseiidae, *Phytoseiulus persimilis*, physiological selectivity, ecological selectivity, IPM

1. Introduction

Predatory mites, mainly from the family Phytoseiidae, are key Biological Control Agents (BCAs), reducing the populations of a range of agricultural pests, including spider mites, thrips, and whiteflies. *Amblyseius swirskii* Athias-Henriot, *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* McGregor, and *N. cucumeris* Oudemans are among the most economically important arthropod BCAs used in augmentative biological control [1].

Due to its economic importance and global distribution, *P. persimilis* is the species attracting the most interest and has been the focus of the largest number of studies among Phytoseiidae predators [2]. *P. persimilis* is a specialist that feeds primarily on the herbivorous Tetranychidae spider mites and has been employed worldwide to control spider mite populations infesting various crops [3].

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a major polyphagous pest in greenhouses and field crops, is the main target of *P. persimilis* [4]. *T. urticae* is characterized by a tremendous reproductive potential, short generation time, and an arrhenotokous mode of reproduction, allowing unfertilized females to lay eggs. *Phytoseiulus persimilis* mediated strategies successfully keep *T. urticae* damage below the economic threshold level, in a range of greenhouse crops. However, chemical control remains an integral component of spider mite management [5,6]. Spider mite outbreaks can often only be contained by the tandem use of acaricides with distinctive modes of action (MoAs) [7]. It is clear that exposure of naive *P. persimilis* to acaricides may reduce the efficacy of augmentative control and, consequently, the success of an integrated pest management (IPM) program [8].

The global acaricide portfolio recommended for spider mite control continues to evolve [9,10]. As a general trend, older chemistries like organophosphates and carbamates are gradually replaced

97 with more selective compounds [11]. Among classical acaricides, electron transport inhibition at the
98 mitochondrial respiratory chain has been a remarkably successful MoA. Several commercial
99 acaricides target these processes at different sites [12]. Mitochondrial electron transport Inhibitors
100 (METI) III, like bifenazate inhibiting cytochrome b at the Q0-site in complex III (IRAC group 20)
101 [13,14], METI II including cyflumetofen specifically inhibiting complex II and most likely interacting
102 with succinate dehydrogenase subunits B (SDhB) and C (SdhC) (IRAC group 25) [15] and acaricides
103 that directly interfere with ATP synthesis at complex V (IRAC group 12), such as the organotin
104 compound fenbutatin oxide [5] are known for their excellent efficacy in controlling spider mites [12].
105 Surprisingly, despite their conserved mode of action in the mitochondria, many of these
106 compounds are fairly selective, not only between insects and mites but also between
107 Tetranychidae and Phytoseiidae [5,16–18].
108 A number of botanical extracts with often non-specified MoA, have been recently included in the
109 IRAC MoA classification scheme as part of the 'biologics' group [19]. Botanicals have gained
110 considerable attention in agriculture, addressing the need for inexpensive, easily sourced, and
111 biodegradable alternatives to classical pesticides [20]. Prev-Am and Requiem are novel botanical
112 products recommended to manage soft-bodied arthropod pests, including spider mites. Prev-
113 Am (60 g/L active ingredient 'organge oil') consists of a naturally occurring essential oil extracted
114 from orange peel rich in D-limonene, the main constituent of the terpenoid fractions [21]. Requiem
115 (153 g/L active ingredient 'terpenoid blend') is a synthetic terpenoid blend composed of D-
116 limonene, p-cymene, and α-terpinene, mimicking the terpene composition of Mexican tea,
117 *Chenopodium ambrosioides* [22].
118 Selective toxicity is an essential quality for acaricides, particularly the ones used in IPM programs.
119 Selectivity can be attained either on the ecological or physiological level [23]. Ecological selectivity
120 builds on the bio-ecology of a given arthropod and exploits its habitat characteristics allowing for

121 the accommodation of compounds essential for plant protection that are otherwise non-selective.
122 The physiological selectivity accounts for similarities and differences in toxicokinetics (TK) and
123 toxicodynamics (TD) pathways. The TK mechanisms encompass physiological pathways that
124 determine if and how much of the pesticide reaches the target site and include penetration,
125 activation, metabolism, transport, and excretion processes. TD mechanisms describe a toxicant's
126 interactions with its target site and the biological consequences of this interaction [24]. In addition
127 to the intrinsic differences between arthropod species, exposure to pesticides and selection leads
128 to the development of resistance in both pests and BCAs, which is generally caused by decreased
129 exposure due to quantitative or qualitative changes in major detoxification enzymes and
130 transporters (TK) and/or mechanisms that decrease sensitivity due to changes in target site
131 sequence (TD) [24,25].
132 Many studies have previously addressed the issue of compatibility between *P. persimilis* and
133 acaricides, some of them simultaneously investigating compounds from different MoA groups,
134 including classical and botanical acaricides [26–28]. Although most studies test only a single
135 population of a certain predatory mite species, it becomes apparent that different strains of *P.*
136 *persimilis* vary in their susceptibility to a given MoA [26,29,30] and are capable of developing acaricide
137 resistance as a result of selection pressure [25,31,32].
138 Compatibility achieved by acquiring heritable insecticide/acaricide resistance has been a highly
139 desirable characteristic for commercial BCAs [25,33,34]. However, the molecular mechanisms behind
140 varying susceptibility to acaricides in Phytoseiidae mites remain largely unknown. In several
141 phytoseiid species, the mechanism of acaricide detoxification has been studied exclusively via
142 biochemical assays and with acaricide synergists, and target site resistance has been studied to
143 an even lesser degree [23,35]. Transcriptomic studies on phytoseiid mites rarely address the
144 molecular mechanism of acaricide adaptation [36]. The general lack of genomic and transcriptomic

145 resources for Phytoseiidae impedes the development of functional genetic tools and
146 ecotoxicological biomarkers assessing acaricide effects and the health status of a predator [33,34].
147 This study attempted to explore the prospect of increasing ecological selectivity and
148 simultaneously investigated the molecular basis of physiological selectivity of three classical
149 selective acaricides, fenbutatin oxide, cyflumetofen, bifenazate, and two plant-based botanical
150 acaricides, Prev-Am and Requiem, towards the predatory mite *P. persimilis*. The survival and
151 fecundity of *P. persimilis* were assessed following acaricide administration via different routes:
152 direct contact, pesticide-laced diet (prey), or a combination of diet, direct and residual contact.
153 The transcriptomic changes affiliated with oral exposure to sublethal doses of fenbutatin oxide
154 and Prev-Am were explored. Further, the transcripts encoding known target sites of acaricides
155 routinely used against *T. urticae* were identified in the search for single nucleotide substitutions
156 (SNP) potentially associated with acaricide target-site based selectivity in *P. persimilis*. Lastly, a
157 comprehensive search of NCBI databases was performed to estimate the incidence of acaricide-
158 target site mutations across predatory mite species within the family Phytoseiidae.

159 # 2. Materials and Methods

160 ## 2.1. Chemicals

161 Commercial formulations of the mitochondrial complex III electron transport inhibitors bifenazate
162 (Floramite® 240 g L^{-1} SC) and cyflumetofen (Scelta® 20 g L^{-1} SC) were purchased from Intergrow
163 (Aalter, Belgium). Fenbutatin oxide (Torque® 550 g L^{-1}) was originally bought from Fyto Vanhulle
164 (Belgium). The commercial formulation of orange oil (Prev-Am® Plus 60 g L^{-1} SL) was purchased
165 from Intergrow (Aalter, Belgium). Orange oil is a common name for an extract from the rind of
166 *Citrus aurantium* of which D-limonene is the active substance at a content of 94.5 – 96.5 %. Bayer
167 CropScience (Diegem, Belgium) provided an experimental sample of terpenoid blend QRD 460

168 (Requiem 152.3 g L^{-1} SC). Terpenoid blend QRD 460 consists of three components: α-terpinene,
169 p-cymene and D-limonene. The nominal concentration of each technical grade component in the
170 active substance as manufactured is as follows: α-terpinene 59.7% w/w, p-cymene 22.4% w/w, D-
171 limonene 17.9% w/w. Both 'orange oil' and 'terpenoid blend QRD 460' are registered and
172 approved active ingredients in the EU (https://ec.europa.eu/food/plants/pesticides/eu-
173 pesticides-database_en)

174 2.2. Predatory mite and spider mite populations

175 A stock culture of *P. persimilis* was obtained from Koppert Biological Systems (Berkel en Rodenrijs,
176 the Netherlands) and reared on detached *T. urticae*-infested bean leaves resting on a moist cotton
177 sheet in a water-filled plastic tray. The London strain of *T. urticae* was used to maintain the *P.*
178 *persimilis* stock culture [37]. The London strain was also used as a diet for *P. persimilis* in orange oil
179 and terpenoid blend tests. In the experiments with bifenazate, cyflumetofen, and fenbutatin oxide
180 *T. urticae* strains BR-VL, Tu008R, and MR-VL, known for their high resistance levels to the
181 respective compounds, were used as a food for *P. persimilis* [38–40].

182 2.3. Survival and fecundity of acaricide treated *Phytoseiulus persimilis*

183 The impact of acaricides on *P. persimilis* fecundity and survival was investigated via three delivery
184 routes (Figure 1A): (1) via direct contact (SCC), (2) via the diet (CSC), and (3) a combination of
185 direct contact, residual contact, and diet (SSS). All treatments consisted of spraying 0.8 ml of spray
186 fluid at 1 bar pressure in a Cornelis spray tower (1.5 ± 0.06 mg aqueous acaricide deposit cm^2) [41].
187 Acaricide solutions were applied at the field rate recommended for spider mite management.
188 These were 96 mg L^{-1} active ingredient (a.i.) bifenazate, 200 mg L^{-1} a. i. cyflumetofen, 247 mg L^{-1}
189 a. i. fenbutatin oxide, 0.4% of commercial formulation orange oil, and 0.6% of commercial

formulation terpenoid blend. A water-sprayed control accompanied each acaricide treatment (CCC).

Acaricide bioassays were performed in experimental units consisting of a 3 cm diameter plastic dish with a lid having a circular opening secured with mite-proof mesh. A 4 cm^2 bean leaf square was placed on cooled but not yet solidified 1% agarose. The abundance of prey for the control (CCC), direct (SCC), and combined (SSS) treatments was ensured by allowing five adult females of *T. urticae* to propagate on the arenas three days before the start of the experiment. Prey for the acaricide-laced diet-treatment (CSC) was sprayed on a separate full-leaf arena and brushed onto clean experimental arenas after drying. Any emerging adult females of *T. urticae* were removed from the arenas to prevent the predatory mites from feeding on unsprayed eggs. The impact of an acaricide via direct contact (SCC) was investigated by spraying adult females of *P. persimilis*, which were then moved to clean arenas with unsprayed *T. urticae* prey. The combined effect of direct, residual contact and diet was investigated by directly spraying the experimental arena with *P. persimilis* and *T. urticae* (SSS).

Once acaricides were administered via one of three routes, single, two-day-old *P. persimilis* females were allowed to feed on the same *T. urticae*-populated arena for four days (Figure 1B). Predatory mite eggs were counted daily and removed from the arena, and the mortality of the predators was recorded. The experiment was kept in a climatically controlled chamber (PHCBI MLR-352H-PE) at 25 ± 0.5 ℃, 70 ± 5% RH, and 16/8 h (light/dark) photoperiod. For practical reasons, experiments were performed in 2-4 time blocks.

Fecundity data were checked for normality and homoscedasticity before statistical analysis and analyzed with a linear mixed-effects model (lmer of the package" lme4") with acaricide-delivery-mode (CCC, CSC, SCC, SSS) as a fixed factor and time block as a random factor. The contrast among treatments was assessed using Tukey's HSD (package" multcomp", [42]). Mortality data were

214 analyzed with a Cox proportional hazards model from package" survival" to test whether
215 differences in *P. persimilis* survival depended on the acaricide delivery mode. Function
216 pairwise_survdiff (package" survminer") was used to perform pairwise comparisons between the
217 different delivery modes. P-values were Benjamini-Hochberg adjusted. Statistical analysis was
218 performed in R, version 3.6.3 [43].

219 ## 2.4. Acaricide treatment for differential gene expression studies

220 Fenbutatin oxide and orange oil were used to investigate gene expression patterns in predatory
221 mites exposed to *T. urticae* diet sprayed with the field rate of acaricide (247 mg L^{-1} and 0.4%
222 respectively) vs. water (Figure 1C, CSC). *T. urticae* prey was obtained from heavily infested bean
223 leaves. They had been mostly abandoned by adult spider mites for their lack of nutrients but were
224 still infested by other life stages. By eliminating adult females, we limited the number of unsprayed
225 eggs in the diet. *T. urticae* infested bean leaves were sprayed until run-off with a hand atomizer
226 and allowed to air-dry. An excess of either acaricide- or water-sprayed *T. urticae* mites was
227 brushed into a plastic container (9.5cm x 7cm) with a tightly fitted lid. The lid had a 4 cm diameter
228 opening secured with mite-proof netting. A moist piece of cotton was placed inside the container
229 to maintain humidity. Approximately one hundred two-day-old female *P. persimilis* were added.
230 Experimental units were kept in a climatically controlled chamber at 25 ± 0.5℃, 70 ± 5% RH, and
231 16/8 h (light/dark) photoperiod. Live predatory mites were collected after a two-day and four-day
232 exposure to orange oil and fenbutatin oxide, respectively. Sampling time was chosen based on
233 the daily fecundity values to maximize the chance of capturing transcriptomic changes related to
234 the sublethal acaricide exposure (Figure 1C, 2A, 2E). A water control accompanied each acaricide
235 treatment to account for the difference in age between the orange oil, and fenbutatin oxide
236 treated samples (Figure 1C). The experiment was repeated four times.

237 ## 2.5. RNA extraction, sequencing, and quality control

238 Total RNA was isolated from 80 live adult females of *P. persimilis* using the RNEasy Plus mini kit
239 (Qiagen, Belgium). RNA quality and quantity were estimated using a DeNovix DS-11
240 spectrophotometer (DeNovix, U.S.A.) and running an aliquot on a 1% agarose gel. Illumina
241 libraries were constructed following a standard protocol with size fractionation (Illumina, USA).
242 The resulting libraries were sequenced on an Illumina NovaSeq 6000, and strand-specific paired
243 reads (2 × 100 bp) were generated. Library construction and sequencing were performed at the
244 Fasteris sequencing facility (Geneva, Switzerland). Before read-mapping, the quality of the reads
245 was verified using FASTQC version 0.11.8 [44].

246 ## 2.6. De novo transcriptome assembly and annotation

247 The *de novo* assembly, transcript annotation, and differential expression analysis were performed
248 according to Alavijeh et al., 2020, with minor deviations from the pipeline. In an attempt to
249 estimate the number of reads sufficient to create a complete assembly of the *P. persimilis*
250 transcriptome, we subsampled a random selection of 2.5 and 5 million reads per replicate, totaling
251 40 and 80 million reads. *De novo* assemblies were created using CLC Genomics Workbench 10.
252 Next, long open reading frames (ORFs) were extracted from the resulting assemblies using
253 Transdecoder v. 5.5.0 [46] with a minimum ORF length of 100 amino acids. CD-HIT-EST [47] was used
254 to filter extracted ORFs at the identity threshold of 98% (−c 0.98) and word size of 10 (−n 10). The
255 transcripts to which the filtered ORFs belonged were retained for further analysis. The transcripts
256 of the two assemblies were then loaded into OmicsBox 1.3.11 and used as a query in a CloudBlast,
257 BLASTx search, using an E-value threshold of E^{-3} and the" fast" setting [48], against the NCBI non-
258 redundant (nr) protein database (the version of 23rd June 2020). Blast2GO was subsequently used
259 to map and annotate gene ontology (GO) terms to transcripts based on the sequences retrieved
260 by the BLASTx search [49]. Finally, the InterProScan pipeline [50] was run, and InterPro identified GO

261 terms were merged to the Blast2GO annotated GO terms. Subsequently, the transcripts having a
262 first BLASTx hit with viruses, bacteria, protozoa, fungi, or *T. urticae* were excluded from the
263 assemblies. Other sequences were removed from the assembly if the mean sequence similarity
264 value was ≥ 98% with members of the genus *Tetranychus*. Based on the overall mapping success
265 rate against an assembly (before removing contamination) and the total amount of sequences
266 with a blast hit (after removing contamination), the" 40 million reads assembly" was selected for
267 further analysis, Table S1.

268 2.7. Expression quantification, principal component analysis (PCA)

269 Once contaminating sequences were removed from the final" 40 million read assembly", paired-
270 end sequences were pseudo-aligned with kallisto (100 bootstraps) to create abundance estimates
271 [51]. Differential expression analyses were performed in R version 4.0.2 using sleuth 0.30.0 running
272 on the default settings and an additional package, gridExtra. The Wald test in sleuth was used to
273 analyze the kallisto bootstrap estimates [52], while the transformation function log2(x + 0.5) [53]
274 option in the sleuth_prep command was used to calculate the effect size (β value) as log2-based
275 fold changes (log2FC). Transcripts with log2FC>0 and a q-value (Benjamini-Hochberg multiple
276 testing corrected p-value) ≤ 0.05 between the fenbutatin oxide and orange oil samples and their
277 respective controls were considered differentially expressed. For each comparison's differentially
278 expressed transcripts (DETs), a GO enrichment analysis was performed using Fisher's exact test in
279 the functional analysis toolbox of OmicsBox 1.3.11. Only the GO categories with an FDR adjusted
280 p-value of <0.05 were considered significantly enriched. Finally, a PCA was created using the
281 interactive sleuth visualization package, Shiny 1.4.0.2.

282 2.8. Identification of target-site mutations in Phytoseiidae

283 The *P. persimilis* transcriptome assembled in this study, the nucleotide collection (nr/ nt), and
284 Transcriptome Shotgun Assembly (TSA) databases available from NCBI, were screened to identify

285 orthologues of acaricide target genes across the family Phytoseiidae. These included known
286 target sites of acaricidal compounds frequently used against *T. urticae:* PSST subunit of
287 Mitochondrial Complex I, cytb, Succinate dehydrogenase, subunits B (SdhB) and C (SdhC), ATP
288 synthase, subunits a and c, Chitin Synthase 1 (CHS1), Glutamate-gated Chloride channel (GluCl),
289 Acetyl CoA carboxylase (ACC), Acetylcholinesterase (AChE), and Voltage-Gated Sodium Channel
290 (VGSC). Only well-documented substitutions, located in the protein most conserved regions
291 across different species, were considered. Although there are no identified target site mutations
292 in arthropods against inhibitors of mitochondrial ATP synthase, conserved domains within a and
293 c subunits were also screened for SNPs.
294 The *P. persimilis* transcriptome and NCBI databases were mined for contigs encoding target-sites
295 of acaricides using tBLASTn (E-value cutoff < $1E^{-5}$) [54] and *T. urticae* protein sequences as a query.
296 When obtaining a complete and continuous target site sequence was impossible, we made sure
297 to identify *P. persimilis* ORFs extending over the regions carrying a known target-site mutation.
298 Cytochrome b sequence was not present in the final" 40 million read assembly" and was instead
299 identified in an earlier version of this assembly with tBLASTx (E-value cutoff < $1E^{-5}$, File S1). Open
300 reading frames were extracted using Transdecoder v. 5.5.0 [46] with TransDecoder.LongORFs. A
301 minimum ORF length of 100 amino acids was set for AChE, GluCl, and VGSC. These genes belong
302 to multimember gene families of Carboxy/Cholinesterases (CCEs), Cys-loop ligand-gated
303 channels, and voltage-gated channels, respectively, and their identification was facilitated by
304 phylogenetic analysis. For the remaining target sites, there was no minimum ORF length specified.
305 To further maximize sensitivity for capturing ORFs that may have functional significance,
306 candidate peptides were screened for homology using BLASTp against *T. urticae* amino acid (aa)
307 sequence (Table 1, E-value cutoff < $1E^{-5}$). Subsequently TransDecoder.Predict was used to predict
308 the likely coding regions, and the best hit per sequence was retained with function –

309 single_best_only. Amino acid sequences were aligned using MAFFT v 7.475 [55], and misaligning
310 sequences were removed.
311 The phylogenetic analysis of cys-loop channels was performed on *P. persimilis* (this study),
312 *Metaseiulus occidentalis* (Nesbitt), and *A. swirskii*. Protein sequences of *T. urticae* and *Drosophila*
313 *melanogaster* (Meigen) were used as a reference. For the phylogenetic analysis of CCEs, a
314 reference set of *T. urticae* CCE protein sequences was included in the alignment with *P. persimilis*
315 (this study), *M. occidentalis, A. swirskii* and *Kampimodromus aberrans* (Oudemans), and
316 representatives of insect CCEs; *D. melanogaster*, *Apis mellifera* L. and *Bombyx mori* L. [56]. As N-
317 and C- termini of CCEs are highly variable, divergent regions were trimmed according to
318 Claudianos et al., 2006. Phylogenetic analysis on voltage-gated channels was performed with
319 sequences of *P. persimilis* (this study), *A. swirskii, M. occidentalis,* and *Neoseiulus barkeri* (Hughes).
320 VGSC protein sequences of *T. urticae* (tetur34g00970) and *Musca domestica* L. (Q25439) were
321 used as a reference. Phylogenetic trees were built using IQ-TREE v 2.0.3 with 1,000 ultrafast
322 bootstrap (-B 1,000) combined with automatic model finding through ModelFinder Plus (-m MFP)
323 [58]. The resulting trees were midpoint rooted and edited with MEGA v 10.2.4 software [59]. Only
324 bootstrap support values ≥ 85% were shown in the tree.

325 3. Results

326 3.1. Survival and fecundity of *P. persimilis* after acaricide treatment

327 Survival and fecundity of predatory mites treated with the acaricides bifenazate, cyflumetofen,
328 bifenazate, fenbutatin oxide, and botanicals terpenoid blend and orange oil, either directly (SCC),
329 only via acaricide-treated diet (CSC) or by a combination of exposure routes (SSS) (Figure 1A),
330 were scored daily for four days (Figure 1B, 2). Figure 3 presents a graphical overview of total
331 fecundity and survival for all acaricide treatments.

332 ### 3.1.1. Daily and total fecundity

333 Except for the first day of cyflumetofen treatment, when the fecundity of the water sprayed control
334 (CCC) in this setup was significantly lower than in the combined acaricide treatment (SSS) (Figure
335 2C), average daily fecundity did not differ between different acaricide delivery routes for the
336 classical acaricides (fenbutatin oxide, bifenazate, and cyflumetofen) and the respective control
337 (Figure 2A, B and C, respectively).

338 However, the negative effect of consuming terpenoid blend-sprayed *T. urticae* (CSC) on daily
339 fecundity became significant on the first day of scoring and remained so throughout the
340 experiment (Figure 2D). The combination of diet, direct and residual contact (SSS) also had
341 immediate and even more pronounced consequences for daily fecundity (Figure 2D). Direct
342 spraying with terpenoid blend (SCC) had a negligible effect on daily fecundity (Figure 2D).
343 Consuming orange oil-treated prey (CSC) had little influence on the fecundity of the predator
344 during the first two days but became significant with time. The direct contact (SCC) and combined
345 treatment (SSS) immediately and acutely affected predator fecundity (Figure 2D). The results of
346 the variance analysis for the daily fecundity are listed in Table S2.

347 There was no significant difference in total fecundity after four days of egg-laying between the
348 different delivery modes and the control for bifenazate (F_3=0.93, $p>0.05$, Figure S1A),
349 cyflumetofen (F_3=1.2, $p>0.05$, Figure S1B) and fenbutatin oxide (F_3= 1.71, $p>0.05$, Figure S1C). Total
350 fecundity differed significantly between the water sprayed control (CCC), terpenoid blend sprayed
351 diet (CSC), and the combined treatment (SSS), and between the direct contact (SCC) and
352 combined treatment (SSS) (F_3=12.68, $p<0.05$, Figure S1E). All comparisons except SSS vs. SCC for
353 orange oil yielded a significant difference in the total number of eggs laid (F_3= 95.50, $p<0.05$,
354 Figure S1D).

3.1.2. Survival

A significant difference in the survival rate of *P. persimilis* mites could not be observed between water sprayed control (CCC) and the treatments involving different routes of acaricide delivery (CSC, SCC, SSS), for either of the classical acaricides, fenbutatin oxide (χ^2 = 0.64 on 3 df, p>0.05), bifenazate (χ^2= 0.09 on 3 df, p>0.05) or cyflumetofen (χ^2 = 0.14 on 3 df, p>0.05).

On the contrary, the survival rate of predatory mites sprayed with a combination treatment of terpenoid blend (SSS) differed significantly from all the other treatments (CCC, CSC, SCC) (χ^2= 8.14 df=3, p<0.05). All comparisons except SSS vs. SCC for orange oil yielded a significant difference in the survival rate (χ^2= 59.02 on 3 df, p<0.05). P-values for the log-rank pairwise comparison between survival rates for orange oil and terpenoid blend can be found in Table S3.

3.2. *Phytoseiulus persimilis* transcriptome and transcriptional responses

RNA from adult *P. persimilis* females exposed via diet (sprayed immature *T. urticae*) to orange oil and fenbutatin oxide and a water-control was isolated and Illumina-sequenced. RNA from predators exposed to orange oil was sampled after two days of acaricide exposure, whereas fenbutatin oxide-exposed predators were sampled after four days. The sampling time was chosen based on the daily fecundity data (Figure 2 A, E) in the hope of detecting transcriptomic changes preceding the statistically significant drop in *P. persimilis* fecundity. Illumina sequenced short reads of each treatment are available in the Gene-Expression Omnibus (GEO) repository with accession number (SRA submission in progress, submission number, SUB10153985). Two transcriptome assemblies were generated, and detailed comparisons of the" 80 million" and" 40 million" assemblies along the preprocessing and annotation pipeline can be found in Table S1. The" 40 million" and" 80 million" assemblies initially consisted of 19,696 (file S2) and 25,076 (file S3) transcripts (Table S1). The average pseudo-alignment mapping rate of reads against the 19,696 and 25,076 transcripts were 87.9% and 86.8%, respectively. A Blast2GO analysis revealed that for

379 the" 40 million read assembly" 16,410 (83.5%) of the 19,696 transcripts had a BLASTx hit, and 3,395
380 (17.2%) transcripts were either protozoan, viral, bacterial, *T. urticae,* or other members of the
381 genus *Tetranychus,* showing a mean sequence similarity value ≥ 98% (Table S4). Consequently,
382 after the 3,395 transcripts were removed, 12,969 (79.6%) reads out of the final assembly of 16,301
383 reads (file S4) had a blast hit (Table S5). For the" 80 million read assembly", 20,100 (80,2%) of the
384 25,076 transcripts had a BLASTx hit, and 7,286 (29.1%) transcripts were either protozoan, viral,
385 bacterial, fungal, *T. urticae,* or other members of the genus *Tetranychus,* showing a mean
386 sequence similarity value ≥ 98% (Table S6). After removing the 7,286 transcripts, 12,755 (71.7%)
387 reads out of 17,790 (file S5) had a blast hit (Table S7). Based on the pseudo alignment mapping
388 rate and the absolute number of transcripts with a blast hit, the" 40 million read assembly" was
389 chosen for further analysis (Table S1). The reads were pseudo-aligned against the remaining 16,301
390 transcripts using kallisto. A principal component analysis (PCA) across all RNAseq samples
391 revealed that 35.0% of the total variation could be explained by PC1, while 16.7% could be
392 explained by PC2 (Figure 4). Clustering by treatment was not apparent, indicating that the
393 treatment is not the primary source of variation. Fenbutatin oxide treated replicates occupied an
394 area mainly overlapping with the control. For the orange oil-treated samples, however, all but
395 one replicate clustered away from the control. We used sleuth to perform a differential transcript
396 expression analysis (logfc2>0, q-value ≤0.05). Transcript expression levels were pairwise
397 compared between acaricide treatment (orange oil and fenbutatin oxide) and the relevant control
398 (orange oil_c and fenbutatin oxide_c). Unsurprisingly only two transcripts were found differentially
399 regulated between fenbutatin oxide and fenbutatin oxide_c. Contig_22124 and contig_24988
400 were upregulated in fenbutatin oxide (log2FC= 3.04 and 3.24, respectively), and neither contig
401 had a BLASTx hit against the NCBI nr protein database at the threshold of E^{-3}. We found only 34
402 DETs between orange oil and orange oil_c, with fold changes being overall modest. Among the

403 34 DETs, 27 were up-, and seven were down-regulated. Eleven DETs did not have a BLASTx hit
404 with the nr protein database. The remaining 23 annotated transcripts are listed in Table S8.
405 Contings upregulated after treatment with orange oil coded for proteins involved in DNA
406 integration and binding, including tigger transposable element (contig_20133, contig_15792,
407 contig_9434), mRNA binding, processing and splicing factors (contig_1302, contig_1223,
408 contig_7063, contig_7578, contig_10832, contig_226), and endoplasmic reticulum activity (ER)
409 (conting_7415). Apart from one transcript blasting with *M. occidentalis* cytochrome P450 1A1
410 (contig_5327) sequence, no representatives of other gene families typically involved in xenobiotic
411 metabolism were found among DETs. There were no significantly enriched GO-terms in orange
412 oil vs. orange oil_c nor fenbutatin oxide vs. fenbutatin oxide_c.

413 ## 3.3. Presence of target site substitutions in Phytoseiidae

414 The transcriptome of *P. persimilis* allowed for successfully identifying sequences coding for CHS1,
415 PSST subunit of Complex I, ACCase, AChE, SdhB and SdhC, cytb, GluCl, ATP synthase subunit c,
416 and a and partial sequence of VGSC (File S6). The IDs of *P. persimilis* contigs coding for the target
417 site sequences can be found in Table S9.

418 Except for cytb, which sequence is routinely used to discriminate between phylogenetically related
419 species, and for which 303 sequences of 51 Phytoseiidae genera could be screened, tBLASTn
420 searches across Phytoseiidae performed on NCBI servers yielded a modest overall outcome with
421 *M. occidentalis* and *A. swirskii* being often the only identified species (Table 1, Table S10).
422 Subsequent phylogenetic analysis identified Phytoseiidae orthologues of AChE (Figure S2), VGSC
423 (Figure S3), and GluCl (Figure S4) within multigene families of cholinesterase, voltage-gated
424 channels, and cys-loop channels, respectively. In many cases, protein sequences were incomplete,
425 preventing screening for target site mutations at know conserved sites (Table 1).

426 We identified an I1017M substitution in the *P. persimilis* transcript coding for the CHS1 gene
427 (Figure 5, Table 1, File S6) [60,61]. NCBI search resulted in 13 hits. Two of these belonged to *M.*
428 *occidentalis* and 11 to *A. swirskii*. Substitution at position 1017 (*T. urticae* numbering) was absent
429 from all the examined sequences (Table 1, Table S10).
430 The H92R substitution associated with Mitochondrial Electron Transport Inhibitors I (METI I)
431 resistant phenotype in *T. urticae* was not detected in the *P. persimilis* transcript, nor were the
432 other mutations reported to debilitate the function of the respiratory Complex I in *Yarrowia*
433 *lypolitica* [62–66], and Human, [67,68]. NCBI search identified three orthologous sequences for *M.*
434 *occidentalis* and five of *A. swirskii* that could be efficiently aligned. None of the mutations were
435 present in the sequences of *M. occidentalis*. Substitutions found in NQO6 (PSST orthologue in
436 bacteria) of *Y. lypolitica* V88I and E140D were identified in *A. swirskii* GHIT01053611.1 [63,65,69]. R145K
437 was present in *A. swirskii* GHIT01033895.1, GHIT01065208.1, GHIT01035679.1 (Table 1, Table S10,).
438 Phylogenetic analysis allowed for high fidelity identification of AChE genes of *M. occidentalis*, *A.*
439 *swirskii, P. persimilis* and *K. aberrans* (Figure S2, Table S10). We did not identify any of the
440 mutations previously associated with resistance to organophosphates and carbamates in the
441 AChE sequence of *P. persimilis* (File S6) [70]. An NCBI search revealed that both *M. occidentalis*
442 sequences (XM018642151.1 and XM018638971.1) and a sequence of chlorpyrifos resistant strain of
443 *K. aberrans* (HF934044.1) carry mutation G119S [71,72] (Table 1).
444 The recently identified A2083V in a highly conserved region of the carboxyltransferase domain
445 (CT) in ACC of a *Bemisia tabaci* (Gennadius) resistant to spiromesifen and spirotetramat [73] was
446 not found in the *P. persimilis* orthologue (File S6). Nineteen hits belonging to *A. swirskii* (12
447 sequences) and *M. occidentalis* (7 sequences) were found via the NCBI. Among five sequences
448 long enough to span the CT domain, three GHIT01022503.1, GHIT01028753.1, GHIT01031955.1 of
449 *A. swirskii* carried A2083V. (Table 1, Table S10).

450 Screening for VGSC mutations (reviewed in Rinkevich et al., 2013) resulted in finding kdr (L1014F)
451 and super-kdr (M918V) mutations (*M. domestica* numbering) in the transcript of *P. persimilis*
452 (Figure 5, Table 1, File S6). Phylogenetic analysis identified five sequences of *A. swirskii*, and a
453 single sequence for *N. barkeri* and *M. occidentalis* (Fig S3, Table 1). In *N. barkeri* KT768110.1,
454 Methionine at super kdr (918) was substituted with Leucine (Table 1, Table S10).
455 The phylogenetic analysis allowed the identification of GluCl genes of *M. occidentalis*, *A. swirskii*
456 and *P. persimilis* supported by high bootstrap values (Fig S4). Contig_4086 of *P. persimilis* (this
457 study) and accession numbers: GHIT01011578.1, GHIT01010960.1, GHIT01043426.1 of *A. swirskii,*
458 and XM_029110899.1 of *M. occidentalis* clustered with GluCl sequences of *T. urticae* and *D.*
459 *melanogaster* (Figure S4). Contig_4086 of *P. persimilis* (this study), transcripts XM_029110899.1 of
460 *M. occidentalis*, and GHIT01011578.1 of *A. swirskii* had sequences long enough to screen for the
461 three target site mutations. However, substitutions G314D, G326E, and I321T previously associated
462 with low resistance levels to abamectin in *T. urticae* were not present [75–77] (File S6, Table S10).
463 Resistance mutations in highly conserved cd1 and ef helices of cytb were not found in the *P.*
464 *persimilis* orthologue of the Koppert strain analyzed in this study (File S6) [13,78,79]. Screening of cytb
465 orthologues within the Phytoseiidae family did not reveal the presence of any of the known
466 mutations in ef helix (Table S10). Substitutions in cd1 helix at the positions corresponding to *T.*
467 *urticae* mutations were present in *Neoseiuella littoralis* (Swirski & Amitai) (G126A, GU938141.1),
468 several species of genus *Typhlodromus; T. pyri* (Scheuten) (A133G, JF279255.1, JF279271.1,
469 JF279279.1, JF279280.1), *T. aestivalis* (Athias-Henriot) (S141T, A MK014109.1, MK014110.1), *T.*
470 *verrucosus* (Wainstein) (I136T, MK014112.1), *T. rhenanoides* (Athias-Henriot) (S141F, MK014067.1,
471 MK014068.1, MK014070.1, MK014073.1), *T. ilicis* (Athias-Henriot) (S141F,V,D, MK014095.1), *T. laurae*
472 (Arutunjan) (S141A, MK014141.1),), *T. exhilaratus* (Ragusa) (S141F, A, MK014144.1, MK014141.1,
473 MK014148.1), *T. setubali* (Dosse) (G132E, MK014116.1), genus *Typhlodromalus*, *T. aripo* (De Leon)

474 (S141T, KU318210.1, KU318209.1, KX610079.1), genus *Amblydromalus*, *A. limonicus* (Garman &
475 McGregor) (S141T in KU318220.1 and KU318219.1) and genus *Euseius*, *E. fustis* (Pritchard & Baker)
476 (S141T in KX610076.1, KX610078.1, KX610077.1), (Table 1, Table S10).
477 *P. persimilis* I260 in SdhB of Complex II was substituted with Methionine (File S6, Figure 5).
478 Similarly, I260M substitution was found in both *A. swirskii* orthologues, GHIT01048799.1 and
479 GHIT01059435.1. Serine substituted the residue R256 [80] in the *P. persimilis* orthologue found in
480 this study (Figure 5, Table 1, File S6), and a proportion of *M. occidentalis* (JL035473.1, JL014348.1,
481 JL012205.1), and *A. swirskii* (GHIT01060452.1) sequences found via NCBI. The S56L in SdhC [15]
482 causing cyenopyrafen resistance in *T. urticae* was not detected in *P. persimilis* and other
483 Phytoseiidae orthologues (File S6). However, residue R74 (*T. urticae* numbering) [81] was substituted
484 by Serine (Table 1, Table S10, File S6). The same substitution was found in both (GHIT01023969.1
485 and GHIT01063425.1) orthologues of *A. swirskii*, identified via NCBI (Figure 5, Table 1, File S6).
486 We have identified V166L, A195S, A213S (*T. urticae* numbering) in the otherwise conserved residue
487 of ATP synthase subunit a of *P. persimilis* (File S6). NCBI search within Phytoseiidae yielded 14
488 sequences that could be effectively aligned against *T. urticae* tetur01g06130. No other sequence
489 carried A195S SNP, but V166L and A213S coincided in a proportion of *A. swirskii* sequences
490 (GHIT01019070.1, GHIT01047891.1) and all available sequences of *M. occidentalis* (XM_003741056.1,
491 JL040066.1, JL020987.1, JL020984.1 - not long enough to confirm the presence of A213S) (Table 1,
492 Table S10). Substitution R109S (*T. urticae,* tetur06g03780) was detected in the conserved C-
493 terminal domain of the ATP synthase subunit c (File S6). Out of 21 sequences, the same
494 substitution has been found at the corresponding position in ATP synthase subunit c of *M.*
495 *occidentalis* orthologue JL013409.1, JL013411.1, JL013412.1, JL028982.1, JL013416.1, JL013415.1,
496 JL021266.1, JL013413.1, and GHIT01045723.1 of *A. swirskii* (Table 1, Table S10).

497 # 4. Discussion

498 This study finds that the classical acaricides targeting different complexes of the mitochondrial
499 electron transport chain, bifenazate (Complex III), cyflumetofen (Complex II), and fenbutatin oxide
500 (complex V) did not adversely affect the fecundity or survival of adult *P. persimilis* in our
501 experimental setup, regardless of whether predators were exposed via diet, by direct contact or
502 a combination of direct contact, residual contact, and diet (Figure 2, Figure 3, Figure S1). The
503 impact of the three acaricides on herbivorous spider mites and predatory mites has been studied
504 extensively [5,17,18,23,27,39]. While very effective against *T. urticae*, there have been conflicting reports
505 of their safety to key phytoseiids [26]. Particularly for fenbutatin oxide, prolonged surveillance of
506 treated predatory mites saw increased mortality in the laboratory tests [18] and fewer *P. persimilis*
507 in the field experiments [82]. Despite that, and in line with the results of this study, the risk of acute
508 toxicity of bifenazate, cyflumetofen, and fenbutatin oxide is generally low, and these MoAs are
509 often recommended for the control of *T. urticae* [17,18,26,83–86].

510 Surprisingly, in contrast with the classical acaricides, the botanical acaricides containing orange
511 oil and terpenoid blend as active ingredients were harmful to *P. persimilis*. When tested in an
512 identical setup, their impact varied depending on their route of administration (Figure 2, 3, S1).
513 Upon contact, lipophilic monoterpene constituents of orange oil and terpenoid blend exert their
514 toxic properties by directly altering epicuticular waxes' components and entering arthropod
515 bodies via cuticle or respiratory tracheoles [87]. D-limonene is efficient as a contact acaricide [88–90].
516 Its high content in orange oil may explain the acute contact toxicity towards *P. persimilis*. Indeed,
517 orange oil and terpenoid blend-based acaricides are thought to act mainly by physical contact,
518 but their target sites can be broad-spectrum from a physiological perspective. Monoterpene
519 mode of action inside the arthropod's body is unclear but most likely relies on interference with

520 biological membrane properties [44,91]. Monoterpenes are also suspected of sharing some of the
521 intracellular target sites with classical insecticides/acaricides acting on the insect nervous system
522 [88,89,92]. The sublethal effect of providing *P. persimilis* with orange oil and terpenoid blend laced-
523 diet could be mediated by the repellent properties of monoterpenes, particularly D-limonene [93,94].
524 Orange oil and terpenoid blend-based acaricides may offer little physiological selectivity towards
525 non-target organisms, interfering with arthropods' essential biochemical and physiological
526 functions. However, our results suggest that the utility of these acaricides in the integrated control
527 of *T. urticae* can be preserved by smart management practices that increase their selectivity on
528 the ecological level [23].

529 *Phytoseiulus persimilis* females surviving ingestion of orange oil-laced diet showed diminished
530 fecundity (Figure 2E and S1D). Although the same could not be seen for fenbutatin oxide in our
531 setup (Figure 2A and S1C), prolonged exposure to this acaricide was harmful to BCAs elsewhere
532 [18,82]. We thus selected fenbutatin oxide and orange oil to investigate differences in gene
533 expression profiles of *P. persimilis*, attempting to identify genetic signatures associated with
534 ingestion of sublethal doses of classical and botanical acaricides. The clustering by treatment was
535 not readily apparent, indicating that the treatment did not cause large reproducible shifts in gene
536 expression (Figure 4, Table S8). In support of the empirical data, differential gene expression in
537 fenbutatin oxide treated samples was negligible, with only two unidentified transcripts being
538 differentially regulated between the treatment and the control. Treatment with orange oil resulted
539 in the 23 annotated DETs ($\log_2 FC > 0$, $qvalue < 0.05$). None but one downregulated contig encoded
540 a member of a classical detoxification gene family, P450 1A1. The remaining differentially
541 regulated transcripts may suggest increased transcriptional regulation [95], modulation of proteome
542 diversity by alternative splicing [96,97], and enhanced protein production and export [98] as a response
543 to orange oil treatment (Table S8). However, overall, the data also suggests that molecular

544 markers of stress responses are difficult to identify in *P. persimilis*, especially when the phenotype
545 resulting from the treatment is subtle. Similarly, Paspati *et al.*, 2019 have found only 39 DETs in *A.*
546 *swirskii* exposed to a challenging host, tomato vs. pepper, suggesting that mild transcriptomic
547 response towards synthetic and natural xenobiotics may characterize Phytoseiidae mites.

548 Phytoseiidae mites, including *P. persimilis*, belong to Parasitiformes, while *T. urticae* belons to
549 Acariformes. These two lineages of Acari separated approximately 400 mln years ago [100,101].
550 Therefore, differences in target sites sequences that might affect the response to acaricide
551 application could result from a phylogenetic distance and be naturally present. However, direct
552 acaricide selection might act in some populations. Different life histories (herbivory versus
553 carnivory) might have also selected for differences in detoxification machinery [102].

554 The prevalence of nucleotide differences (mutations) in target-site sequences potentially involved
555 in natural tolerance or evolved resistance was screened for in the assembled here *P. persimilis*
556 transcriptome and sequences of other Phytoseiidae species available via public sequence
557 repositories. We encountered a limited availability of genetic data that prevents a realistic
558 estimate of the frequency of mutations in acaricide target sites in Phytoseiidae. However, even in
559 the small sequence pool, we identified SNPs previously validated for their role in resistance to at
560 least six out of nine acaricide/insecticide MoA groups considered in this study (Table 1, Figure 5).
561 In several cases, a target site substitution was present in a proportion of all sequences available
562 for a given Phytoseiidae species, suggesting that these SNPs could be the result of acaricide
563 selection in some populations (Table 1).

564 In pro-acaricides such as bifenazate and cyflumetofen that must undergo metabolic conversion
565 before becoming pharmacologically active [40,103], differential metabolism can result in selectivity
566 [104]. As the screening of the cytb did not reveal any SNPs associated with resistant phenotype in *P.*
567 *persimilis*, the excellent selectivity of bifenazate observed in this study (Figure 2, Figure 3) might

568 rely on the inability to convert the pro-acaricide to a biologically active metabolite [86]. Interestingly,
569 the NCBI searches found bifenazate target site mutations in 12 other Phytoseiidae species.
570 Mutation of a conserved and strongly validated residue S141 in the cd1 helix of cytb was especially
571 ubiquitous across the genera [14,75]. In the genus Typhlodromus, S141 is substituted with
572 Phenylalanine as in *T. urticae*. But substitutions with Alanine, Valine, and Aspartic acid could also
573 be found (Table 1). All the identified cytb sequences of *T. aripo*, *A. limonicus,* and *E. fustis* carried
574 S141T (Table 1). Whether different substitutions of S141 are reflected in bifenazate resistance levels
575 is not known.

576 Cyflumetofen, cyenopyrafen, and pyflubumide are relatively novel compounds with a new mode
577 of action in the arthropods as inhibitors at Complex II (succinate dehydrogenase; Sdh) in the
578 mitochondrial electron transport chain (METI II) [39,105]. The genetic basis of METI II resistance in *T.*
579 *urticae* was clarified using quantitative trait locus (QTL) analysis, revealing that cyflumetofen and
580 pyflumubide resistance was associated with I260T and I260V in SdhB, respectively. In contrast,
581 cyenopyrafen resistance was mapped to SdhC carrying S56L substitution [15,106]. We have identified
582 the substitution I260 (I260M) in SdhB of *P. persimilis,* which may explain the high selectivity of
583 cyflumetofen seen in this study (Figure 2, Figure 3, File S6). The same substitution has been found
584 in sequences of *A. swirskii* available via NCBI (Table 1). The cyenopyrafen resistance mutation,
585 S56L in SdhC was absent in all the examined Phytoseiidae species, but residue R74 (*T. urticae*
586 numbering) known to bind ubiquinone at the Q2-site in *Escherichia coli* [81] was substituted with
587 Serine in *P. persimilis,* and *A. swirskii*, suggesting that exact residues interacting with METI II
588 acaricides may differ between *T. urticae* and *Phytoseiidae* species (Figure 5).

589 Newly discovered SNPs in ATP synthase subunits a (A195S, V116L and A213S, *T. urticae*
590 numbering), and c (R109S, *T. urticae* numbering) of *P. persimilis*, *A. swirskii* and *M. occidentalis*
591 are present at the conserved positions within proteins previously identified as binding sites of

592 organotin compounds [107,108]. They could potentially decrease the efficiency of organotin binding,
593 leading to resistance. However, their contribution to fenbutatin oxide insensitivity in *P. persimilis*
594 must be verified beyond this article's scope.

595 In mites and insects, mutations in CHS1 were previously reported and validated to be responsible
596 for cross-resistance between acaricides Mite Growth Inhibitors (IRAC 10) and insecticides Chitin
597 Synthase Inhibitors (IRAC 15) in *T. urticae* (I1017F), *P. xylostella* (I1017M) and *Culex pipiens* L.
598 (I1043L,M,F) [60,61,109]. We identified an I1017M substitution in *P. persimilis* transcript coding for the
599 CHS1 gene, the first report of this SNP in Phytoseiidae (Figure 5). However, no SNP was found at
600 the corresponding positions in *M. occidentalis* and *A. swirskii* CHS1 sequences found via NCBI.

601 The kdr (L1014F) and super-kdr (M918V,L,T) found in VGSC of *P. persimilis* are well-known
602 pyrethroid resistance mutations present in a wide range of insects. Within Tetranychidae, only *T.*
603 *evansi* is known to carry the super-kdr, but no kdr mutation [110]. In *T. urticae*, instead of kdr and
604 super kdr, target-site resistance relies on the presence of L1024V or F1538I [111,112]. *P. persimilis* used
605 in this study carried both insect kdr (L1014F) and super-kdr (M918V) instead of the mutations
606 typical for pyrethroid-resistant *T. urticae* (Figure 5). Interestingly Benavent-Albarracin et al., 2020
607 have reported commercial strains of *P. persimilis* with super-kdr M918L but no kdr. Instead*,* L925V
608 identified in *Varroa destructor* Anderson & Truema [114] and previously not described mutations
609 A1536T, S1539T, were present. VGSC sequences identified via NCBI belonging to *A. swirskii* were
610 free of known pyrethroid-resistance mutations, but super-kdr substitution M918L was found in
611 VGSC of a fenpropathrin-resistant *N. barkeri* [36,74] (Table 1).

612 Interestingly, we have not found any known AChE mutations [70] in the *P. persimilis* orthologue.
613 However, a common organophosphate/carbamate resistance mutation, G119S [115], was identified
614 in AChE sequences belonging to *M. occidentalis* and *K. aberrans* resistant to the carbaryl-
615 organophosphate-sulfur and chlorpyrifos, respectively [71,72] (Table 1). The lack of

616 organophosphate/carbamate resistance mutations in *P. persimilis* is surprising considering the
617 widespread use of organophosphates/carbamates that has continued since the 1950s. It is
618 perhaps a consequence of modern agricultural practices, increasingly relying on more selective
619 compounds [11].

620 NCBI searches resulted in identifying two interesting SNPs in a Spanish commercial Koppert strain
621 of *A. swirskii* that were absent in this study's *P. persimilis* strain. A2083V in ACC and V88I in the
622 PSST subunit of mitochondrial respiratory Complex I are reported for the first time in Chelicerata
623 and Eukaryota, respectively (Table 1). The A2083V substitution was previously identified in Spanish
624 field populations of *B. tabaci* and caused resistance to spirotetramat, which was thoroughly
625 validated [73]. Given that *A. swirskii* is often used to control whitefly populations and hence exposed
626 to the same insecticides, the presence of the A2083V in both species is probably reflecting strong
627 selection. In *T. urticae,* resistance to METI I acaricides depends partly on the presence of H92R in
628 the PSST subunit [116,117]. Although H92R was not identified in any Phytoseiidae species, residing
629 four residues away, V88I makes a plausible candidate for the METI I target site resistance in *A.*
630 *swirskii*. The residue is located in a highly conserved aa stretch previously photoaffinity labeled
631 with METI I derivatives [118] and has undergone extensive site-directed mutagenesis in *Y. lipolytica,*
632 revealing its crucial role in quinone/inhibitor binding [62,63].

633 To conclude, bifenazate, cyflumetofen and fenbutatin oxide, the representatives of acaricide
634 classes targeting Complex III, II, and V of the mitochondrial electron transport chain, appear safe
635 for the predatory mite *P. persimilis* irrespective of the route of exposure. In contrast, the botanical
636 acaricides, orange oil and terpenoid blend seem much more toxic. Acute and sublethal effects
637 were present via direct exposure, diet, and a combination of direct exposure, diet, and residual
638 contact. Orange oil appears to be particularly harmful to *P. persimilis.* The transcriptomic analysis
639 of selected combinations of acaricides and exposure routes revealed only a limited molecular

stress response, impeding the development of ecotoxicological biomarkers to detect signs of failing biological control. However, transcriptome analysis also allowed the identification of *P. persimilis* orthologues coding for acaricide target sites of acaricide classes frequently used to control *T. urticae*. Surprisingly, many known insecticide/acaricide target site mutations were readily identified in the studied *P. persimilis* strain, potentially explaining its resilience to the tested acaricides. The search for target-site mutations was extended by the Phytoseiidae sequences available via public databases, revealing that an insensitive target site may be a common resistance mechanism in predatory mites of agricultural importance. Several target site mutations reported here have not been previously identified in Phytoseiidae and await functional validation.

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Conflict of Interest Statement

The authors state no conflict

Figure legends

Graphical abstract-text

Selectivity and molecular stress responses to classical and botanical acaricides in the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae).

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662 Acaricide selectivity towards Phytoseiidae may be achieved by limiting exposure routes and

663 exploiting differences in acaricides' molecular targets.

664 Figure 1. An overview of the experiments performed in this study. The predatory mite *Phytoseiulus*

665 *perimilis* and *Tetranychus urticae* spider mites are depicted by red and green arthropods, not

666 drawn to scale. A field dose of either bifenazate, fenbutatin oxide, cyflumetofene, orange oil or

667 terpenoid blend was sprayed on two-day-old *P. persimilis* directly (SCC), or on *T. urticae* mites

668 serving as a diet to the predator (CSC), or the whole experimental arena was sprayed while *P.*

669 *persimilis* and *T. urticae* mites were residing on it (SSS). A water control accompanied each

670 acaricide treatment (CCC). B. Fecundity and survival of individual predatory mites was monitored

671 over four consecutive days. C. Orange oil and fenbutatin oxide were chosen to investigate

672 differential gene expression in response to acaricide treatment. Two-day-old *P. persimilis* mites

673 were fed with an acaricide-laced diet for two (orange oil) or four days (fenbutatin oxide).

674 Subsequently, RNA was isolated from acaricide, and water-treated samples, and Ilumina-

675 sequenced. We assembled the transcriptome of *P. persimilis,* and differential gene expression

676 analysis was performed between the acaricide and its relevant water-spray control.

677 Figure 2. Mean daily fecundity per female of *Phytoseiulus persimilis*, depending on acaricide

678 treatment. A- fenbutatin oxide, B- bifenazate, C- cyflumetofen, D- terpenoid blend, E- orange oil,

679 and the route of exposure, yellow – water sprayed control (CCC), gray – exposure via

680 contaminated diet (CSC), green – direct exposure (SCC), brown – combined diet, direct and

681 residual exposure (SSS). The numbers of eggs per day were compared between different delivery

682 routes. Error bars represent standard error. Means labeled with the same letter are not

683 significantly different.

684 Figure 3. Mean total fecundity of *Phytoseiulus persimilis* females vs. % surviving females after four

685 days of exposure for all acaricides and acaricide delivery modes tested in this study. Circle-

686 bifenazate, triangle- cyflumetofen, square- fenbutatin oxide, polygon- orange oil, star- terpenoid
687 blend. Yellow – water sprayed control (CCC), gray – exposure via contaminated diet (CSC), green
688 – direct exposure (SCC), brown – combined diet direct, and residual exposure (SSS). Error bars
689 represent standard error

690 Figure 4. PCA plot of gene expression levels of *Phytoseiulus persimilis* mites feeding on spider
691 mite prey laced with fenbutatin oxide and orange oil and their corresponding water-treated
692 controls. For each treatment, convex hulls were added for a more straightforward visual
693 interpretation.

694 Figure 5. Comparison of partial amino acid sequences of acaricide target site proteins. CHS1:
695 *Phytoseiulus persimilis* (contig_1573), *Tetranychus urticae* (tetur03g08510), *Musca domestica*
696 (AFP61787.1), *Anopheles gambiae* (AAL23627.1), *Saccharomyces cerevisiae* (NP_009579.1),
697 *Tribolium castaneum* (NP001034491.1), *Plutella xylostella* (BAF47974.1); VGSC: *M. domestica*
698 (ATZ81482.1), *T. urticae* (tetur34g00970), *Metaseiulus occidentalis* (XP_028966827.1), *Bos taurus*
699 (DAA30006.1), *Homo sapiens* (AAA18895.1), *P. persimilis* (contig__4927), *A. gambiae* (CAM12801.1);
700 SdhB: *T. urticae* (tetur01g15710), *P. persimilis* (contig_236), *Escherichia coli* (REE06640.1), *B. taurus*
701 (NP_001035573.1), *Homo sapiens* (NP_002991.2), *D. melanogaster* (NP_477101.1), *S. cerevisiae*
702 (GAX71644.1); SdhC: *T. urticae* (tetur30g00210), *P. persimilis* (contig_7732), *H. sapiens*
703 (CAG33383.1), *B. taurus* (NP_787008.1), *D. melanogaster* (NP_001262472.1), *S. cerevisiae*
704 (AAT93043.1), *E. coli* (VWQ01513.1). Universally conserved residues are marked in bold. The
705 position of the I1017M in CHS1, kdr and super-kdr in VGSC, R256S and I260M in SdhB, R74S in
706 SdhC in *P. persimilis* are indicated in gray. Resistance mutations in SdhB and SdhC against
707 fungicidal SDH inhibitors reported in a previous study [80] are indicated by asterisks. Solid squares
708 indicate the residues in SdhB and SdhC that may bind with ubiquinone at the Q2-site in *E. coli* [81].

709 Open squares indicate resistance mutations previously reported in *T. urticae* [15,60]. The insect Kdr

710 and super-kdr are indicated with open and closed circles, respectively [74].

711 Figure S1. The total number of eggs laid by female predatory mites over four days, depending on

712 the acaricide they were exposed to. A- bifenazate, B- cyflumetofen, C- fenbutatin oxide, D-

713 orange oil, E- terpenoid blend, and the route of exposure. Values in each graph represent means,

714 and error bars represent the standard error. Means labeled with the same letter are not

715 significantly different.

716 Figure S2. Phylogenetic Identification of Phytoseiidae putative acetylcholinesterase sequences

717 (AChE). Maximum likelihood tree of *Kampimodromus aberrans, Amblyseius swirskii, Metaseiulus*

718 *occidentalis, Phytoseiulus persimilis,* and *Tetranychus urticae, Drosophila melanogaster, Apis*

719 *mellifera, Bombyx mori* carboxylesterase (CCE) protein sequences. The phylogenetic tree was built

720 using IQ-TREE v 2.0.3 with 1,000 ultrafast bootstrap combined with automatic model finding

721 through ModelFinder Plus [58]. The resulting trees were midpoint rooted and edited with MEGA v

722 10.2.4 software. Only bootstrap support values > 85% were indicated in the tree. Colour and shape

723 codes are as follows: *K. aberrans*, blue circle, *A. swirskii*, purple circle, *M. occidentalis*, yellow circle,

724 *P. persimilis*, green circle, *T. urticae*, red square, *D. melanogaster*, pink diamond, *A. mellifera*, black

725 diamond, *B. mori*, gray diamond. The tree branch drew in green contains identified AChE

726 sequences. Accession numbers and IDs of sequences that appear in the tree can be found in

727 Table S10.

728 Figure S3. Phylogenetic Identification of Phytoseiidae putative voltage-gated sodium channels

729 (VGSC). Maximum likelihood tree of *Neoseiulus barkeri*, *Amblyseius swirskii, Metaseiulus*

730 *occidentalis,* and *Phytoseiulus persimilis* VGSC protein sequences. VGSC sequences of

731 *Tetranychus urticae* and *Musca domestica* were included as a reference. The phylogenetic tree

732 was built using IQ-TREE v 2.0.3 with 1,000 ultrafast bootstrap combined with automatic model

733 finding through ModelFinder Plus [58]. The resulting trees were midpoint rooted and edited with
734 MEGA v 10.2.4 software. Only bootstrap support values > 85% were indicated in the tree. Colour
735 and shape codes are as follows: *N. barkeri*, brown circle, *A. swirskii,* purple circle, *M. occidentalis*,
736 yellow circle, *P. persimilis*, green circle, *T. urticae*, red square, *M. domestica*, black diamond shape.
737 Black arrow points at the branch with identified VGSC sequences. Accession numbers and IDs of
738 sequences that appear in the tree can be found in Table S10.
739 Figure S4. Phylogenetic Identification of Phytoseiidae putative glutamate-gated chloride channels
740 (GluCls). Maximum likelihood tree of *Amblyseius swirskii*, *Metaseiulus occidentalis*, *Phytoseiulus*
741 *persimilis,* and *Tetranychus urticae* cys-loop ligand-gated ion channel protein sequences. The
742 phylogenetic tree was built using IQ-TREE v 2.0.3 with 1,000 ultrafast bootstrap combined with
743 automatic model finding through ModelFinder Plus [58]. The resulting trees were midpoint rooted
744 and edited with MEGA v 10.2.4 software. Only bootstrap support values > 85% were indicated in
745 the tree. Colour and shape codes are as follows: *D. melanogaster*, pink diamond shape, *A. swirskii*,
746 purple circle, *M. occidentalis*, yellow circle, *P. persimilis*, green circle, *T. urticae*, red square. Black
747 arrow points at the branch with identified GluCl sequences. Accession numbers and IDs of
748 sequences that appear in the tree can be found in Table S10.

749 Tables

750 Table 1. Occurrence and frequency of acaricide target site mutations in Phytoseiidae. Only SNPs
751 that occur in at least one of the examined predatory mite species were listed. Mutations previously
752 validated for their role in arthropod resistance to insecticides/acaricides are marked in bold.
753 Table S1. Comparison of the two *Phytoseiulus persimilis* transcriptome assemblies.
754 Table S2. Variance analysis of the daily fecundity data.

755 Table S3. P values of the log-rank pairwise comparisons between survival rates after treatment
756 with orange oil and terpenoid blend. P values are Benjamini-Hochberg adjusted.
757 Table S4. List of contigs having a BLASTx hit (E^{-3}) with protozoa, viruses, bacteria, *Tetranychus*
758 *urticae,* and other members of genus *Tetranychus* showing a mean sequence similarity value ≥
759 98%, removed from the" 40 million reads assembly".
760 Table S5. Annotation of the" 40 million reads assembly" of *Phytoseiulus persimilis*.
761 Table S6. List of contigs having a BLASTx hit (E^{-3}) with protozoa, viruses, bacteria, *Tetranychus*
762 *urticae,* and other members of genus *Tetranychus* showing a mean sequence similarity value ≥
763 98%, removed from the" 80 million reads assembly".
764 Table S7. Annotation of the" 80 million reads assembly" of *Phytoseiulus persimilis*.
765 Table S8. Transcripts differentially expressed in *Phytoseiulus persimilis* females (log2FC>0,
766 qvalue<0.05) after two days of consuming orange oil treated *Tetranychus urticae* mites,
767 compared to the water-sprayed control (orange oil_c).
768 Contig names written in bold indicate upregulated transcripts, while contig names written with
769 regular letters indicate the downregulated transcripts.
770 Table S9. Target sites of acaricides commonly used against *Tetranychus urticae*, their orthologues
771 in *Phytoseiulus persimilis*, and the IRAC acaricide classes that target these proteins.
772 Table S10. Accession numbers and IDs of arthropod acaricide target-site proteins included in the
773 alignments and phylogenetic analysis.

774 ## Files

775 File S1. Preprocessed" 40 million assembly" of *Phytoseiulus persimilis* used to identify cytb
776 sequence. Requires FASTA file reader.

777 File S2." 40 million reads" assembly of *Phytoseiulus persimilis* transcriptome before removing the

778 contaminating sequences. Requires FASTA file reader

779 File S3." 80 million reads" assembly of *Phytoseiulus persimilis* transcriptome before removing the

780 contaminating sequences. Requires FASTA file reader

781 File S4. Final" 40 million reads" assembly of *Phytoseiulus persimilis* transcriptome. Requires FASTA

782 file reader

783 File S5. Final" 80 million reads" assembly of *Phytoseiulus persimilis* transcriptome. Requires FASTA

784 file reader

785 File S6. Nucleotide sequences of acaricide target sites, identified in *Phytoseiulus persimilis*

786 transcriptome. Requires FASTA file reader.

787 References

788 1. Knapp M, van Houten J, van Baal E, Groot T. Use of predatory mites in commercial

789 biocontrol: current status and future prospects. Acarologia 58, 72–82 (2018).

790 2. Migeon, A., Tixier, M.-S., Navajas, M., Litskas, V. D. & Stavrinides, M. C. A predator-prey

791 system: *Phytoseiulus persimilis* (Acari: Phytoseiidae) and *Tetranychus urticae* (Acari:

792 Tetranychidae): worldwide occurrence datasets. *Acarologia* 59, 301–307 (2019).

793 3. Bale JS, van Lenteren JC, & Bigler F. Biological control and sustainable food production.

794 Philos Trans R Soc Lond B Biol Sci 363, 761–776 (2007).

795 4. van de Vrie M, McMurtry J, Huffaker C. Ecology of tetranychid mites and their natural

796 enemies: A review: III. Biology, ecology, and pest status, and host-plant relations of

797 tetranychids. Hilgardia 41, 343–432 (1972).

798 5. Van Leeuwen, T., Vontas, J., Tsagkarakou, A. & Tirry, L. Mechanisms of acaricide resistance

799 in the two-spotted spider mite *Tetranychus urticae*. in *Biorational Control of Arthropod*

800 *Pests: Application and Resistance Management* (eds. Ishaaya, I. & Horowitz, A. R.) 347–393
801 (Springer Netherlands, 2009). doi:10.1007/978-90-481-2316-2_14.
802 6. Van Leeuwen T, Vontas J , Tsagkarakou A, Dermauw W, Tirry L. Acaricide resistance
803 mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari:
804 A review. Insect Biochem Molec 40, 563–572 (2010).
805 7. Nauen R, Elbert A, & McCaffery A. IRAC: Insecticide resistance, and mode of action
806 classification of insecticides. in *Modern Crop Protection Compounds: Insecticides* 935–955
807 (Wiley-VCH, 2012).
808 8. Desneux, N., Decourtye, A. & Delpuech, J.-M. The sublethal effects of pesticides on
809 beneficial arthropods. Ann Rev Entomol 52, 81–106 (2007).
810 9. Sparks, T. C. & Lorsbach, B. A. Perspectives on the agrochemical industry and agrochemical
811 discovery. Pest Manag Sci 73, 672–677 (2017).
812 10. Phillips McDougall. *Evolution of the crop protection industry since 1960*. 18 p.
813 info@phillipsmcdougall.com (2019).
814 11. Hertz-Picciotto, I. *et al.* Organophosphate exposures during pregnancy and child
815 neurodevelopment: Recommendations for essential policy reforms. PLOS Medicine 15,
816 e1002671 (2018).
817 12. Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, & Dermauw W. The economic importance
818 of acaricides in the control of phytophagous mites and an update on recent acaricide
819 mode of action research. Pestic Biochem Physiol 121, 12–21 (2015).
820 13. Van Leeuwen T *et al.* Mitochondrial heteroplasmy and the evolution of insecticide
821 resistance: Non-Mendelian inheritance in action. P Natl Acad Sci USA 105, 5980–5985
822 (2008).

823 14. Van Nieuwenhuyse, P., Van Leeuwen, T., Khajehali, J., Vanholme, B. & Tirry, L. Mutations in
824 the mitochondrial cytochrome b of *Tetranychus urticae* Koch (Acari: Tetranychidae) confer
825 cross-resistance between bifenazate and acequinocyl. Pest Manag Sci 65, 404–412 (2009).

826 15. Sugimoto, N. *et al.* QTL mapping using microsatellite linkage reveals target-site mutations
827 associated with high levels of resistance against three mitochondrial complex II inhibitors
828 in *Tetranychus urticae*. Insect Biochem Mol Biol 123, 103410 (2020).

829 16. Kishimoto, H., Yaginuma, K. & Toyama, M. Effects of pesticides on four native generalist
830 phytoseiid specier Acari: Phytoseiidae. Jpn J Appl Entomol Z 62, 29–39 (2018).

831 17. Yoo SS & Kim SS. Comparative toxicity of some pesticides to the predatory mite,
832 *Phytoseiulus persimilis* (Acarina: Phytoseiidae) and the twospotted spider mite,
833 *Tetranychus urticae* (Acarina: Tetranychidae). Korean J Entomol 30, 235–241 (2000).

834 18. Kim, S. S. & Yoo, S. S. Comparative toxicity of some acaricides to the predatory mite,
835 *Phytoseiulus persimilis* and the twospotted spider mite, *Tetranychus urticae* . BioControl
836 47, 563–573 (2002).

837 19. Sparks, T. C. *et al.* Insecticides, biologics and nematicides: Updates to IRAC's mode of
838 action classification - a tool for resistance management. Pestic Biochem Physiol 167,
839 104587 (2020).

840 20. Isman, M. B. Botanical insecticides in the twenty-first century-fulfilling their promise? Annu
841 Rev Entomol 65, 233–249 (2020).

842 21. Chaieb I *et al.* Chemical composition and aphicidal potential of *Citrus aurantium* peel
843 essential oils. Entomol Gen 37, 063–075 (2018).

844 22. Chiasson, H., Vincent, C. & Bostanian, N. J. Insecticidal properties of a *Chenopodium*-based
845 botanical. J Econ Entomol 97, 1378–1383 (2004).

846 23. Duso C, Van Leeuwen T, & Pozzebon A. Improving the compatibility of pesticides and
847 predatory mites: recent findings on physiological and ecological selectivity. Curr Opi Insect
848 Sci 39, 63–68 (2020).
849 24. Van Leeuwen, T. & Dermauw, W. The molecular evolution of xenobiotic metabolism and
850 resistance in Chelicerate mites. Annu Rev Entomol 61, 475–498 (2016).
851 25. Bielza P. Insecticide resistance in natural enemies. in *Advances in insect control and*
852 *resistance management* (eds. Horowitz R & Ishaaya I) 313–329 (Springer, 2016).
853 26. Bergeron, P. E. & Schmidt-Jeffris, R. A. Not all predators are equal: miticide non-target
854 effects and differential selectivity. Pest Manag Sci 76, 2170–2179 (2020).
855 27. Si YK, Hee GA, Pan JH, Un TL, & Joon-Ho L. Toxicities of 26 pesticides against 10 biological
856 control species. J Asia-Pac Entomol 21, 1–8 (2018).
857 28. Ahn KS, Lee SY, & Lee KY. Selective toxicity of pesticides to the predatory mite,
858 *Phytoseiulus persimilis* and control effects of the two-spotted spider mite, *Tetranychus*
859 *urticae* by predatory mite and pesticide mixture on rose. Koren J Appl Entomol 43, 71–79
860 (2005).
861 29. Markwick, N. P. Detecting variability and selecting for pesticide resistance in two species of
862 phytoseiid mites. Entomophaga 31, 225–236 (1986).
863 30. Lee SG, Hilton SA, Broadbent B, & Kim JH. Insecticide resistance in phytoseiid predatory
864 mites, *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Acarina: Phytoseiidae). J Asia-Pac
865 Entomol 5, 123–129 (2002).
866 31. Salman SY, Aydınlı F, & Ay R. Selection for resistance: Cross-resistance, inheritance,
867 synergists and biochemical mechanisms of resistance to acequinocyl in *Phytoseiulus*
868 *persimilis* A.H. (Acari: Phytoseiidae). Crop Prot 67, 109–115 (2015).

869 32. Sibel Yorulmaz Salman & Cenk Keskin. The effects of milbemectin and spirodiclofen
870 resistance on *Phytoseiulus persimilis*A.single bondH. (Acari:Phytoseiidae) life table
871 parameters. Crop Prot 124, 104751 (2019).
872 33. Bielza, P., Balanza, V., Cifuentes, D. & Mendoza, J. Challenges facing arthropod biological
873 control: identifying traits for genetic improvement of predators in protected crops. Pest
874 Manag Sci 76, 3517–3526 (2020).
875 34. Leung, K. *et al.* Next-generation biological control: the need for integrating genetics and
876 genomics. Biol Rev 95, 1838–1854 (2020).
877 35. İnak, E. & Yorulmaz Salman, S. Insecticide resistance mechanisms in predatory mites. Int J
878 Pest Manag 1–7 (2020) doi:10.1080/09670874.2020.1817619.
879 36. Cong, L. *et al.* Transcriptome and difference analysis of fenpropathrin resistant predatory
880 mite, *Neoseiulus barkeri* (Hughes). Int J Mol Sci 17, (2016).
881 37. Grbić, M. *et al.* The genome of *Tetranychus urticae* reveals herbivorous pest adaptations.
882 Nature 479, 487–492 (2011).
883 38. Van Leeuwen, T., Van Pottelberge, S. & Tirry, L. Comparative acaricide susceptibility and
884 detoxifying enzyme activities in field-collected resistant and susceptible strains of
885 *Tetranychus urticae* . Pest Manag Sci 61, 499–507 (2005).
886 39. Khalighi, M., Tirry, L. & Van Leeuwen, T. Cross-resistance risk of the novel complex II
887 inhibitors cyenopyrafen and cyflumetofen in resistant strains of the two-spotted spider
888 mite *Tetranychus urticae*. Pest Manag Sci 70, 365–368 (2014).
889 40. Van Leeuwen, T., Tirry, L. & Nauen, R. Complete maternal inheritance of bifenazate
890 resistance in Tetranychus urticae Koch (Acari: Tetranychidae) and its implications in mode
891 of action considerations. Insect Biochem Mol Biol 36, 869–877 (2006).

892 41. Laecke, K. V. & Degheele, D. Effect of insecticide- synergist combinations on the survival of
893 *Spodoptera exigua*. Pest Sci 37, 283–288 (1993).
894 42. Hothorn, T., Bretz, F. & Westfall, P. Simultaneous inference in general parametric models.
895 Biom J 50, 346–363 (2008).
896 43. R Core Team. *R: A language and environment for statistical computing*. (R Foundation for
897 Statistical Computing, 2020).
898 44. Andrews S. *FastQC: a quality control tool for high throughput sequence data.* (2010).
899 45. Alavijeh, E. S. *et al.* Molecular and genetic analysis of resistance to METI-I acaricides in
900 Iranian populations of the citrus red mite *Panonychus citri*. Pestic Biochem Physiol 164, 73–
901 84 (2020).
902 46. Haas, B. J. *et al.* De novo transcript sequence reconstruction from RNA-seq using the
903 Trinity platform for reference generation and analysis. Nat Protoc 8, 1494–1512 (2013).
904 47. Li, W. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of
905 protein or nucleotide sequences. Bioinformatics 22, 1658–1659 (2006).
906 48. Matsunaga, A., Tsugawa, M. & Fortes, J. CloudBLAST: Combining MapReduce and
907 virtualization on distributed resources for bioinformatics applications. in *2008 IEEE Fourth*
908 *International Conference on eScience* 222–229 (IEEE, 2008). doi:10.1109/eScience.2008.62.
909 49. Gotz, S. *et al.* High-throughput functional annotation and data mining with the Blast2GO
910 suite. Nucleic Acids Res 36, 3420–3435 (2008).
911 50. Finn, R. D. *et al.* InterPro in 2017—beyond protein family and domain annotations. Nucleic
912 Acids Res 45, D190–D199 (2017).
913 51. Bray, N. L., Pimentel, H., Melsted, P. & Pachter, L. Near-optimal probabilistic RNA-seq
914 quantification. Nat Biotechnol 34, 525–527 (2016).

915 52. Pimentel, H., Bray, N. L., Puente, S., Melsted, P. & Pachter, L. Differential analysis of RNA-
916 seq incorporating quantification uncertainty. Nat Methods 14, 687–690 (2017).
917 53. Sahraeian, S. M. E. *et al.* Gaining comprehensive biological insight into the transcriptome
918 by performing a broad-spectrum RNA-seq analysis. Nat Commun 8, 59 (2017).
919 54. Altschul, S. Gapped BLAST and PSI-BLAST: a new generation of protein database search
920 programs. Nucleic Acids Res 25, 3389–3402 (1997).
921 55. Katoh, K. & Standley, D. M. MAFFT Multiple sequence alignment software version 7:
922 Improvements in performance and usability. Mol Biol Evol 30, 772–780 (2013).
923 56. Wei, P. *et al.* Overexpression of an alternative allele of carboxyl/choline esterase 4 (CCE04)
924 of *Tetranychus urticae* is associated with high levels of resistance to the keto-enol acaricide
925 spirodiclofen. Pest Manag Sci 76, 1142–1153 (2020).
926 57. Claudianos, C. *et al.* A deficit of detoxification enzymes: pesticide sensitivity and
927 environmental response in the honeybee. Insect Mol Biol 15, 615–636 (2006).
928 58. Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S.
929 ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14,
930 587–589 (2017).
931 59. Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis
932 Version 7.0 for bigger datasets. Mol Biol Evol 33, 1870–1874 (2016).
933 60. Van Leeuwen, T. *et al.* Population bulk segregant mapping uncovers resistance mutations
934 and the mode of action of a chitin synthesis inhibitor in arthropods. Proc Natl Acad Sci
935 USA 109, 4407–4412 (2012).
936 61. Douris, V. *et al.* Resistance mutation conserved between insects and mites unravels the
937 benzoylurea insecticide mode of action on chitin biosynthesis. Proc Natl Acad Sci USA 113,
938 14692–14697 (2016).

939 62. Angerer, H. *et al.* Tracing the tail of ubiquinone in mitochondrial complex I. Biochim
940 Biophys Acta Bioenerg 1817, 1776–1784 (2012).
941 63. Tocilescu, M. A., Fendel, U., Zwicker, K., Kerscher, S. & Brandt, U. Exploring the ubiquinone
942 binding cavity of respiratory Complex I. J Biol Chem 282, 29514–29520 (2007).
943 64. Fendel, U., Tocilescu, M. A., Kerscher, S. & Brandt, U. Exploring the inhibitor binding pocket
944 of respiratory complex I. Biochim Biophys Acta Bioenerg 1777, 660–665 (2008).
945 65. Ahlers, P. M., Zwicker, K., Kerscher, S. & Brandt, U. Function of conserved acidic residues in
946 the PSST homologue of Complex I (NADH:Ubiquinone Oxidoreductase) from *Yarrowia*
947 *lipolytica*. J Biol Chem 275, 23577–23582 (2000).
948 66. Ahlers, P. M., Garofano, A., Kerscher, S. J. & Brandt, U. Application of the obligate aerobic
949 yeast *Yarrowia lipolytica* as a eucaryotic model to analyse Leigh syndrome mutations in
950 the complex I core subunits PSST and TYKY. Biochim Biophys Acta Bioenerg 1459, 258–265
951 (2000).
952 67. Triepels, R. H. *et al.* Leigh syndrome associated with a mutation in the NDUFS7 (PSST)
953 nuclear encoded subunit of complex I. Ann Neurol 45, 787–790 (1999).
954 68. Lebon, S. *et al.* A novel mutation in the human complex I NDUFS7 subunit associated with
955 Leigh syndrome. Mol Gent Metab 90, 379–382 (2007).
956 69. Garofano, A., Zwicker, K., Kerscher, S., Okun, P. & Brandt, U. Two aspartic acid residues in
957 the PSST-homologous NUKM subunit of Complex I from *Yarrowia lipolytica* are essential
958 for catalytic activity. J Biol Chem 278, 42435–42440 (2003).
959 70. Feyereisen, R., Dermauw, W. & Van Leeuwen, T. Genotype to phenotype, the molecular
960 and physiological dimensions of resistance in arthropods. Pestic Biochem Physiol 121, 61–
961 77 (2015).

962 71. Cassanelli, S., Ahmad, S., Duso, C., Tirello, P. & Pozzebon, A. A single nucleotide
963 polymorphism in the acetylcholinesterase gene of the predatory mite *Kampimodromus*
964 *aberrans* (Acari: Phytoseiidae) is associated with chlorpyrifos resistance. Biological Control
965 90, 75–82 (2015).
966 72. Pomerantz, A. F. & Hoy, M. A. Expression analysis of *Drosophila* doublesex, transformer-2,
967 intersex, fruitless-like, and vitellogenin homologs in the parahaploid predator *Metaseiulus*
968 *occidentalis* (Chelicerata: Acari: Phytoseiidae). Exp Appl Acarol 65, 1–16 (2015).
969 73. Lueke, B. *et al.* Identification and functional characterization of a novel acetyl-CoA
970 carboxylase mutation associated with ketoenol resistance in *Bemisia tabaci*. Pestic Biochem
971 Physiol 166, 104583 (2020).
972 74. Rinkevich, F. D., Du, Y. & Dong, K. Diversity and convergence of sodium channel mutations
973 involved in resistance to pyrethroids. Pestic Biochem Physiol 106, 93–100 (2013).
974 75. Riga, M. *et al.* The relative contribution of target-site mutations in complex acaricide
975 resistant phenotypes as assessed by marker assisted backcrossing in *Tetranychus urticae*.
976 Sci Rep 7, 9202 (2017).
977 76. Xue, W. *et al.* Untangling a G ordian knot: the role of a GLUCL3 I321T mutation in
978 abamectin resistance in *Tetranychus urticae*. Pest Manag Sci 77, 1581–1593 (2021).
979 77. Dermauw, W. *et al.* The cys-loop ligand-gated ion channel gene family of *Tetranychus*
980 *urticae*: Implications for acaricide toxicology and a novel mutation associated with
981 abamectin resistance. Insect Biochem Mol Biol 42, 455–465 (2012).
982 78. Kim, S. I. *et al.* Acequinocyl resistance associated with I256V and N321S mutations in the
983 two-spotted spider mite (Acari: Tetranychidae). J Econ Entomol 112, 835–841 (2019).

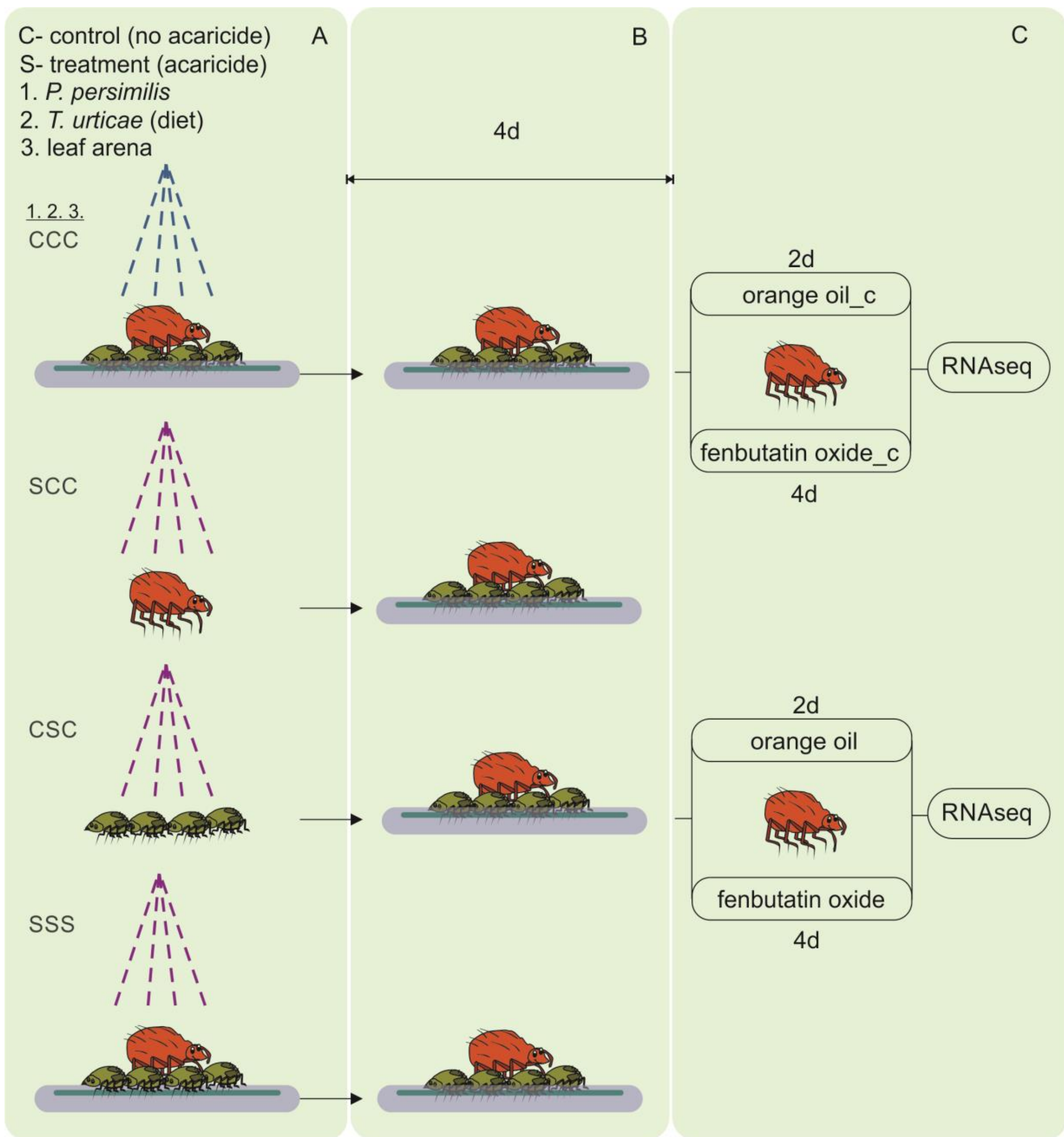
984 79. Van Leeuwen, T. *et al.* Parallel evolution of cytochrome b mediated bifenazate resistance in
985 the citrus red mite *Panonychus citri*: QoI resistance in *Panonychus citri*. Insect Mol Biol 20,
986 135–140 (2011).
987 80. Fraaije, B. A. *et al.* Risk assessment studies on succinate dehydrogenase inhibitors, the new
988 weapons in the battle to control *Septoria* leaf blotch in wheat: SDHI fungicides and
989 *Septoria* leaf blotch control. Mol Plant Pathol 13, 263–275 (2012).
990 81. Horsefield, R. *et al.* Structural and computational analysis of the quinone-binding site of
991 Complex II (Succinate-Ubiquinone Oxidoreductase). J Biol Chem 281, 7309–7316 (2006).
992 82. Barbar, Z. Evaluation of three pesticides against phytophagous mites and their impact on
993 phytoseiid predators in an eggplant open-field. Acarologia 57, 529–539 (2017).
994 83. Rhodes, E. M., Liburd, O. E., Kelts, C., Rondon, S. I. & Francis, R. R. Comparison of single
995 and combination treatments of *Phytoseiulus persimilis, Neoseiulus californicus* , and
996 Acramite (bifenazate) for control of twospotted spider mites in strawberries. Exp Appl
997 Acarol 39, 213–225 (2006).
998 84. Qiao Yan *et al.* Integrating *Phytoseiulus persimilis* and cyflumetofenin for control of
999 *Tetrangchus urticae* on strawberry. Chinese J Biol Cont 34, 514–519 (2018).
1000 85. Atef MMS, Ibrahim MMS, & Xuenong Xu. Comparative selectivity of acaricides to the
1001 predatory mites of *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari:
1002 Phytoseiidae). Egyptian J of Plant Prot Res Inst 2, 586–598 (2019).
1003 86. Ochiai, N. *et al.* Toxicity of bifenazate and its principal active metabolite, diazene, to
1004 *Tetranychus urticae* and *Panonychus citri* and their relative toxicity to the predaceous
1005 mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*. Exp Appl Acarol 43, 181–197
1006 (2007).

1007 87. Dambolena, J. S. *et al.* Terpenes: natural products for controlling Insects of importance to
1008 human health—a structure-activity relationship study. Psyche 2016, 1–17 (2016).
1009 88. Abdelgaleil, S. A. M., Badawy, M. E. I., Mahmoud, N. F. & Marei, A. E.-S. M. Acaricidal
1010 activity, biochemical effects and molecular docking of some monoterpenes against two-
1011 spotted spider mite (*Tetranychus urticae* Koch). Pestic Biochem Physiol 156, 105–115 (2019).
1012 89. Badawy, M. E. I., El-Arami, S. A. A. & Abdelgaleil, S. A. M. Acaricidal and quantitative
1013 structure activity relationship of monoterpenes against the two-spotted spider mite,
1014 *Tetranychus urticae* . Exp Appl Acarol 52, 261–274 (2010).
1015 90. Roh, H. S., Lee, B. H. & Park, C. G. Acaricidal and repellent effects of myrtacean essential
1016 oils and their major constituents against *Tetranychus urticae* (Tetranychidae). J Asia Pac
1017 Entomol 16, 245–249 (2013).
1018 91. Sikkema, J., de Bont, J. A. & Poolman, B. Interactions of cyclic hydrocarbons with biological
1019 membranes. J Biol Chem 269, 8022–8028 (1994).
1020 92. Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N. & Shaaya, E. Activation of
1021 octopaminergic receptors by essential oil constituents isolated from aromatic plants:
1022 possible mode of action against insect pests. Pest Manag Sci 58, 1101–1106 (2002).
1023 93. Odalo, J. O. *et al.* Repellency of essential oils of some plants from the Kenyan coast against
1024 *Anopheles gambiae*. Acta Tropica 95, 210–218 (2005).
1025 94. da Camara, C. A. G., Akhtar, Y., Isman, M. B., Seffrin, R. C. & Born, F. S. Repellent activity of
1026 essential oils from two species of Citrus against *Tetranychus urticae* in the laboratory and
1027 greenhouse. Crop Prot 74, 110–115 (2015).
1028 95. Villanueva-Cañas, J. L., Horvath, V., Aguilera, L. & González, J. Diverse families of
1029 transposable elements affect the transcriptional regulation of stress-response genes in
1030 *Drosophila melanogaster*. Nucleic Acids Res 6842–6857 (2019) doi:10.1093/nar/gkz490.

1031 96. Agafonov, D. E. *et al.* Molecular architecture of the human U4/U6.U5 tri-snRNP. Science
1032 351, 1416–1420 (2016).
1033 97. Riddell, C. E. *et al.* Differential gene expression and alternative splicing in insect immune
1034 specificity. BMC Genomics 15, 1031 (2014).
1035 98. Niu, L. *et al.* Atlastin-mediated membrane tethering is critical for cargo mobility and exit
1036 from the endoplasmic reticulum. Proc Natl Acad Sci USA 116, 14029–14038 (2019).
1037 99. Paspati, A. Living on an unfriendly plant host: impact of tomato on the predatory mite
1038 *Amblyseius swirskii*. (Universitat Jaume I, 2019). doi:10.6035/14104.2019.692822.
1039 100. Lozano-Fernandez, J. *et al.* Increasing species sampling in chelicerate genomic-scale
1040 datasets provides support for monophyly of Acari and Arachnida. Nat Commun 10, 2295
1041 (2019).
1042 101. Xue, X.-F., Dong, Y., Deng, W., Hong, X.-Y. & Shao, R. The phylogenetic position of
1043 eriophyoid mites (superfamily Eriophyoidea) in Acariformes inferred from the sequences of
1044 mitochondrial genomes and nuclear small subunit (18S) rRNA gene. Mol Phylogenetics
1045 Evol 109, 271–282 (2017).
1046 102. Wu, G., Miyata, T., Kang, C. Y. & Xie, L. H. Insecticide toxicity and synergism by enzyme
1047 inhibitors in 18 species of pest insect and natural enemies in crucifer vegetable crops. Pest
1048 Manag Sci 63, 500–510 (2007).
1049 103. Hayashi, N., Sasama, Y., Takahashi, N. & Ikemi, N. Cyflumetofen, a novel acaricide - its
1050 mode of action and selectivity: Mode of action and selectivity of the novel acaricide
1051 cyflumetofen. Pest Manag Sci 69, 1080–1084 (2013).
1052 104. Van Nieuwenhuyse, P. *et al.* On the mode of action of bifenazate: New evidence for a
1053 mitochondrial target site. Pestic Biochem Physiol 104, 88–95 (2012).

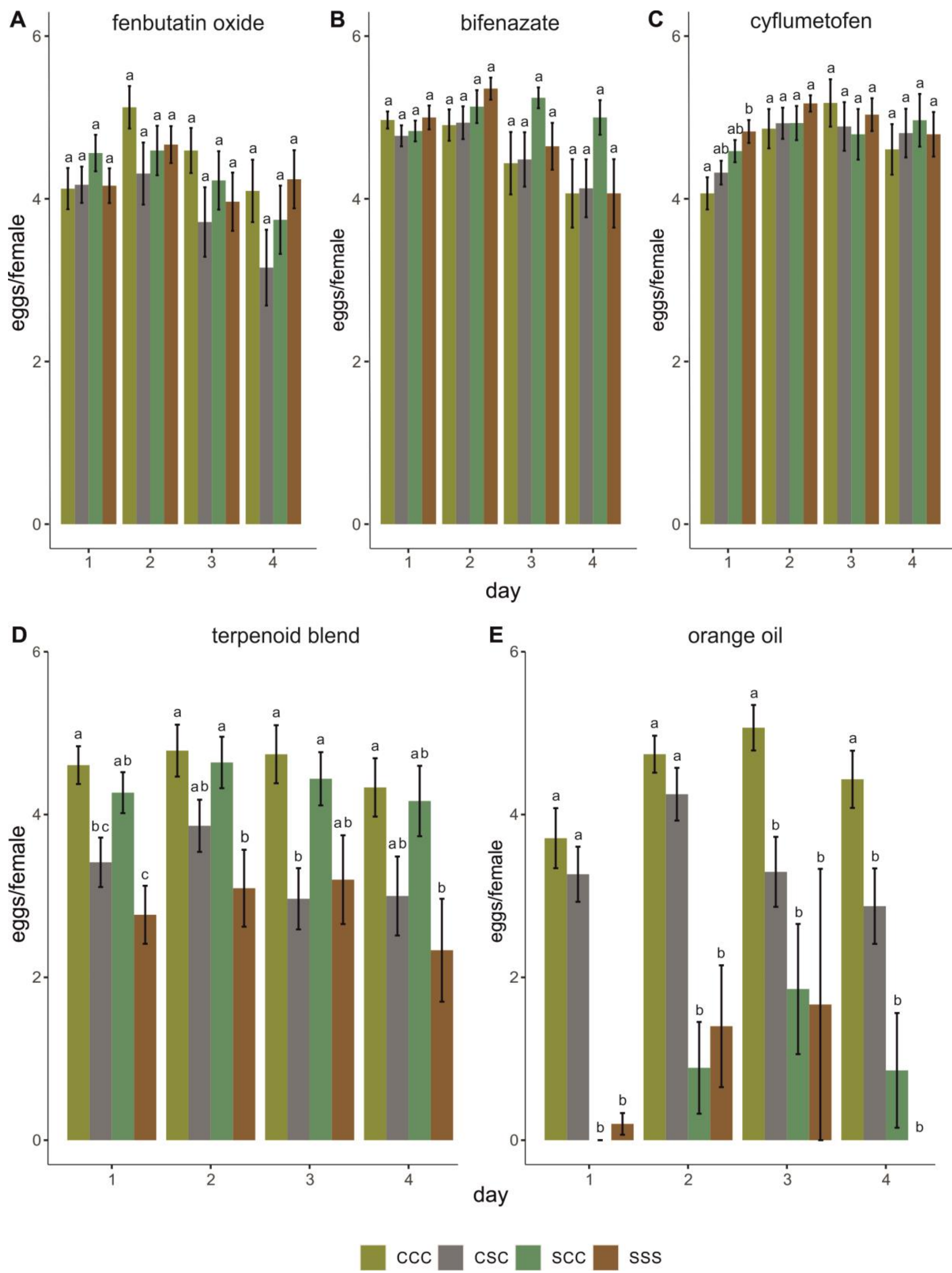
1054 105. Furuya, T., Machiya, K., Fujioka, S., Nakano, M. & Inagaki, K. Development of a novel
1055 acaricide, pyflubumide. Pestic Sci 42, 132–136 (2017).
1056 106. Maeoka, A. & Osakabe, M. Co-occurrence of subunit B and C mutations in respiratory
1057 complex II confers high resistance levels to pyflubumide and cyenopyrafen in the two-
1058 spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Pest Manag Sci ps.6555
1059 (2021) doi:10.1002/ps.6555.
1060 107. von Ballmoos, C., Wiedenmann, A. & Dimroth, P. Essentials for ATP Synthesis by F_1F_0 ATP
1061 Synthases. Annu Rev Biochem 78, 649–672 (2009).
1062 108. Partis, M. D., Griffiths, D. G. & Beechey, R. B. Discrimination between the binding sites of
1063 modulators of the H+-translocating ATPase activity in rat liver mitochondrial membranes.
1064 Arch Biochem Biophys 232, 610–615 (1984).
1065 109. Fotakis, E. A. *et al.* Identification and detection of a novel point mutation in the Chitin
1066 Synthase gene of *Culex pipiens* associated with diflubenzuron resistance. PLoS Negl Trop
1067 Dis 14, e0008284 (2020).
1068 110. Nyoni, B. N. *et al.* Pyrethroid resistance in the tomato red spider mite, *Tetranychus evansi* ,
1069 is associated with mutation of the para-type sodium channel. *Pest. Manag. Sci.* 67, 891–897
1070 (2011).
1071 111. Tsagkarakou, A. *et al.* Identification of pyrethroid resistance associated mutations in the
1072 *para* sodium channel of the two-spotted spider mite *Tetranychus urticae* (Acari:
1073 Tetranychidae). Insect Mol Biol 18, 583–593 (2009).
1074 112. Kwon, D. H., Clark, J. M. & Lee, S. H. Cloning of a sodium channel gene and identification
1075 of mutations putatively associated with fenpropathrin resistance in *Tetranychus urticae* .
1076 Pestic Biochem Physiol 97, 93–100 (2010).

1077 113. Benavent-Albarracín, L. *et al.* Mutations in the voltage-gated sodium channel gene
1078 associated with deltamethrin resistance in commercially sourced *Phytoseiulus persimilis*.
1079 Insect Mol Biol 29, 373–380 (2020).
1080 114. González-Cabrera, J., Davies, T. G. E., Field, L. M., Kennedy, P. J. & Williamson, M. S. An
1081 amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa*
1082 *destructor* . PLoS ONE 8, e82941 (2013).
1083 115. Weill, M. *et al.* The unique mutation in ace-1 giving high insecticide resistance is easily
1084 detectable in mosquito vectors. Insect Mol Biol 13, 1–7 (2004).
1085 116. Bajda, S. *et al.* A mutation in the PSST homologue of complex I (NADH:ubiquinone
1086 oxidoreductase) from *Tetranychus urticae* is associated with resistance to METI acaricides.
1087 Insect Biochem Mol Biol 80, 79–90 (2017).
1088 117. Snoeck, S. *et al.* High-resolution QTL mapping in *Tetranychus urticae* reveals acaricide-
1089 specific responses and common target-site resistance after selection by different METI-I
1090 acaricides. Insect Biochem Mol Biol 110, 19–33 (2019).
1091 118. Shiraishi, Y., Murai, M., Sakiyama, N., Ifuku, K. & Miyoshi, H. Fenpyroximate binds to the
1092 interface between PSST and 49 kDa subunits in Mitochondrial NADH-Ubiquinone
1093 Oxidoreductase. Biochemistry 51, 1953–1963 (2012).



1094

1095 Figure 1.



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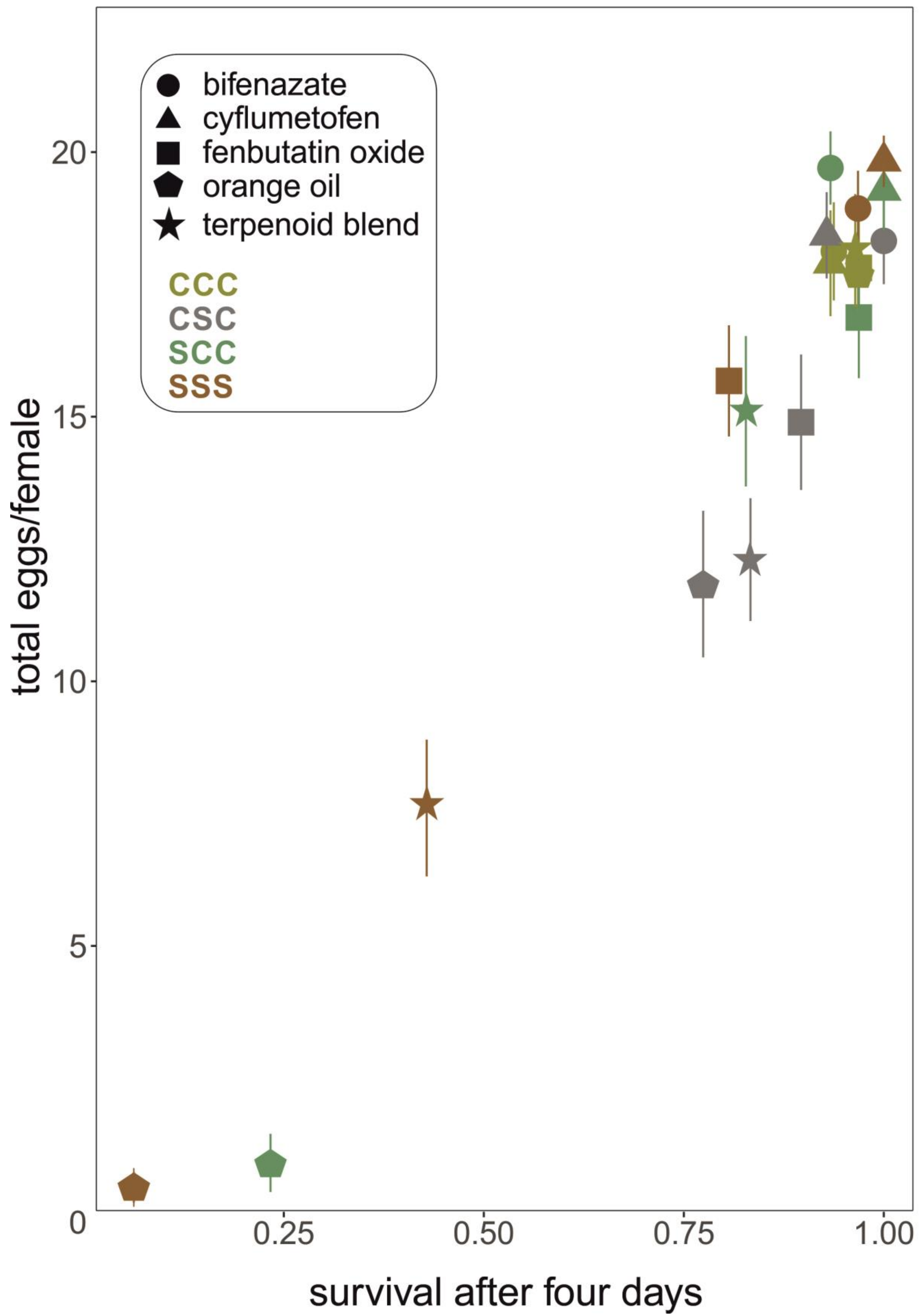
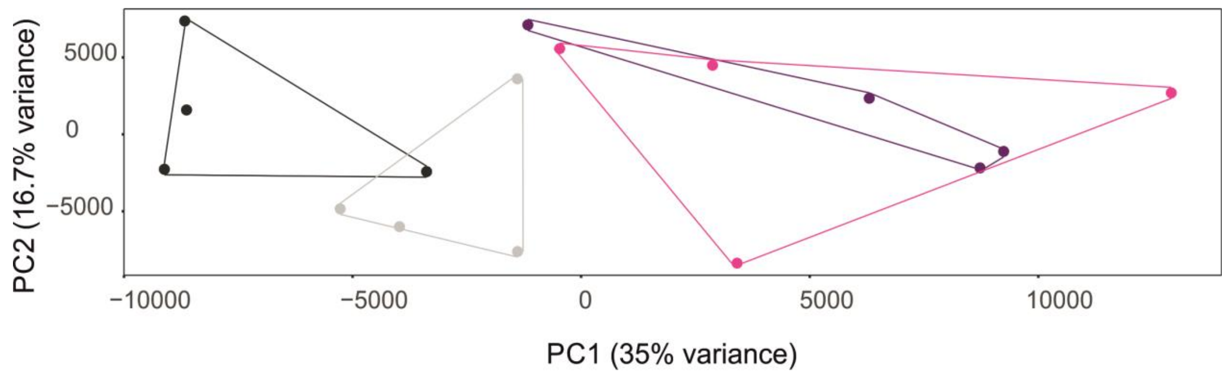
1097 Figure 2.

1098



1099 Figure 3.

1100



1101 Figure 4.

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CHS1
Phytoseiulus persimilis   1025 ALMHPQEFSCVMPGFIYFLSMPSMYLLLILYSLINLNVV 1063

Saccharomyces cerevisiae  987  T---ATRWSYLWWMCVYICALPIWNFVLPSYAYWKFDDF 1022
Anopheles gambiae         675  GVLHPQEMEALPAGLVYYITIPSMYMLLVIYSVFNMNDV 713
Tetranychus urticae       997  ALLHPQEFHCLYPCLLYFLSIPCMYLLLMIYSLVNLNVV 1035
Musca domestica           1025 ACLHPQEFWCISCGLIYLLSIPSMYLLLILYSIINLNVV 1063
Tribolium castaneum       1019 ACLHPQEFWCIVPGIIYLLSIPSMYLLLILYSIINLNVV 1057
Plutella xylostella       1022 ACLHPQEFWCIVPGIIYLLSIPSMYLLLILYSTINLNVV 1060

SdhB
Phytoseiulus persimilis   237  FSVYSCHTMMNCSKTCPKGLNPGKSIAELKKLMSGIAKK 275

Escherichia coli          201  FSVFRCHSIMNCVSVCPKGLNPTRAIGHIKSMLLQRNA- 238
Tetranychus urticae       252  FSLYRCHTIMNCSRTCPKNLNPGRAIGELKKLLAGWSKK 290
Saccharomyces cerevisiae  231  MSLYRCHTIMNCTRTCPKGLNPGLAIAEIKKSLAFA--- 266
Drosophila melanogaster   247  FSVYRCHTIMNCTRTCPKGLNPGRAIAEIKKLLSGLASK 285
Bos taurus                238  FSLYRCHTIMNCTQTCPKGLNPGKAIAEIKKMMATYKEK 276
Homo sapiens              238  FSLYRCHTIMNCTRTCPKGLNPGKAIAEIKKMMATYKEK 276
                                   * * *
SdhC
Phytoseiulus persimilis   43 DLGSPLSPHLSIYKPQMTTVLSITHSITGLGLTAGVYTI 81

Escherichia coli          11 KKQRPVNLDLQTIRFPVTAIASILHRVSGVITFVAVGIL 49
Saccharomyces cerevisiae  72 RAKRPISPHLTIYQPQLTWYLSSLHRISLVLMGLGFYLF 110
Drosophila melanogaster   49 RLGRELSPHLTIYQPQLTSMLSICHRGTGLALGVGVWGL 87
Homo Sapiens              47 GSNRPLSPHITIYSWSLPMAMSICHRGTGIALSAGVSLF 85
Bos taurus                47 TLNRPLSPHISIYGWSLPMAMSICHRGTGIALSAGVSLF 85
Tetranychus urticae       50 RLNRPISP-YTIYQPQLTSVLSISHRVSGVALSVGIYAM 87
                                                    ***  *
VGSC
Phytoseiulus persimilis   289 LNLLISIVGKTIGAL 303        384 LATVVIGNFVVLNLFLA 400

Bos taurus                861 LNMLIKIIGNSVGAL 875        958 MMVMVIGNLVVLNLFLA 974
Homo sapiens              867 LNMLIKIIGNSVGAL 881        964 MMVMVIGNLVVLNLFLA 980
Tetranychus urticae       901 LNLLITIMGKTLGDL 916        996 LATVIIGHLVMLNLFLA 1012
Metaseiulus occidentalis  943 LNLLISIMGKTIGAL 957       1038 LATVVIGNLVVLNLFLA 1054
Musca domestica           911 LNLLISIMGRTMGAL 925       1006 LATVVIGNLVVLNLFLA 1022
Anopheles gambiae         916 LNLLISIMGRTMGAL 930       1011 LATVVIGNLVVLNLFLA 1027
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1106

1107 Figure 5.

1108

1109 Table 1.

IRAC group	Target gene	SNP	*P. persimilis*	NCBI species (spanning the region/total seqs)	No of seq. With SNP, SNP
21 Mitochondrial complex I electron transport inhibitors	PSST	V88M, L, F[62–64]	-	*M. occidentalis* (2/2)	-
				A. swirskii (5/5)	1, V88I
		E140Q[65]	-	*M. occidentalis* (1/3)	-
				A. swirskii (4/5)	1, E140D

		R145H[68]	-	*M. occidentalis* (1/3)	-
				A. swirskii (4/5)	3, R145K
25 Mitochondrial complex II electron transport inhibitors	SdhB	I260T/V[15]	I260M	*M. occidentalis* (3/8)	-
				A. swirskii (4/5)	2, I260M
		R256P[80]	R256S	*M. occidentalis* (4/8),	3, R256S
				A. swirskii (4/5)	1, R256S
	SdhC	R74S[81]	R74S	*M. occidentalis* (2/4)	-
				A. swirskii (2/2)	2, R74S
20 Mitochondrial complex III electron transport inhibitors	cytb	G126S[14]	-	*N. litoralis* (5/5)	1, G126A
		A133T[79]	-	*T. pyri* (22/36)	4, A133G
		I136T[14]	-	*T. verrucosus* (2/2)	1, I136T
		S141F[14]	-	*T. aripo* (3/3),	3, S141T,
				A. limonicus (2/2)	2, S141T
				E. fustis (3/5)	3, S141T
				T. rhenanoides (15/15)	4, S141F
				T. ilicis (14/14)	3, S141F,V,D
				T. aestivalis (2/2)	2, S141F,A
				T. laurae (2/2)	1, S141A
				T. exhilaratus (34/34)	3, S141F,A
		G132A[119]	-	*T. setubali* (9/9)	1, G132E
1 Acetylcholinesterase (AChE) inhibitors	AChE	G119S[115]	-	*M. occidentalis* (2/2)	2, G119S
				K. aberrans (2/2)	1, G119S
				A. swirskii (1/1)	-

23 Inhibitors of acetyl CoA carboxylase	ACC	A2083V[73]	-	*M. occidentalis* (2/7)	-
				A. swirskii (5/12)	3, A2083V
10 Mite growth inhibitors affecting CHS1, 15 Inhibitors of chitin biosynthesis affecting CHS1	CHS1	I1017L,M,F[60,61,109]	I1017M	*A. swirskii* (2/11)	-
				M. occidentalis (2/2)	-
3 Sodium channel modulators	VGSC	L1014F (kdr)[74]	L1014F	*M. occidentalis* (0/1)	-
				A. swirskii (1/5)	-
				N. barkeri (1/1)	-
		M918 (super-kdr)[74]	M918V	*M. occidentalis* (1/1)	-
				A. swirskii (2/5)	-
				N. barkeri (1/1)	1, M918V
12 Inhibitors of mitochondrial ATP synthase	ATP synthase subunit c	-	R109S	*A. swirskii* (8/8)	1, R109S
				M. occidentalis (11/13)	8, R109S

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