1 **The host plant strongly modulates acaricide resistance levels to mitochondrial complex II**
2 **inhibitors in a multi-resistant field population of *Tetranychus urticae***

3 Christine Njiru [1*], Marilou Vandenhole [1*], Wim Jonckheere [1], Nicky Wybouw [2], Thomas Van
4 Leeuwen [1]

5 [1] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent,
6 Belgium

7 [2] Terrestrial Ecology Unit, Department of Biology, Faculty of Science, Ghent University, K.L.
8 Ledeganckstraat 35, 9000 Ghent, Belgium
9 *Christine Njiru and Marilou Vandenhole contributed equally to this work
10 correspondence to: Nicky Wybouw (nicky.wybouw@ugent.be) or Thomas Van Leeuwen
11 (thomas.vanleeuwen@ugent.be)

12 **Abstract**

13 The two-spotted spider mite *Tetranychus urticae* is a polyphagous pest with an extraordinary
14 ability to develop acaricide resistance. Here, we characterize the resistance mechanisms in a
15 *T. urticae* population (VR-BE) collected from a Belgian tomato greenhouse, where the grower
16 was unsuccessful in chemically controlling the mite population resulting in crop loss. Upon
17 arrival in the laboratory, the VR-BE population was established both on bean and tomato
18 plants as hosts. Toxicity bioassays on both populations confirmed that the population was
19 highly multi-resistant, recording resistance to 12 out of 13 compounds tested from various
20 mode of action groups. DNA sequencing revealed the presence of multiple target-site
21 resistance mutations, but these could not explain resistance to all compounds. In addition,
22 striking differences in toxicity for six acaricides were observed between the populations on
23 bean and tomato. The highest difference was recorded for the complex II inhibitors
24 cyenopyrafen and cyflumetofen, which were 4.4 and 3.3-fold less toxic for VR-BE mites on
25 tomato versus bean. PBO synergism bioassays suggested increased P450 based detoxification
26 contribute to the host-dependent toxicity. Given the involvement of increased detoxification,
27 we subsequently determined genome-wide gene expression levels of VR-BE on both hosts, in
28 comparison to a reference susceptible population, revealing overexpression of a large set of
29 detoxification genes in VR-BE on both hosts compared to the reference. In addition, a number
30 of mainly detoxification genes with higher expression in VR-BE on tomato compared to bean

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31 was identified, including several cytochrome P450s. Together, our work suggests that multi-
32 resistant field populations can accumulate a striking number of target-site resistance
33 mutations. We also show that the host plant can have a profound effect on the P450-
34 associated resistance levels to cyenopyrafen and cyflumetofen.

35 **Key words:** Host plant adaptation, multi-resistance, P450 metabolism, arthropod viruses,
36 gene expression, detoxification

37

38 1 Introduction

39 The spider mite *Tetranychus urticae* is one of the most polyphagous arthropod herbivores,
40 with the ability to feed on a wide range of plant species including several economically
41 important agricultural crops (Jeppson et al., 1975; Migeon et al., 2010). The major control
42 method for *T. urticae* is based on the use of acaricides that are classified based on the mode
43 of action (MOA), including but not limited to, sodium channel modulators such as bifenthrin;
44 glutamate-gated chloride channel modulators such as abamectin; mite growth inhibitors
45 affecting chitin synthase I such as etoxazole; mitochondrial electron transport complex I
46 inhibitors such as pyridaben, fenpyroximate and tebufenpyrad; complex II inhibitors such as
47 cyenopyrafen and cyflumetofen; complex III inhibitors such as acequinocyl and bifenazate; ATP
48 synthase inhibitors such as fenbutatin oxide; and acetylCoA carboxylase inhibitors such as
49 spiromesifen (Sparks and Nauen, 2015). However, *T. urticae* is able to quickly develop
50 resistance regardless of the chemical class, with the first case of resistance often reported a
51 few years after introduction of a new acaricide (De Rouck et al., 2023).

52 Utilizing a broad host range comes with a significant challenge as polyphagous herbivores are
53 exposed to divergent mixtures of plant produced defense compounds. These compounds can
54 be extremely diverse and/or highly toxic. The ability to metabolize and detoxify plant chemicals
55 is considered one of the major responses that arthropod herbivores have evolved (Després et
56 al., 2007; Futuyma and Agrawal, 2009; Simon et al., 2015). For spider mites, this is reflected in
57 an exceptionally strong toolkit to detoxify xenobiotic compounds, including laterally acquired
58 genes from microorganisms with novel metabolic abilities, expansion of detoxification gene
59 families and a fine-tuned transcriptional plasticity in response to host plant transfer (Dermauw
60 et al., 2013; Grbić et al., 2011; Snoeck et al., 2018; Wybouw et al., 2016, 2015). Even the mite's

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61 salivary composition may be tailored to its current host (Jonckheere et al., 2017, 2016),
62 potentially to optimize interactions with the plant's defense response (Blaazer et al., 2018;
63 Villarroel et al., 2016). Transcriptional plasticity and genetic variation determining gene-
64 expression regulation of these adaptation genes might be a key factor in allowing polyphagous
65 herbivores to colonize diverse host plant species (Brattsten, 1988; Castle et al., 2009; Kurlovs
66 et al., 2022; Liang et al., 2007; Yu, 1986). It has been suggested that the evolutionary history
67 of polyphagy might have led to the ability to better cope with anthropogenic pesticides
68 (Alyokhin and Chen, 2017; Dermauw et al., 2018). Indeed, several studies have shown that
69 adaptation to different host plants or even short term exposure to different plant chemicals
70 alters the herbivore's sensitivity to pesticides (Castle et al., 2009; Gould et al., 1982; Liang et
71 al., 2007; Pym et al., 2019; Yang et al., 2001).

72 Resistance mechanisms to natural and synthetic toxins can be broadly classified into (i)
73 toxicodynamic changes that involve a reduction in the sensitivity or availability of the target-
74 site due to point mutations, gene knockout or amplification; and (ii) toxicokinetic changes that
75 reduce the amount of toxic chemicals that reach the target-site through changes in
76 metabolism, penetration, transportation, exposure and excretion (Feyereisen et al., 2015).
77 Metabolic resistance to natural and synthetic xenobiotics is known to commonly rely on
78 increased expression of genes that belong to large multi-gene families such as cytochrome
79 P450 monooxygenases (CYPs), carboxyl/cholinesterases (CCEs), glutathione-S-transferases
80 (GSTs), UDP-glycosyl transferases (UGTs) and xenobiotic transporters such as ABC-
81 transporters (De Rouck et al., 2023; Van Leeuwen and Dermauw, 2016). Novel gene families
82 have also been implicated in xenobiotic metabolism and transport, including: intradiol ring
83 cleavage dioxygenases (DOGs), lipocalins, short chain dehydrogenases (SDRs) and the major
84 facilitator superfamily (MFS) (Dermauw et al., 2013; Wybouw et al., 2015; Zhurov et al., 2014).
85 Recent work has provided formal evidence that some of these detoxification enzymes can
86 metabolize plant allelochemicals (Njiru et al., 2022).

87 In *T. urticae* and other agricultural pests, high levels of pesticide resistance has often been
88 attributed to a combination of target site mutations and detoxification enzymes, which
89 suggests that resistance traits can involve multiple genetic factors (for a review see De Rouck
90 et al., 2023). Here, we used various approaches to characterize a population of *T. urticae*
91 collected from a tomato greenhouse near Antwerp (Belgium) that could no longer be

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92 controlled by the registered available acaricides, resulting in crop failure. Upon arrival in the
93 laboratory, we created two sub-populations: one on tomato as the original host and one on
94 bean as the standard laboratory host of *T. urticae*. First, the efficacy of 13 commercially
95 important acaricides from different MOA groups was investigated in toxicity assays performed
96 on both hosts. Next, molecular assays were used to uncover known target-site mutations. To
97 explain the observed patterns, synergism experiments were performed together with
98 transcriptome sequencing of the resistant population on both hosts and a susceptible
99 reference. The results were discussed in the light of a pest management strategy.

100 **2 Materials and methods**

101 **2.1 *T. urticae* populations**

102 The German susceptible strain (GSS) is a reference strain that has been reared without
103 pesticide exposure for more than five decades (Stumpf et al., 2001). The very resistant Belgian
104 (VR-BE) population was collected from a tomato greenhouse near Antwerp (Belgium) in 2021
105 where the grower was unsuccessful in controlling it using commercial formulations of
106 abamectin, hexythiazox, bifenazate, spirodiclofen and cyflumetofen. Upon arrival in the
107 laboratory, VR-BE was transferred to unsprayed potted tomato plants cv. 'Moneymaker' and
108 bean plants cv. 'Prelude' and maintained separately on these two hosts throughout the
109 experiments. Both populations will be referred to as VR-BE $_{tomato}$ and VR-BE $_{bean}$, respectively.
110 All mite populations were reared in climatically controlled chambers maintained at 25 ± 1°C
111 and 60% relative humidity (RH) with a 16:8 light:dark photoperiod.

112 **2.2 Chemicals**

113 Thirteen formulated acaricides/insecticides were used for toxicity bioassays: abamectin
114 (Vertimec 1.8% EC), acequinocyl (Kanemite 164 g L^{-1} SC), bifenazate (Floramite 240 g L^{-1} SC),
115 bifenthrin (Talstar 80 g L^{-1} SC), cyenopyrafen (Kunoichi 30% SC), cyflumetofen (Scelta 20% SC),
116 etoxazole (Borneo 110 g L^{-1} SC), fenbutation oxide (Acrimite 550 g L^{-1} SC), fenpyroximate (Kiron
117 51.2 g L^{-1} SC), azadirachtin A (NeemAzal-T/S 10 g L^{-1} EC), pyridaben (Sanmite 150 g L^{-1} SC),
118 spiromesifen (Oberon 240 g L^{-1} SC) and tebufenpyrad (Masai 20 WP).

119 **2.3 Toxicity and synergism assays**

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120 Dose-response assays on adults or larvae were conducted with a slight modification of the
121 standard method described by Van Leeuwen et al., (2004). Briefly, 20-25 adult females of VR-
122 BE $_{bean}$ and GSS were placed on the upper side of a 9 cm^2 kidney bean leaf disc prepared on
123 wet cotton wool. Using a custom-built spray tower (Van Laecke and Degheele, 1993), plates
124 were sprayed with 870 µl of at least five serial dilutions of each acaricide and a control
125 (distilled water) at 1 bar pressure to obtain a homogenous spray film (2 mg aqueous
126 deposit/cm^2). At least four replicates were used for each acaricide dose. Female adults of VR-
127 BE $_{tomato}$ were assayed in a similar way but using 9 cm^2 tomato leaf discs. For the larval
128 bioassays with spiromesifen and etoxazole, 20-30 adult females were placed on 9 cm^2 tomato
129 or bean leaf discs and allowed to lay eggs for 6 h in a climatically controlled chamber (25 ± 1
130 ^{o}C and 60% RH with a 16:8 light: dark photoperiod). After hatching (3-4 days), larvae were
131 counted and sprayed with 870 µL of at least five serial dilutions of each acaricide and a water
132 control as previously described. Mortality was assessed after one day for abamectin,
133 cyflumetofen, cyenopyrafen and tebufenpyrad, after two days for azadirachtin A, acequinocyl,
134 bifenthrin, fenpyroximate and pyridaben, after three days for fenbutatin oxide and bifenazate,
135 and after four days for etoxazole and spiromesifen. Mortality on the control replicates never
136 exceeded 10%. For adulticidal assays, mites were considered dead when not being able to
137 walk their own body length within 10 seconds after prodding with a fine brush. For larval
138 bioassays, mites were considered unaffected if they displayed the same development stage
139 as a water treated control at the time of scoring. When 5000 mg L^{-1} acaricide did not cause
140 50% mortality, higher concentrations were not tested. Lethal concentration killing 50% of the
141 population (LC_{50}) values, slopes, resistance ratios (RR) and 95% confidence intervals (CI) were
142 calculated by probit analysis using Polo Plus 2.0 software. The RR was considered significant if
143 the 95% CI did not include the value 1 (Robertson et al., 2017).

144 Synergism assays were conducted as described in Van Pottelberge et al., (2009). Briefly, the
145 synergist piperonyl butoxide (PBO), a P450 mono-oxygenase inhibitor, was dissolved in a 200
146 µL mixture of N,N-dimethylformamide and emulsifier W (alkarylpolyglycoether) in a 3:1 w/w
147 ratio and subsequently diluted 100-fold in demineralized water to 1000 mg L^{-1} . Adult female
148 mites of VR-BE $_{bean}$ and VR-BE $_{tomato}$ were transferred to 9 cm^2 leaf discs and sprayed with the
149 synergist mixture. After 24 h, the surviving mites (about 90% of PBO treated mites) were
150 transferred to fresh leaf discs and used in cyflumetofen toxicity bioassays as described above.

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151 The synergism ratios (SR, calculated as LC_{50} obtained after cyflumetofen treatment alone
152 divided by the LC_{50} obtained after cyflumetofen and synergist pretreatment) and 95% CI were
153 calculated by probit analysis using Polo Plus 2.0 software (LeOra Software, USA). The SR was
154 considered significant if the 95% CI did not include the value 1.

155 **2.4 Detection of target-site mutations**

156 Upon arrival and establishment in the laboratory, approximately 200 adult female mites were
157 collected from the VR-BE $_{tomato}$ population. Genomic DNA was extracted using the DNA blood
158 and tissue kit (Qiagen, Belgium), according to the manufacturer's instructions, and was used
159 as a template for PCR amplification. PCR amplification was used to screen for known or novel
160 target-site mutations, with a set of well validated primers (De Beer et al., 2022b; İnak et al.,
161 2022; Khalighi et al., 2015; Simma et al., 2020). Primers used for the amplification and
162 sequencing of different target-site regions are provided in Table S1.

163 For all PCR setups except for *cytb*, the reactions were performed using the Promega GoTaq®
164 G2 DNA polymerase kit in 50 µl reactions containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3
165 µM forward and reverse primer, 1.25 u GoTaq DNA polymerase and 1-2 µL gDNA template.
166 Cycling conditions were 2 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 52-55°C and 30-120 s
167 at 72 °C; and final extension of 7 min at 72°C. For *cytb*, the Expand Long Range dNTPack was
168 used in 50 µL reactions containing 1.25 mM $MgCl_2$, 0.5 mM of each dNTP, 0.3 µM forward and
169 reverse primer, 3.5 u Expand Long Range Enzyme mix and 2 µl gDNA template. Cycling
170 conditions were 2 min at 92°C; 40 cycles of 30 s at 92°C, 15 s at 54°C and 2 min at 58°C (with
171 extension time increasing 10 s/cycle after the 10th cycle); and final extension of 7 min at 58°C.

172 Amplicon purification and sequencing was performed at LGC Genomics GmbH (Germany). The
173 sequencing data were analyzed using BioEdit v.7.0.5 software (Hall, 1999), while visual
174 inspection of chromatograms for segregating SNPs was performed using Unipro UGENE v.37.0
175 (Okonechnikov et al., 2012). To check the persistence of these target site mutations over time,
176 200 adult females of VR-BE population were collected after one year on bean. DNA extraction
177 and mutation screening was carried out as described above.

178 **2.5 RNA isolation**

179 RNA was extracted from a pool of 150-200 adult females using the RNeasy plus mini kit
180 (Qiagen, Belgium), according to the manufacturer's instructions. Five independent extractions

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181 were performed for each population (GSS, VR-BE $_{bean}$ and VR-BE $_{tomato}$). VR-BE $_{bean}$ was sampled
182 a second time after six months using five replicates. The concentration and integrity of RNA
183 samples were assessed by a DeNovix DS-11 spectrophotometer (DeNovix, USA) and by running
184 a 2 µL aliquot on 1% and 2% agarose gel.

185 **2.6 RNA sequencing, mapping and principal component analysis (PCA)**

186 From all RNA samples, Illumina libraries were constructed using the NEBNext Ultra II RNA
187 Library Prep Kit for Illumina. Libraries were sequenced using Illumina NovaSeq6000 generating
188 an output of paired reads of 2 × 150 bp (library construction and sequencing was performed
189 at Genewiz (Germany)). The quality of the RNA reads was verified using FASTQC v.0.11.9
190 (Andrews, 2010) and reads that passed the quality control were aligned to the annotated *T.*
191 *urticae* three-chromosome genome assembly using the two-pass alignment mode of STAR
192 v.2.7.9a with a maximum intron size set to 20 kb (Dobin et al., 2013; Wybouw et al., 2019).
193 Resulting BAM files were sorted by chromosomal coordinate and indexed using SAMtools
194 v.1.11 (Li et al., 2009). HTSeq v.0.11.2 performed read-counting on a per-gene basis with the
195 default settings (Anders et al., 2015). The total read-counts per gene were used as an input
196 for the R-package (R v.4.2.0) DESeq2 v.1.36.0 to perform a PCA analysis; VR-BE $_{bean}$ month 1,
197 VR-BE $_{bean}$ month 6 (referring to samples collected six months apart), VR-BE $_{tomato}$ and GSS.
198 Read counts were normalized via the regularized-logarithm (rlog) transformation function of
199 the DESeq2 package. Using these values, a PCA was performed and plotted for the 5000 most
200 variable genes across all RNA samples using the DESeq2 function PlotPCA (Love et al., 2014).

201 **2.7 Identification of viral contaminants**

202 As a drastically lower number of reads of all VR-BE $_{bean}$ samples mapped against the *T. urticae*
203 genome, unmapped reads across all ten VR-BE $_{bean}$ samples were pooled and used as an input
204 for Trinity (v.2.13.2) to construct a *de novo* assembly under default conditions. An NCBI
205 BLASTn search against the non-redundant nucleotide collection database was performed
206 using a random subset of 100 unmapped reads in order to identify the most abundant
207 contaminants present in the RNA samples. Based on relative abundance of the best blast hits
208 with > 95% query cover and > 95% identities with the read, we could identify three main virus
209 species present, and their respective genomes were used for further analysis*;* *Tetranychus*
210 *urticae*-associated picorna-like virus 1 isolate Lisbon, complete genome (MK533157.1),

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211 *Tetranychus urticae*-associated dicistrovirus 1 isolate Lisbon, partial genome (MK533147.1);
212 *Aphis glycines* virus 1 isolate Lisbon, partial genome (MK533146.1). These viral genomes were
213 at their turn used for a BLASTn search against the *de novo* assembly to verify presence of the
214 full-length genomes. All reads that could not be mapped against the *T. urticae* genome were
215 mapped against the three viral genomes using the same methods as described above but
216 without setting the maximum intron size.

217 **2.8 Differential expression analysis and Gene ontology (GO) enrichment analysis**

218 Differential expression (DE) analysis was performed with DESeq2 v.1.36.0 using the total per-
219 gene read counts generated by HTSeq as input (Love et al., 2014). In first instance, gene
220 expression changes associated with different hosts compared to GSS as a reference was
221 assessed by identifying significantly differentially expressed genes (DEGs, Log_2 Fold Change
222 $|Log_2FC| > 1$, Benjamini-Hochberg adjusted p value < 0.05) in the VR-BE $_{bean}$ vs GSS and VR-BE
223 $_{tomato}$ vs GSS comparisons (Benjamini and Hochberg, 1995). From these lists of DEGs, subsets
224 of genes belonging to important detoxification families were made (Kurlovs et al., 2022). A
225 plot showing commonly overexpressed genes in both VR-BE $_{tomato}$ and VR-BE $_{bean}$ was produced
226 with the ggplot2 package v.3.3.6 (Wickham, 2009). To assess the intrinsic expression change
227 of the VR-BE population due to the host plant change from tomato to bean, the same method
228 was used to identify DEGs in the VR-BE $_{bean}$ vs VR-BE $_{tomato}$ comparison of which a volcano plot,
229 color coded by detoxifying gene family was made using ggplot2 package.

230 A gene ontology (GO) enrichment analysis was performed on the DEGs in the pairwise
231 comparisons between VR-BE $_{bean}$ and GSS and VRBE $_{tomato}$ and GSS using the R function
232 “enricher” from the package clusterProfiler (v.4.2.2). The GO terms for Biological Processes
233 (BP) and Molecular Functions (MF) were collected based on the *T. urticae* annotation (v
234 20190125) from the Orcae database (Sterck et al., 2012). Benjamini-Hochberg correction for
235 multiple testing was done by assigning the argument “pAdjustMethod = ‘BH’”.

236 **3 Results**

237 **3.1 VR-BE has a multi-resistant profile on bean and tomato hosts**

238 Toxicity bioassays revealed that in comparison to GSS, VR-BE $_{tomato}$ and VR-BE $_{bean}$ exhibited
239 resistance to all compounds, except for azadirachtin (Table 1). Resistance ratios ranged
240 between 28-8300 fold on tomato and 8.6-3900 fold on bean. Resistance to the mite growth

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241 inhibitor etoxazole was extremely high in populations on both hosts, with a RR of > 13000.
242 Comparing the LC_{50} values of VR-BE $_{tomato}$ and VR-BE $_{bean}$ (RR $_{host}$), clear differences could be
243 seen with the mitochondria complex II inhibitors cyflumetofen and cyenopyrafen (> 3-fold RR
244 $_{host}$), complex III inhibitors acequinocyl and bifenazate (~2 fold RR $_{host}$) and the acetyl-coA
245 carboxylase inhibitor spiromesifen (2-fold RR $_{host}$), suggesting a (strong) effect of the host plant
246 on acaricide toxicity.

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Table 1. Probit analysis of mortality data of 13 acaricides on GSS and VR-BE populations.

Acaricide	GSS susceptible strain			VR-BE $_{bean}$				VR-BE $_{tomato}$				
	Slope ± SE	LC_{50}[a] (95%CI)	χ^2 (df)[b]	Slope ± SE	LC_{50}[a] (95%CI)	χ^2 (df)[b]	RR[c] (95%CI)	Slope ± SE	LC_{50}[a] (95%CI)	χ^2 (df)[b]	RR[c] (95%CI)	RR^{d}_{host}
Abamectin	5.1 ± 0.73	0.76 (0.67 - 0.86)	30 (25)	2.3 ± 0.17	66 (55 - 79)	30 (21)	87 (73 - 100)	4.6 ± 0.76	91 (70 - 110)	32 (22)	120 (99 - 150)	1.4 (1.1-1.7)
Acequinocyl	3.4 ± 0.25	9.3 (8.3 - 10)	22 (18)	3.7 ± 0.28	240 (210 - 270)	24 (18)	26 (23 - 30)	1.8 ± 0.16	440 (360 - 560)	26 (18)	47 (39 - 58)	1.8 (1.5-2.2)
Azadirachtin	2.6 ± 0.21	120 (100 -140)	18 (18)	4.1 ± 0.64	120 (90 - 150)	28 (18)	1.0 (0.80 - 1.2)	4.0 ± 0.34	120 (110 -140)	19 (16)	1.0 (0.84 - 1.2)	1.0 (0.8-1.2)
Bifenazate	2.7 ± 0.41	2.2 (1.3 - 2.8)	27 (18)	0.83 ± 0.05	180 (130 - 250)	45 (33)	84 (59 - 120)	1.2 ± 0.11	440 (330 - 570)	36 (30)	200 (140 - 280)	2.4 (1.7-3.4)
Bifenthrin	1.4 ± 0.11	3.3 (2.7 - 4.1)	34 (26)	0.95 ± 0.08	360 (250 - 490)	26 (26)	110 (73 - 150)	0.83 ± 0.07	310 (210 - 440)	36 (30)	92 (63 - 130)	0.9 (0.5-1.4)
Cyenopyrafen	9.7 ± 1.2	0.64 (0.58 - 0.70)	20 (18)	1.8 ± 0.10	39 (34 - 45)	26 (25)	52 (44 - 62)	2.3 ± 0.23	170 (130 - 210)	32 (21)	230 (190 - 280)	4.4 (3.5-5.5)
Cyflumetofen	2.1 ± 0.17	6.2 (5.4 - 7.3)	16 (22)	3.5 ± 0.23	54 (48 - 59)	27 (22)	8.6 (7.2 – 10)	1.6 ± 0.16	180 (140 - 220)	14 (25)	28 (22 - 37)	3.3 (2.6-4.1)
Etoxazole	1.1 ± 0.18	0.38 (0.27 - 0.55)	5.3 (16)	1.1 ± 0.21	>5000		>13000	0.06 ± 0.05	>5000		>13000	
Fenbutatin oxide	2.7 ± 0.23	80 (68 - 93)	19 (18)	1.1 ± 0.21	>5000		> 63	1.1 ± 0.19	>5000		> 63	
Fenpyroximate	1.4 ± 0.15	61 (49 - 79)	13 (18)	1.5 ± 0.59	>5000		>82	0.65 ± 0.21	>5000		>82	
Pyridaben	3.2 ± 0.24	50 (46 - 56)	14 (18)	0.63 ± 0.38	>5000		>100	2.6 ± 0.41	>5000		>100	
Spiromesifen	3.4 ± 0.38	0.28 (0.22 - 0.33)	28 (18)	0.89 ± 0.13	1100 (460 - 1800)	33 (24)	3900 (2300-6700)	1.6 ± 0.23	2300 (1700 - 2800)	16 (18)	8300 (6300 - 11000)	2.1 (1.2-3.7)
Tebufenpyrad	2.8 ± 0.23	30 (27 - 33)	19 (30)	1.2 ± 0.17	>5000		>167	2.3 ± 0.66	>5000		>167	

[a] LC_{50} is expressed in mg active ingredient L^{-1}

[b] χ^2 is the Chi square goodness of fit value and (df) is the degrees of freedom

[c] Resistance ratio = LC_{50} VR-BE/ LC_{50} GSS

[d] Resistance ratio host = LC_{50} VR-BE $_{tomato}$ / LC_{50} VR-BE $_{bean}$

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251 ## 3.2 Target-site resistance mutations of VR-BE

252 PCR screening of VR-BE $_{tomato}$ upon establishment in the laboratory revealed the presence of
253 several known target site resistance mutations (Table 2, see Table S1 for all target-site
254 mutations screened). Specifically, the I1017F substitution in chitin synthase 1, which is
255 associated with high resistance to mite growth inhibitors (Demaeght et al., 2014; Van Leeuwen
256 et al., 2012), was fixed. Similarly, the acetylcholinesterase mutation F331W associated with
257 resistance to organophosphates and carbamates (Anazawa et al., 2003; Khajehali et al., 2010;
258 Kwon et al., 2010); the PSST homologue mutation H92R, associated with high resistance to
259 METI-I acaricides (Xue et al., 2022); the ATP synthase mutation V89A, associated with
260 resistance to fenbutatin oxide (De Beer et al., 2022b) were also found to be fixed in the 200
261 mites sampled from VR-BE $_{tomato}$. The recently identified abamectin resistance mutation I321T
262 (Xue et al., 2020) found in subunit 3 of the glutamate chloride channel was segregating in the
263 population. The screening did not reveal any known or novel candidate non-synonymous
264 resistance mutations in the voltage gated sodium channel, mitochondrial succinate
265 dehydrogenase subunits (complex II) and acetyl-CoA carboxylase. After one year on bean,
266 estimated allele frequencies of the fixed mutations I1017F, F331W, H92R and V89A remained
267 at 100%, while allele frequency of the segregating I321T mutation decreased from 50% to
268 20% (Table 2).

269 **Table 2.** Target site mutations identified in VR-BE.

Target gene	Substitution	Frequency (%)		Status	Compounds
		Initial	After one year		
tetur03g08510 (CHS1)[a]	I1017F	100	100	Fixed	Etoxazole, clofentezine, hexythiazox
tetur10g03090 (GluCl3)[a]	I321T	50	20	Segregating	Abamectin
tetur06g03780 (ATP synthase)[a]	V89A	100	100	Fixed	Fenbutatin oxide
tetur07g05240 (PSST)[b]	H92R	100	100	Fixed	Fenpyroximate, pyridaben, tebufenpyrad
tetur19g00850 (AChE)[c]	F331W	100	100	Fixed	Organophosphates

[a] Numbering of the substitution according to the reference species *Tetranychus urticae*

[b] Numbering of the substitution according to the reference species *Yarrowia lipolytica*

[c] Numbering of the substitution according to the reference species *Torpedo californica*

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271 **3.3 PBO synergizes cyflumetofen toxicity on VR-BE $_{tomato}$**

272 To assess whether metabolic detoxification, in particular cytochrome P450 metabolism, could
273 be at least partially responsible for the observed decrease in sensitivity to some acaricides in
274 VR-BE $_{tomato}$ relative to VR-BE $_{bean}$, synergism assays with PBO were carried out. Pre-treatment
275 with PBO enhanced the toxicity of cyflumetofen by 3-fold in VR-BE $_{tomato}$ but not in VR-BE $_{bean}$
276 (Table 3), suggesting the increased metabolic detoxification by P450s of cyflumetofen in the
277 population on tomato.

278 **Table 3.** Probit mortality of cyflumetofen in the VR-BE populations after pretreatment with synergist PBO

Population	Treatment	Slope ± SE	LC_{50} [a] (95% CI)	χ^2 [b] (df)	SR [c] (95% CI)
VR-BE$_{bean}$	Cyflumetofen	3.5 ± 0.23	54 (48-59)	27 (22)	
	PBO + cyflumetofen	3.8 ± 0.33	45 (39 - 50)	25 (18)	1.2 (1.0 - 1.3)
VR-BE$_{tomato}$	Cyflumetofen	1.6 ± 0.16	180 (140 - 220)	14 (25)	
	PBO + cyflumetofen	6.2 ± 0.75	52 (46 - 58)	28 (18)	3.4 (2.7 - 4.2)

[a] LC_{50} is expressed in mg active ingredient L^{-1}

[b] χ^2 is the Chi square goodness of fit value and (df) is the degrees of freedom

[c] Synergism ratio = LC_{50} without PBO treatment/ LC_{50} after PBO treatment

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280 **3.4 RNA sequencing reveals presence of viruses in VR-BE $_{bean}$ and GSS populations**

281 Illumina sequencing resulted on average in approximately 30 million paired reads per sample
282 (raw reads were deposited on NCBI SRA database under BioProject PRJNA1006202, will be
283 provided upon acceptance). Alignment of RNA-seq reads against the *T. urticae* annotation
284 resulted in an overall mapping rate of uniquely mapped reads of 73.59% for VR-BE $_{tomato}$,
285 64.66% for GSS and 46.27% for VR-BE $_{bean}$ (Table S2). Noteworthy, a large fraction of the reads
286 for VR-BE $_{bean}$ (~ 40%) and GSS (~ 18%) did not map against the *T. urticae* genome which hints
287 towards a potential contamination. An NCBI BLASTn search of a random subset of the
288 unmapped reads was performed in order to identify the most abundant contaminants present
289 in the RNA samples which identified three virus species; *Tetranychus urticae*-associated
290 picorna-like virus 1 isolate Lisbon, complete genome (MK533157.1), *Tetranychus urticae*-
291 associated dicistrovirus 1 isolate Lisbon, partial genome (MK533147.1) and *Aphis glycines*
292 virus 1 isolate Lisbon, partial genome (MK533146.1) as the main reason for the lower mapping
293 rates against the *T. urticae* genome. Next, a *de novo* transcriptome assembly of the unmapped
294 reads from VR-BE $_{bean}$ was built under default conditions and a BLASTn search of the viral

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295 genomes against the *de novo* assembly identified multiple contigs of > 4 kb that give hits with
296 > 95% identity for each of the three viral genomes studied. For the *Tetranychus urticae*-
297 associated dicistrovirus 1 isolate Lisbon, partial genome there is even a contig spanning the
298 genome (8290 bp) with 96% identities and with an additional 564 bp in the assembly
299 (Supplementary file 1). All reads that did not map against the *T. urticae* genome in both VR-
300 BE $_{bean}$ and GSS samples were then mapped against the three viral genomes to estimate the
301 relative abundance of each virus species (Figure 1, Table S2). Interestingly, the largest fraction
302 of reads mapped against *Tetranychus urticae*-associated picorna-like virus 1 for VR-BE $_{bean}$,
303 whereas for GSS the largest fraction mapped against *Tetranychus urticae*-associated
304 dicistrovirus 1. *Aphis glycines* virus 1 was only present in VR-BE $_{bean}$ and none of the identified
305 viruses were present in VR-BE $_{tomato.}$ Re-sampling and sequencing of the VR-BE $_{bean}$ population
306 six months after arrival in the laboratory and transfer to bean yielded a similar result, with a
307 44.64% uniquely mapped reads matching to the three viruses identified in the first sequencing
308 (Table S2).

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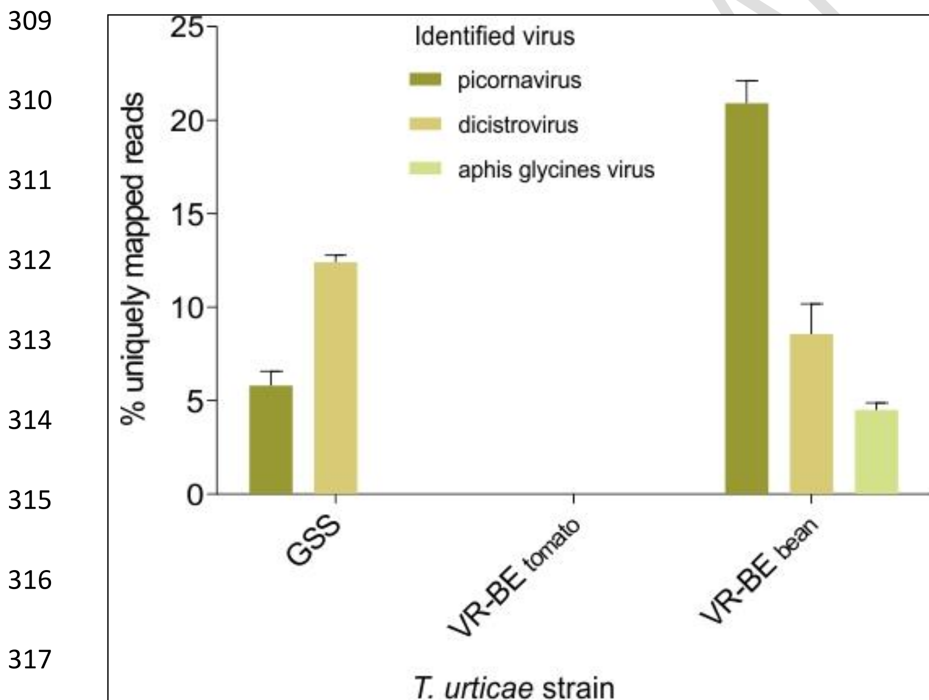

318

319 **Figure 1.** Percentage frequency of uniquely mapped reads. All genes in VR-BE $_{bean}$ that could not be mapped to the *T. urticae*
320 transcriptome were mapped to three viral genomes: picornavirus which was the highest contaminant, followed by the
321 dicistrovirus and aphis glycines virus. GSS was also contaminated with picornavirus and dicistrovirus while VR-BE $_{tomato}$ was
322 not contaminated with the viruses. Error bars represent standard error of the mean. N=5 for GSS and N= 10 for VR-BE $_{bean}$.

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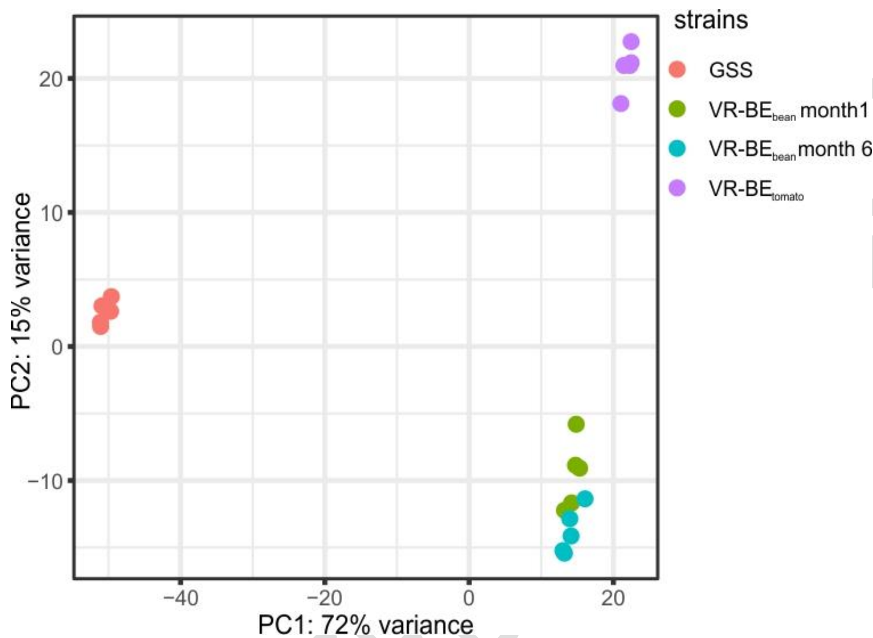
324 **3.5 Effect of the host plant on gene expression**

325 Principal component analysis revealed that 72% of the total variation could be explained by
326 principal component 1 (PC1) while 15% could be explained by PC2 (Figure 2). Replicates
327 clustered by population and the groups were clearly separated from each other. The two
328 batches of VR-BE $_{bean}$ samples collected six months apart clustered together on PC1. VR-BE
329 $_{tomato}$ was positioned far away from VR-BE $_{bean}$ on PC1, which clearly indicates the dramatic
330 effect the host plant has on gene expression.



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340 **Figure 2.** Principal component analysis (PCA) of gene expression among GSS, VR-BE $_{bean}$ and VR-BE $_{tomato}$ populations of *T.*
341 *urticae*.

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343 Gene expression patterns in the resistant VR-BE populations were compared to the
344 susceptible GSS. Differential expression analysis identified 1423 upregulated genes in VR-BE
345 $_{bean}$ population compared to GSS, and 80% of these genes (1145 genes) overlapped with VR-
346 BE $_{tomato}$ vs GSS comparison (Figure 3a, Table S3). A total of 1693 genes were downregulated
347 in VR-BE $_{bean}$ vs GSS, with 82% of these genes (1392 genes) overlapping with the VR-BE $_{tomato}$
348 vs GSS comparison. 301 genes were overexpressed specifically in the VR-BE $_{tomato\ vs}$ GSS
349 comparison only, while 650 genes were downregulated in this comparison only (Figure 3a,
350 Table S3). The overexpression plot shown in Figure 3a clearly indicates that multiple gene
351 families that have been implicated in detoxification (CYPs, CCEs, GSTs, UGTs and DOGs) or

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352 transport of xenobiotics (MFS and ABCs) were amongst the overexpressed genes in both
353 comparisons, with CYPs showing the highest Log_2 fold changes. Moreover, a gene ontology
354 (GO) enrichment analysis revealed a statistically significant (p adj < 0.05) enrichment of GO
355 terms associated with detoxification and metabolic processes. Results in Table S4 show
356 several GO terms associated with cytochrome P450s amongst the enriched GO terms with
357 highest significance in both comparisons (e.g. GO:0055114 "*oxidation-reduction process*";
358 GO:0016705 "*oxidoreductase activity, acting on paired donors, with incorporation or reduction*
359 *of molecular oxygen*"; GO:0020037 "*heme binding*"; GO:0005506 "*iron ion binding*"). To
360 identify the genes differentially expressed in the population on bean versus tomato, gene
361 expression patterns in VR-BE $_{tomato}$ were directly compared to VR-BE $_{bean}$. Similar to the
362 comparison with GSS, multiple detoxification genes and transporters were differentially
363 expressed in VR-BE $_{tomato}$ vs VR-BE $_{bean}$ (Figure 3b). Overexpressed detoxification genes and
364 transporters with a Log_2FC of ≥2 in VR-BE $_{tomato}$ vs VR-BE $_{bean}$ are shown in (Table 4).

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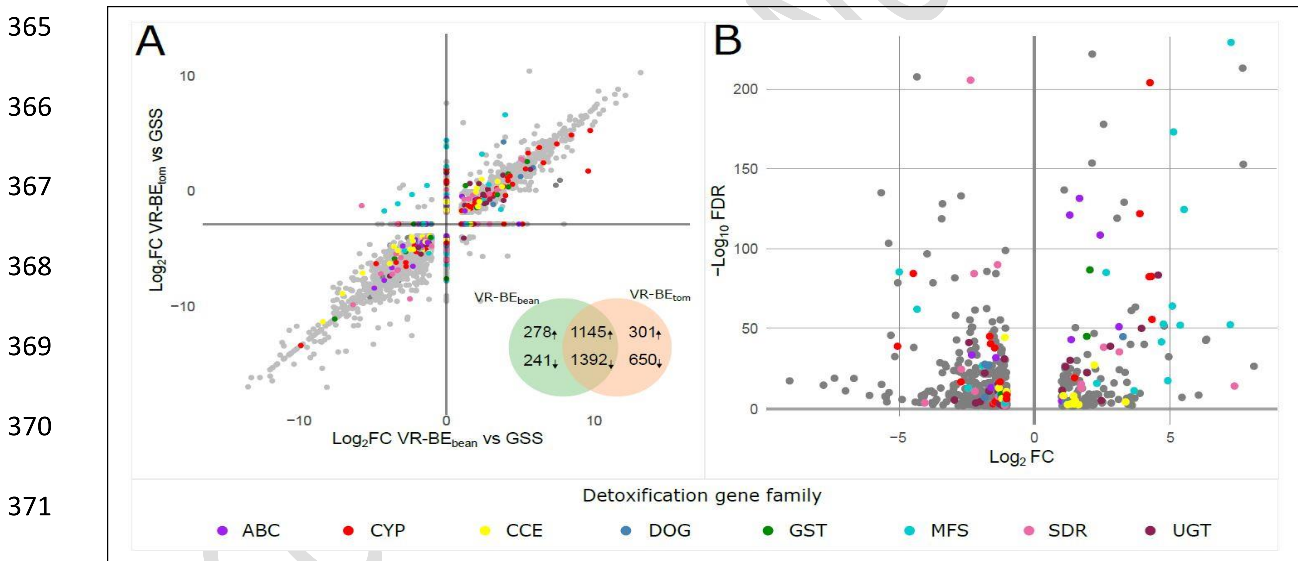

372

373 **Figure 3.** Overview of differentially expressed genes (|Log_2 FC| ≥ 1.0, p adj < 0.05). A) Overexpression plot of differentially
374 expressed genes in VR-BE $_{bean}$ and VR-BE $_{tomato}$ compared to the susceptible GSS; Venn diagram depicting overlap among
375 differentially expressed genes from VR-BE $_{bean}$ vs GSS and VR-BE $_{tomato}$ vs GSS comparisons. B) A volcano plot of the
376 differentially expressed genes in a pairwise comparison of the VR-BE $_{tomato}$ vs VR-BE $_{bean}$. Genes known to be implicated in
377 detoxification and xenobiotic transport are shown in colours in the two plots: red, cytochrome P450 monooxygenases (CYPs);
378 yellow, carboxyl choline esterases (CCEs); dark blue, intradiol ring cleavage dioxygenases (DOGs); green, glutathione-s-
379 transferases (GSTs); blue, major facilitator superfamily transporters (MFS); pink, short chain dehydrogenases/reductases
380 (SDRs); maroon, UDP-glycosyl transferases (UGTs); and purple, ATP-binding cassette transporters (ABC transporters).

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382 **Table 4.** List of highly overexpressed (Log_2FC ≥ 2) detoxification genes and transporters in VR-BE $_{tomato}$ compared to VR-BE
383 $_{bean}$

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tetur ID	Description	Log_2 FC
tetur11g05520	*CYP385C4*	4.2
tetur20g00290	*CYP392B3*	4.3
tetur11g05540	*CYP385C3*	3.9
tetur03g02810	*CCEincTu04*	3.3
tetur11g05760	*TuCCE34*	2.2
tetur05g00060	*UGT20*	4.5
tetur22g00270	*UGT59*	3.9
tetur13g04550	*TuDOG11*	3.3
tetur29g00220	*TuGSTd14*	2.0
tetur28g01720	SDR	2.5
tetur46g00180	MFS	4.7
tetur40g00030	MFS	3.7
tetur03g09800	*TuABCC-10*	3.1
tetur03g09880	*TuABCC-11*	2.4

394

395 **4 Discussion**

396 In this study, we characterized VR-BE, a *T. urticae* field population collected from a tomato
397 greenhouse in Belgium. The grower had reported loss of efficacy after treatment with
398 commercial formulations of abamectin, hexythiazox, bifenazate, spirodiclofen and
399 cyflumetofen, and the crop was lost to spider mites. Occasionally, low efficacy of acaricides
400 results from operational factors such as incorrect spray technique, the use of tank mixes or
401 inappropriate application time (Khajehali et al., 2011). We therefore first confirmed resistance
402 in the laboratory with toxicity bioassays with acaricides of different mode of action groups,
403 revealing that VR-BE was indeed resistant to almost all acaricides tested.

404 Resistance to a mite growth inhibitor etoxazole and the METI-I acaricides pyridaben,
405 tebufenpyrad and fenpyroximate may be fully explained by the target site mutations I1017F
406 and H92R respectively, which were fixed in VR-BE. However, other mechanisms might also
407 contribute to the high cross-resistance observed with METI-Is. High resolution QTL mapping
408 in *T. urticae* has revealed that cross resistance to METI-Is is not only associated with the target-
409 site resistance mutation, but also possibly cytochrome P450 metabolism (Snoeck et al., 2019).
410 Validation of the mutation in *T. urticae* by marker assisted back-crossing indeed revealed that
411 the mutation alone only contributes up to 22-fold resistance to fenpyroximate and 30-60 fold

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412 resistance to tebufenpyrad and pyridaben (Bajda et al., 2017). A study with resistant field
413 populations has also revealed that additive or synergistic effects of multiple mechanisms most
414 likely determine the phenotypic strength (Xue et al., 2022). As CYPs were among the most
415 highly overexpressed detoxification genes in both VR-BE populations, they might be
416 contributing to the extremely high resistance levels to METI-Is in addition to the H92R
417 mutation.

418 High resistance to the mitochondrial ATP synthase inhibitor (fenbutation oxide) was recorded
419 in both VR-BE populations, and can be attributed to the fixed mutation V89A but also to
420 metabolic detoxification by CYPs. Using QTL mapping, De Beer et al., (2022b) recently
421 characterized resistance to fenbutatin oxide, revealing that high resistance is likely achieved
422 by a combination of V89A and metabolic detoxification by the P450s *CYP392E4* and *CYP392E6*,
423 which were overexpressed in both VR-BE populations. Similarly, resistance to abamectin may
424 be attributed to I321T mutation in the GluCl3 subunit, which we identified in VR-BE. Since the
425 mutation was not fixed in VR-BE, additional mechanisms might contribute to the observed
426 abamectin resistance. Previous studies have indicated that detoxification enzymes, especially
427 CYPs and UGTs are also involved in abamectin resistance (Çağatay et al., 2018; Riga et al.,
428 2014; Xue et al., 2020). Specifically, functionally expressed CYP392A16 has been shown to
429 hydroxylate abamectin *in vitro* (Riga et al., 2014), but was only moderately overexpressed in
430 both VR-BE populations (~1.6-fold in VR-BE $_{bean}$ and ∼1.4-fold in VR-BE $_{tomato}$) and therefore
431 other P450s might be involved. Similarly, recombinant UGT10 (*tetur02g09830*) and UGT29
432 (*tetur05g05060*) enzymes have been shown to glycosylate abamectin *in vitro* (Xue et al.,
433 2020). The genes encoding these enzymes were also overexpressed in both VR-BE populations
434 (2-fold in VR-BE $_{tomato}$ for both UGTs, 2-fold for UGT10 and 3.8-fold for UGT29 in the VR-BE
435 $_{bean}$), and might be contributing to resistance.

436 The high levels of resistance to bifenthrin and spiromesifen can likely be exclusively attributed
437 to metabolic detoxification as no target-site mutations were identified in the target sites of
438 these acaricides. De Beer et al., (2022a) recently showed that recombinant CCEinc18 could
439 metabolize bifenthrin, and UGT10 could glycosylate bifenthrin-alcohol. The genes encoding
440 these two enzymes were overexpressed in both VR-BE populations (2-fold in VR-BE $_{tomato}$ for
441 both genes, 1.5-fold for CCEinc18 and 2-fold for UGT10 in VR-BE $_{bean}$), and potentially
442 contribute to the observed resistance. Previous studies have shown that the P450 enzyme

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CYP392E10 can metabolize spirodiclofen and spiromesifen (Demaeght et al., 2013), but *CYP392E10* was not among the differentially expressed P450s in our study, suggesting that other mechanisms might be responsible for the observed resistance. Synergism studies have indicated that, in addition to CYPs, CCEs also play an important role in resistance to tetronic/tetramic acid derivatives (İnak et al., 2022; Van Pottelberge et al., 2009b; Wei et al., 2020). Several CCEs were overexpressed in both VR-BE populations (*TuCCE48, TuCCE42, TuCCE04, TuCCE71, TuCCE05, TuCCE49, TuCCE33, TuCCE50* and *TuCCE27*) and might play a role in spiromesifen resistance.

We did not identify any mutation in *cytb* in spite of the moderate resistance recorded with acequinocyl and bifenazate. Although most often associated with maternal inheritance and point mutations in *cytb*, some genetic studies in combination with genome-wide gene expression analysis have revealed that acequinocyl and bifenazate resistance can also have a polygenic inheritance pattern, involving both mutations in the mitochondrial *cytb* gene and overexpression of detoxification genes, especially CYPs (Lu et al., 2023). The involvement of P450-based increased detoxification is further supported by strong synergism with the P450 inhibitor piperonyl butoxide (Sugimoto and Osakabe, 2019). Functionally expressed CYP392A11 has been shown to metabolize bifenazate (Lu et al., 2023), but this P450 was downregulated in both VR-BE populations, likely suggesting alternative mechanisms.

VR-BE also showed moderate resistance to cyflumetofen and cross-resistance to cyenopyrafen, which is not yet registered in Europe. None of the previously reported resistance mutations were detected in VR-BE (mutations reviewed in De Rouck et al., 2023). In contrast, synergism assays with PBO indicated the involvement of P450 detoxification in cyenopyrafen resistance, which is in line with previous studies (Khalighi et al., 2014, 2015; Riga et al., 2015). Functional expression studies have shown that at least CYP392A11 can hydroxylate cyenopyrafen (Riga et al., 2015). However, in VR-BE *CYP392A11* was downregulated versus GSS, and no significant expression differences between hosts were detected. Other P450s from the 392A subfamily including *CYP392A14, CYP392A9, CYP392A13, CYP392A15* and *CYP392A10*, next to *CYP392D8* and *CYP392D7,* were highly expressed in both VR-BE populations and should be functionally characterized to understand their role in resistance. In addition, TuGST05, a GST enzyme shown to metabolize cyflumetofen (Pavlidi et al., 2017) was not overexpressed in VR-BE, but other GSTs were highly overexpressed in VR-

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474 BE on both hosts. These include: *TuGSTd08* (5.5-fold on both hosts), *TuGSTd12* (4.2-fold on
475 bean and 3.2-fold on tomato), *TuGSTd10* (3.5-fold on bean and 2.5-fold on tomato), and
476 *TuGSTd14* (1.3-fold on bean and 3.3-fold on tomato). The functional role of these GSTs should
477 be further investigated.

478 Although both VR-BE $_{tomato}$ and VR-BE $_{bean}$ were highly resistant to acaricides of different MOA
479 groups, we still observed a strikingly decreased toxicity for six acaricides in the tomato
480 population compared to bean. A caveat of the population comparisons within this study is the
481 lack of replication, since drift and other factors might result in different responses to
482 acaricides. Since specifically the bean population (and not tomato) was contaminated with
483 viruses, we considered the possibility that the presence of viruses might have an influence on
484 acaricide toxicity. Previous studies have identified the presence of viruses in arthropods
485 (Berman et al., 2023; Niu et al., 2019; Wu et al., 2020). Even so, the presence of such large
486 amounts of viral RNA reads has not been reported in previous RNA sequencing datasets of *T.*
487 *urticae* (De Beer et al., 2022a, 2022b; Fotoukkiaii et al., 2021; Kurlovs et al., 2022; Lu et al.,
488 2023). As such, whether infection occurred by chance or is related to the host plant shift,
489 remains unclear and little is known on how these viruses interact with their hosts. However,
490 all viruses require the hosts machinery to be able to synthesize viral proteins. Indeed, some
491 RNA viruses such as dicistroviridae have evolved elegant strategies to hijack the hosts
492 ribosome (Warsaba et al., 2019). By redirecting the hosts translation machinery, the entire
493 cellular response to stress is compromised, which can include the response to xenobiotic
494 stress. However, synergism assays showed that piperonyl butoxide (PBO), a P450 inhibitor,
495 synergized cyflumetofen toxicity in the tomato population and had no significant effect on the
496 bean population, suggesting that the increased resistance in VR-BE $_{tomato}$ is more likely due to
497 increased detoxification, even though the VR-BE populations were not replicated on both
498 hosts. Indeed, detoxification gene response with host change between bean and tomato has
499 previously been associated with altered acaricide toxicity in *T. urticae* (Dermauw et al., 2013).
500 Moreover, the fact that cyenopyrafen and cyflumetofen are vulnerable to metabolic attack
501 was indeed already documented in a previous study, where some multi-resistant field
502 populations of *T. urticae* showed cross-resistance to cyenopyrafen and cyflumetofen, without
503 prior to exposure to these compounds in the field. Cyenopyrafen cross-resistance was
504 specifically linked to the overexpression of P450s (Khalighi et al., 2015, 2014). Interestingly, of

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all P450s differentially expressed in VR-BE in comparison to GSS, only *CYP392B3, CYP385C3 and CYP385C4* were specially upregulated in the VR-BE $_{tomato}$ vs VR-BE $_{bean}$ comparison. These P450s were indeed also previously shown to be induced upon mite transfer from bean to tomato (Wybouw et al., 2015). Despite the fact that there is no functional validation at present, these P450s might further elevate resistance levels to complex II inhibitors conferred by other P450s.

Because of the effect of the host on detoxification enzyme activity and acaricide toxicity, we also including a plant derived acaricide containing azadirachtin in toxicity bioassays. Surprisingly, this was the only compound effective on both populations, suggesting that secondary plant metabolites are not necessarily more vulnerable to metabolic attack by higher detoxification associated with different host plants in *T. urticae*.

It has been proposed that, due to their ability to cope with diverse plant defense chemicals encountered during feeding, generalist herbivores such as *T. urticae* are pre-adapted to evolve pesticide resistance (Alyokhin and Chen, 2017; Dermauw et al., 2013). But, resistance is also known to mainly result from a strong selection imposed by intensive pesticide use, and the relative importance of the evolutionary history associated with polyphagy on resistance development is still a matter of debate (Dermauw et al., 2018). Dermauw et al., (2013) observed highly coordinated changes in gene expression for many genes in tomato-adapted mites and in pesticide-resistant strains, suggesting that adaptation to tomato would also increase tolerance to pesticides. In the current study, a multi-resistant field population of *T. urticae* showed remarkable differences in gene expression when maintained on tomato or bean, and toxicity of some acaricides was reduced in the population on tomato. Similar to Dermauw et al., (2013), most of the differentially expressed genes belonged to gene families that have been commonly implicated in detoxification (CCEs, P450s, GSTs and UGTs) or xenobiotic transportation (ABC transporters). This shows that the host plant influences gene expression in *T. urticae*, and these host-specific changes in transcript levels of detoxification enzymes influence acaricide toxicity and resistance levels. This observation is further supported by synergism assays, where we show that inhibiting P450s in the tomato population increases toxicity of cyflumetofen, reaching the same level of toxicity as the bean population. Host plant responses have also been shown to affect the toxicity of insecticides to insects. In relation to this, a study with the polyphagous whitefly *Trialeurodes vaporariorum* revealed

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considerable differences in transcriptional responses to various host plants, and these changes in gene expression were associated with significant shifts in tolerance of the host-adapted *T. vaporariorum* lines to pesticides (Pym et al., 2019). Additionally, the role of detoxification enzymes in pesticide resistance and tolerance to plant allelochemicals is well established in insects and mites (Dermauw and Van Leeuwen, 2014; Després et al., 2007; Feyereisen et al., 2015; Heidel-Fischer and Vogel, 2015), especially the functionally diverse P450s which are expressed in response to phytochemicals (Vandenhole et al., 2021), and whose role in detoxification of xenobiotics has been widely studied (Feyereisen, 2012; Nauen et al., 2021).

Similar to Dermauw et al., (2013), we observed strong differential expression of genes not previously implicated in detoxification. These included lipocalins, small extracellular proteins with the ability to bind hydrophobic molecules (Ahnström et al., 2007; Flower et al., 2000). Therefore, they may bind acaricides or plant toxins, resulting in sequestration of these normally hydrophobic molecules (Dermauw et al., 2013). Genes belonging to the major facilitator superfamily (MFS) were especially highly upregulated in the VR-BE $_{\text{tomato}}$ compared to the VR-BE $_{\text{bean}}$. Upregulation of these single polypeptide carriers might result in a higher efflux of acaricides or toxic plant metabolites out of spider mite cells as previously suggested by Dermauw et al., (2013). Additionally, two intradiol ring cleavage dioxygenases (DOGs): *TuDOG1* (*tetur01g00490*) and *TuDOG11* (*tetur13g04550)* were upregulated in VR-BE $_{\text{tomato}}$ relative to VR-BE $_{\text{bean}}$ (1.6-fold and 7-fold respectively). TuDOG11 has recently been shown to detoxify the tomato metabolites caffeic acid and chlorogenic acid (Njiru et al., 2022), and is therefore important in adaptation to tomato. We also observed upregulation of two transcription factors *tetur07g01800* and *tetur36g00260*. The latter belongs to the nuclear receptors family, that is known to be involved in response to stress and xenobiotics in vertebrates and insects (Misra et al., 2011; Pascussi et al., 2008). The two transcription factors were also upregulated in resistant strains and upon adaptation to tomato in Dermauw et al., (2013), and could be playing a role in regulation of gene expression in response to plant allelochemicals or acaricides. Indeed, a recent study quantifying the extent of *cis-* versus *trans-*regulation on a genome-wide basis in a collection of multi-resistant *T. urticae* strains revealed that trans-effects are most abundant, especially for P450s and DOGs (Kurlovs et al., 2022).

566 To conclude, we confirmed that field failure of a tomato crop to spider mites was due to high
567 levels of resistance to all tested registered acaricides. The presence of target-site mutations
568 could explain resistance to some acaricides, but not all. In addition, resistance levels differed
569 between the population kept on bean or on tomato. This was likely not associated with the
570 presence of large amount of virus in the bean population, but with the induction of
571 detoxification genes on tomato. Further, RNA sequencing revealed large transcriptional
572 differences between the population grown on bean or on tomato, and P450s were shown to
573 contribute to increased resistance levels on tomato.

574 **Acknowledgement**

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580 the experimental period.

581 **Declaration of competing interest**

582 The authors declare that they have no competing interests.

583 **Supplementary information**

584 All supplementary data can be found at https://doi.org/10.1016/j.pestbp.2023.105591

585 Supplementary file S1. Partial genome sequence of *Tetranychus urticae* associated dicistrovirus 1 isolate Belgium.

586 Supplementary Table S1. An overview of target site mutations that the VR-BE population was screened for.

587 Supplementary Table S2. Percentage number of uniquely mapped reads and unmapped reads after RNA sequencing.

588 Supplementary Table S3. Normalized read-counts of all samples and Log_2 fold changes of all differentially expressed genes in
589 all studied comparisons.

590 Supplementary Table S4. Significantly enriched GO terms in VR-BE bean and tomato populations in comparison to GSS.

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