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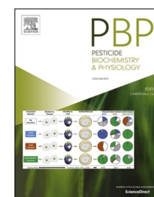

The role of the *Bemisia tabaci* and *Trialeurodes vaporariorum* cytochrome-P450 clade *CYP6DPx* in resistance to nicotine and neonicotinoids

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ABSTRACT

The alkaloid, nicotine, produced by tobacco and other Solanaceae as an anti-herbivore defence chemical is one of the most toxic natural insecticides in nature. However, some insects, such as the whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* show strong tolerance to this allelochemical and can utilise tobacco as a host. Here, we used biological, molecular and functional approaches to investigate the role of cytochrome P450 enzymes in nicotine tolerance in *T. vaporariorum* and *B. tabaci*. Insecticide bioassays revealed that feeding on tobacco resulted in strong induced tolerance to nicotine in both species. Transcriptome profiling of both species reared on tobacco and bean hosts revealed profound differences in the transcriptional response these host plants. Interrogation of the expression of P450 genes in the host-adapted lines revealed that P450 genes belonging to the CYP6DP subfamily are strongly upregulated in lines reared on tobacco. Functional characterisation of these P450s revealed that *CYP6DP1* and *CYP6DP2* of *T. vaporariorum* and *CYP6DP3* of *B. tabaci* confer resistance to nicotine *in vivo*. These three genes, in addition to the *B. tabaci* P450 *CYP6DP5*, were also found to confer resistance to the neonicotinoid imidacloprid. Our data provide new insight into the molecular basis of nicotine resistance in insects and illustrates how divergence in the evolution of P450 genes in this subfamily in whiteflies may have impacted the extent to which different species can tolerate a potent natural insecticide.

1. Introduction

Herbivory in insects is thought to have evolved over 300 million years ago, with insect herbivores accounting for approximately a quarter of all described eukaryotic species (Bernays, 1998; War et al., 2018). The close association between plants and insects has led to diversification and speciation of both phytophagous insects and their hosts (Mitter et al., 1988; Ramos and Schiestl, 2019). To prevent insect damage, potential host plants often produce defensive compounds which can reduce pest fitness, recruit natural enemies and influence behaviour (Inbar and Gerling, 2008). These secondary metabolites may be constitutively expressed and/or upregulated upon herbivory (Paré and Tumlinson, 1999), and are highly diverse encompassing alkaloids, phenolics, glucosinolates, steroids, terpenoids, glucosides and polypeptides and polypeptides (Mithöfer and Boland, 2012).

In response to host plant defences, herbivorous insects have evolved varying mechanisms to circumvent, detoxify and/or excrete these toxic compounds (Alyokhin and Chen, 2017). These evolutionary adaptations most commonly involve either hard-wired genetic changes that provide constitutive defence against host plant toxins, or induced mechanisms that are mobilised as required. In the case of the former, genetic adaptations can allow the insect herbivore to detoxify or sequester defensive compounds more efficiently. A well characterised example of this is in adaption of the aphid *Myzus persicae* to tobacco (Bass et al., 2013). Transcriptomic and genomic analysis of tobacco-adapted and non-adapted subspecies of *M. persicae* uncovered a remarkable series of

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mutations in the tobacco-adapted subspecies that led to dramatic qualitative and quantitative changes in the expression of the cytochrome P450 *CYP6CY3* (Bass et al., 2013). This allowed this subspecies to efficiently detoxify nicotine, the potent natural insecticide produced by tobacco efficiently overcoming this anti-herbivore defence chemical. Other genomic signatures associated with host plant feeding transitions, such as gene gain and loss have been identified in insects (Gloss et al., 2019; Xue et al., 2014), with such alterations generally associated with longer term adaptation.

Upon transition to a novel host, insects may also rely on large scale changes in gene expression to enable them to adapt to the new environment, a process known as 'transcriptional plasticity'. This mechanism typically results in greater insect fitness on the new host (Yu, 1986), and is more strongly associated with generalist rather than specialist herbivores (Birnbaum and Abbot, 2020). Many of the genes which allow herbivores to rapidly adapt to the host plant are detoxification enzymes, capable of directly metabolising or sequestering toxic compounds (Heidel-Fischer and Vogel, 2015). Of these detoxification enzymes, cytochrome P450 monooxygenases (P450s) constitute the largest and most functionally diverse superfamily (Li et al., 2007). The CYP3 clade of P450s has been frequently implicated in detoxification of a wide range of plant secondary metabolites including alkaloids and furanocoumarins (Mao et al., 2006; Snyder and Glendinning, 1996; Feyereisen, 1999).

Importantly, insect adaption to plant secondary metabolites by either constitutive or induced mechanisms can also lead to a reduction in sensitivity to synthetic insecticides, as the enzymes that metabolise natural plant compounds may also have the capacity to metabolise synthetic insecticides (Pym et al., 2019; Bass et al., 2013; Dermauw et al., 2013; Gould et al., 1982). This is known as pre-adaptation (Gordon, 1961) and may be more common in generalist herbivores. For example, the overexpression of *CYP6CY3* in tobacco-adapted *M. persicae,* confers resistance to both nicotine and several neonicotinoid insecticides (Troczka et al., 2021; Puinean et al., 2010; Bass et al., 2013). Similarly, work on the two-spotted spider mite identified an increased tolerance to acaricides in response to tomato feeding and this was associated with the upregulation of genes encoding detoxification enzymes (Dermauw et al., 2013).

Another family of generalist herbivores is that of the Aleyrodoidea or whiteflies. Many of these Hemipterans are global crop pests with both nymph and adult stages feeding on a wide variety of crops leading to substantial reductions in yield (Lloyd, 1922). Whiteflies cause plant damage by feeding on the phloem of hosts, depriving them of nutrients and acting as vectors for >100 different plant viruses (Byrne and Bellows, 1991). The two most economically important species of whiteflies worldwide, and the focus of the current study, are the greenhouse whitefly, *Trialeurodes vaporariorum*, and the tobacco whitefly, *Bemisia tabaci*. Both species are highly polyphagous, cause large scale agricultural damage and are among the world's most destructive insect pest species (Choi et al., 2003; Lowe et al., 2000). The economic importance of these species, results, in part, from their ability to feed on a wide range of host plants (Van Lenteren and Noldus, 1990), many considered hostile to herbivorous insects. One such hostile host on which both species are able to feed is the tobacco plant *Nicotiana tabacum* which forms part of the family *Solanaceae* or nightshades. The members of this plant family produce a wide range of alkaloids, glycoalkaloids, terpenoids, organic acids and alcohols (Chowański et al., 2016). The most well-known of these toxic allelochemicals is nicotine, which exhibits high insecticidal activity due to its ability to mimic acetylcholine and intensify synaptic transmission (Chowański et al., 2016). The toxic chemistry of nicotine has led to its use as a natural insecticide (Tomizawa and Casida, 2005) although, due to its lack of host specificity and high mammalian toxicity, it is not widely-used today (Tomizawa and Casida, 2005).

Intriguingly, previous research has identified a difference in susceptibility to nicotine between *T. vaporariorum* and *B. tabaci.* Despite its common name, the tobacco whitefly, *B. tabaci* has been recorded as ~10-fold more susceptible to nicotine than *T. vaporariorum* (Gorman et al., 2002). While survival on tobacco in *B. tabaci* has been linked to an increased body volume and muscle content (Xia et al., 2017), the mechanism(s) underpinning the greater intrinsic tolerance to nicotine of *T. vaporariorum* have not been resolved. However, two P450s have been identified in this species that confer low but statistically significant levels of resistance to nicotine (Pym et al., 2019). Interestingly, these two P450s, *CYP6CM3* and *CYP6CM4*, belong to the same subfamily as *CYP6CM1*, a P450 in *B. tabaci* that confers strong resistance to several neonicotinoid insecticides including imidacloprid (Jones et al., 2011; Karunker et al., 2008; Karunker et al., 2009; Nauen et al., 2013). *B. tabaci*, while more susceptible to nicotine, can still tolerate high doses of this alkaloid and tobacco-reared lines have shown elevated resistance to nicotine (Kliot et al., 2014). As with the case of *T. vaporariorum,* the mechanisms by which *B. tabaci* detoxifies nicotine have not been fully resolved. However RNA interference of *CYP6CM1* was shown to result in modest but statistically significant increases in the sensitivity of this species to nicotine (Li et al., 2015). Taken together, these findings suggest that P450s of the CYP6CM subfamily may play a role in determining nicotine sensitivity but are unlikely to fully explain the marked resistance of both whitefly species to this allelochemical, or the differences in nicotine sensitivity between *T. vaporariorum* and *B. tabaci.*

Here, we quantified detoxification gene expression in both *T. vapoariorum* and *B. tabaci* when feeding on either tobacco or a host plant with a less challenging secondary metabolite profile and correlated this with changes in nicotine sensitivity. We identified a subfamily of cytochrome P450 genes present in both whitefly species that are strongly upregulated upon tobacco feeding. Ectopic expression of members of this gene subfamily in *Drosophila melanogaster* provide functional evidence of their role in resistance to nicotine and subsequently the neonicotinoid imidacloprid. Our data provides new insight into how these important crop pests are able to colonise the toxic host plant tobacco and the mechanistic basis of the greater resistance of *T. vaporariorum* to nicotine than *B. tabaci.*

2. Material and methods

2.1. Insect strains

The *T. vaporariorum* strain (Tv1) used in this study is a long-term, insecticide-susceptible laboraratory culture that is normally reared on French bean (*Phaseolus vulgaris* L., cv. 'Canadian Wonder'). The *B. tabaci* strain (Sud-R), usually reared on cotton, is a long-term laboratory strain of the MED species and susceptible to most known insecticides. Colonies of both species were established on two different host plants, French bean (*Phaseolus vulgaris*) and tobacco (*Nicotiana tabacum*). All colonies were reared on these host plants for >7 generations before bioassays were performed. All whitefly cultures in this study were reared at 24 °C, 55% relative humidity, with a 16/8 h. (day/night) light cycle.

2.2. Insecticide bioassays

Artificial feeding assays were conducted to identify changes in sensitivity of *T. vaporariorum* and *B. tabaci* to insecticides. This method avoided potential confounding effects caused by performing bioassays directly on host plant material, where the attributes of the host can influence insecticide sensitivity. Insecticides tested included nicotine ((−)-1-Methyl-2-(3-pyridyl)pyrrolidine, Sigma >99%) and imidacloprid (3-[(2*S*)-1-methylpyrrolidin-2-yl]pyridine, Sigma, > 99.0%). Compounds were dissolved in de-ionised water and then diluted to the required concentration in a 15% sucrose solution. 200 μL of nicotine or imidacloprid was subsequently applied between two sheets of parafilm stretched over 55 mm petri dishes to make a feeding sachet, as described previously (Pym et al., 2019; Rauch and Nauen, 2003). Control samples using 15% sucrose without insecticide were also included. Adult

whiteflies (2–7 days old) were then removed from each host plant and anaesthetised using carbon dioxide. Twenty adults of mixed sex were added to each petri dish with every insecticide concentration tested in triplicate. Mortality was then recorded after 48 h and a Probit analysis used to calculate LC_{50} values and 95% confidence limits using the 'drc' package (Ritz et al., 2016) in RStudio v1.3.1056 (Allaire, 2012).

2.3. RNA extraction and sequencing

RNA was extracted from each whitefly line described above in four biological replicates (pooled homogenates of 30 insects) using the Bioline Isolate II RNA Mini Kit (Bioline, UK) according to the manufacturer's instructions. RNA quality and quantity was assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA), a Qubit Fluorometer (Invitrogen) and by running an aliquot on a 1.5% gel. For the latter, RNA was mixed with 2× loading buffer (95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 18 mM ethylenediamintetraacetic acid, 0.025% sodium dodecyl sulphate), heated for 5 min at 65 °C and briefly chilled on ice prior to loading. RNA samples were subsequently used for the creation of barcoded libraries (TrueSeq RNA library preparation, Illumina, San Diego, California, USA) which were run on an Illumina HiSeq2500 flowcell (125 bp paired end reads) at the Earlham Institute (Norwich, UK).

2.4. RNASeq. analysis

The quality of the RNA sequencing reads was assessed using FastQC v0.11.8 (Andrews, 2010). Adaptor regions and low quality base calls were removed using TrimGalore v0.6.4 (Krueger, 2012). Sequenced reads were subsequently aligned to the *T. vaporariorum* (Pym et al., 2019) and *B. tabaci* (Chen et al., 2016) genomes using HISAT2 v2.1.0 (Kim et al., 2019) and Samtools v1.9 (Li et al., 2009). Significantly differentially-expressed genes were called using the EdgeR analysis tool (Robinson et al., 2010) using a corrected *p*-value of <0.05 and a fold change of >2. Analysis plots were generated using the ggplot2 (Wickham, 2016), pheatmap (Kolde and Kolde, 2015) and ggsci (Xiao et al., 2018) packages in RStudio. Phylogenetic trees were constructed using MEGA X (Kumar et al., 2018) from aligned cytochrome-P450s using a maximum likelihood model with a bootstrap value of 1000.

2.5. Creation and bioassay of transgenic Drosophila melanogaster expressing whitefly CYP6DP genes

CYP6DP gene sequences were synthesised and subcloned into the pUASTattB plasmid (GenBank: EF362409.1). Constructs were transformed into the germline of a *D. melanogaster* strain, containing an attP docking site on chromosome 2 (attP40) and the phiC41 integrase gene under the control of the vasa regulatory region on the X chromosome (y w M (eGFP, vas-int, dmRFP)ZH-2 A; P [CaryP]attP40) (Markstein et al., 2008), using the PhiC31 system. The resultant lines were subsequently balanced and gene integration confirmed by PCR and sequencing using Phusion DNA polymerase, as described previously (Manjon et al., 2018).

Virgin females were collected from the Act5C-GAL4 strain and crossed with UAS-GOI males. Bioassays were used to test the susceptibility of the lines to the synthetic insecticide imidacloprid in addition to the natural plant compound nicotine. Multiple concentrations of the insecticide were overlaid onto agar vials (1.5% agar, 1% sucrose, 4% acetic acid, 5 vials/dose) and allowed to dry at room temperature overnight. Twenty adult female flies (2–7 days post-eclosion) were added to each vial and mortality assessed at a specific timepoint. LC_{50} values and 95% confidence limits were calculated using the drc package (Ritz et al., 2016) in RStudio (Allaire, 2012).

The expression of transgenes was confirmed using quantitative PCR (qPCR) (Fig. S1). For this, RNA was extracted from four biological replicates of five female adults of each transgenic fly strain and the progeny of crosses of these strains with the Act5C-GAL4 strain using the Isolate II RNAMini Kit (Bioline) as detailed above. cDNA was synthesised from 1 μg of RNA per replicate using the Maxima H Minus First Strand cDNA Synthesis Kit (ThermoFisher) using both random hexamer and oligo (dT) primers. qPCR analysis was performed using the primers detailed in Table S1, with the efficiency of PCR for each primer pair assessed using a serial dilution of 100 ng to 0.01 ng of cDNA. PCR reactions (15 μL) contained 10 ng of cDNA, 7.5 μL of SYBR Green JumpStart Taq Readymix (Sigma), and 0.25 μM of each primer. Each qPCR experiment consisted of two technical replicates and four biological replicates. Reactions were run on a BioRad Real-Time PCR System (BioRad) using temperature cycling conditions of 3 min at 95 °C followed by 40 cycles of 95 °C for 30 s, 58 °C for 20 s and 72 °C for 15 s. A melt curve step was included (ranging from 65 °C to 95 °C by 1 °C every 5 s) to confirm the absence of non-specific amplification. Data were analysed using the ΔΔCT method (Livak and Schmittgen, 2001) and normalised using the geometric mean of two *Drosophila melanogaster* housekeeping genes (RPL32 and SDHA, Table S1). Graphical analysis was performed using RStudio (Allaire, 2012) and BioRender (Biorender, 2022).

3. Results

3.1. Insecticide bioassays reveal whitefly species and host plant related differences in nicotine and imidacloprid sensitivity

To compare the relative sensitivity of *B. tabaci* and *T. vaporariorum* to the natural insecticide nicotine, and examine the impact of host plant on the sensitivity of the two species to this allelochemical, cultures of *B. tabaci* and *T. vaporariorum* were reared on tobacco, a challenging host, and bean, a less challenging host. After >7 generations, the sensitivity of the two cultures of *B. tabaci* and *T. vaporariorum* to nicotine was examined using full dose-response bioassays. Both cultures of *T. vaporariorum* exhibited greater tolerance to nicotine than the respective *B. tabaci* cultures on the same host plant (Fig. 1A). The *T. vaporariorum* culture displaying a 29-fold increase in tolerance on French bean and a 4-fold increase on tobacco. The tobacco-reared cultures also showed a greater tolerance to nicotine than those on French bean. The rearing on tobacco induced a 3-fold increase in tolerance in *T. vaporariorum* and a 23-fold increase in *B. tabaci*.

These findings are consistent with previous reports (Gorman et al., 2002) that show *T. vaporariorum* displays a greater intrinsic tolerance to nicotine than *B. tabaci*. Additionally, they demonstrate that, in the case of both whitefly species, nicotine tolerance is strongly influenced by host plant, with feeding on tobacco resulting in a higher tolerance to nicotine than feeding on bean. Finally, this host-plant related difference in nicotine sensitivity was more pronounced for *B. tabaci* than *T. vaporariorum* suggestive of a greater induced adaptive response to this host plant in the former than the latter.

To investigate whether the whitefly species and host plant related differences in sensitivity to the plant secondary metabolite nicotine were also observed for a chemically related synthetic insecticide, we conducted bioassays on the same whitefly lines using the neonicotinoid imidacloprid. Both *B. tabaci* and *T. vaporariorum* individuals reared on tobacco showed a modest but statistically significant ($p < 0.05$) ~1.5-fold increase in tolerance to imidacloprid when compared to the same lines reared on French bean (Fig. 1B).

3.2. Nicotine and imidacloprid tolerance are associated with changes in the expression of genes encoding detoxification enzymes

To explore the mechanism(s) underpinning the variation in pesticide susceptibility of the two whitefly species when reared on different host plants, and gain insight into the differences in sensitivity of the two species to nicotine, we performed replicated RNA sequencing (RNAseq) of the *B. tabaci* and *T. vaporariorum* lines reared on tobacco and bean. RNAseq data was used to conduct differential gene expression analyses, comparing the cultures of each whitefly species reared on tobacco with

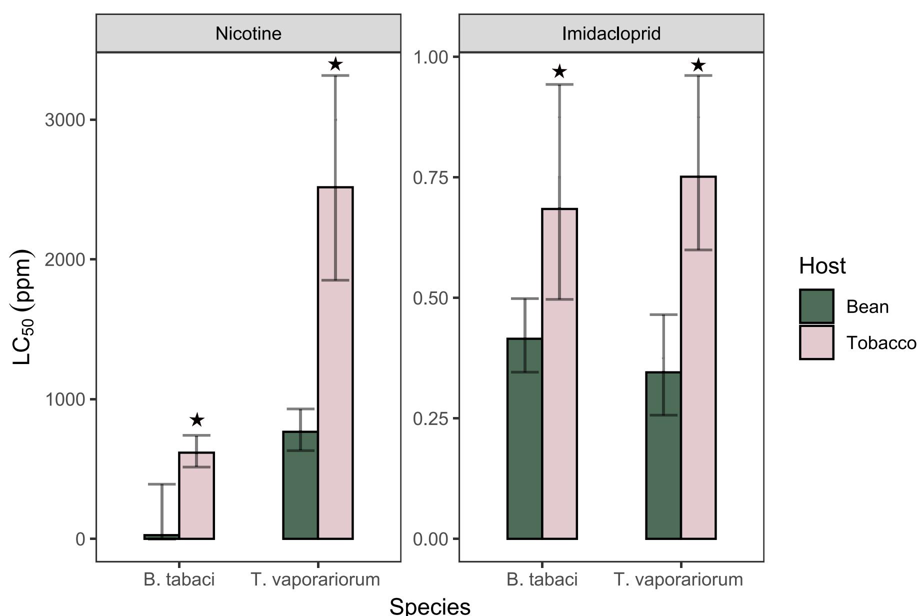

Fig. 1. Sensitivity of whitefly lines reared on tobacco and bean to nicotine. Lethal concentration (LC_{50}) values of *T. vaporariorum* and *B. tabaci* colonies reared on French bean (*Phaesolus vulgaris)* and tobacco (*Nicotiana tabacum*) to (A) the natural insecticide nicotine and (B) the synthetic neonicotinoid imidacloprid. Error bars denote 95% confidence limits.

those reared on bean. This analysis revealed a dramatic transcriptional response induced by feeding on different host plants in the case of both species (Fig. 2). A higher number of significantly differentially expressed (DE) genes was observed in *B. tabaci* (4647) of which 3721 were over-expressed in the culture reared on tobacco. A lower number of DE genes, 2974, was observed for *T. vaporariorum*, of which 1671 were over-expressed in the culture reared on tobacco.

As previous work has implicated P450 enzymes in resistance to nicotine in both *B. tabaci* and *T. vaporariorum*, and other insect species (Bass et al., 2013; Pym et al., 2019; Li et al., 2015; Snyder and Glendinning, 1996), we examined the expression of genes in this detoxification enzyme superfamily in our dataset in more detail. A large number of P450 genes were found to be differentially-expressed in response to nicotine feeding (Fig. 3). Of these, a total of 41 *B. tabaci* P40 genes, and 8 *T. vaporariorum* P450 genes were found to be significantly upregulated. The expression data of P450s obtained for both whitefly species was subsequently overlaid onto a phylogenetic tree of the P450 genes (Fig. 4) to identify clades of genes commonly over-expressed upon tobacco feeding. P450s belonging to the CYP4 clade were generally under-expressed or showed low levels of over-expression. Similarly, P450s found in the mitochondrial or CYP2 clade showed low-moderate levels of overexpression associated with tobacco feeding. In contrast, several P450s belonging to the CYP3 clade, frequently instrumental in the detoxification of toxic compounds (Nauen et al., 2022), exhibited large-scale changes in expression associated with tobacco feeding. Within this clade, multiple genes in several subclades were identified as significantly over-expressed. Somewhat surprisingly, this did not include P450s belonging to the CYP6CM subfamily. As detailed in the introduction, *CYP6CM3* and *CYP6CM4* of *T. vaporariorum* have been previously implicated in resistance to nicotine. However, of the four CYP6CM subfamily P450 genes identified in the two whitefly species, only *CYP6CM4* was upregulated 1.7-fold in whitefly lines reared on tobacco, suggesting that upregulation of these P450s is unlikely to fully explain the increased tolerance to nicotine following feeding on tobacco. In contrast to these findings, members of the *CYP6DP* subfamily, comprising *CYP6DP1* and *CYP6DP2* in *T. vaporariorum* and *CYP6DP3* and *CYP6DP5* in *B. tabaci*, exhibited much stronger and more consistent upregulation upon tobacco feeding (Fig. 4). *CYP6DP1* and *CYP6DP2* were ~4-fold and ~19-fold over-expressed in the tobacco feeding *T. vaporariorum* line whereas *CYP6DP3* and *CYP6DP5* were 8-fold and 25-fold over-expressed in the tobacco feeding *B. tabaci* line. Taken together, these findings suggest that P450 genes belonging to the CYP6DP subfamily are strong candidate genes for a role in nicotine tolerance in the two species.

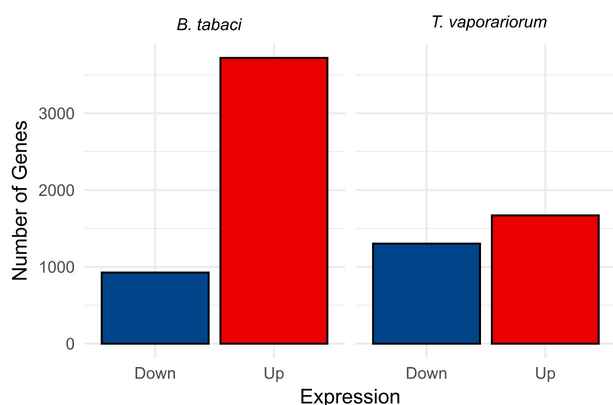

Fig. 2. Summary of significantly over- and under- expressed genes in lines of *T. vaporariorum* and *B. tabaci* reared on tobacco, compared to the same strains reared on French bean. Genes were identified by RNASeq analysis using the edgeR package.

3.3. CYP6DP subfamily P450s confer tolerance to nicotine in vivo

To functionally validate the role of the candidate P450 genes identified by transcriptome profiling in nicotine tolerance, four transgenic lines of *D. melanogaster* were created each expressing one of the *CYP6DPx* genes. The sensitivity of these lines to nicotine was examined using full-dose response nicotine bioassays and compared to a control fly line of the same genetic background but lacking a transgene (Fig. 5, Table S2). The transgenic lines expressing *CYP6DP1* and *CYP6DP2* of *T. vaporariorum* displayed significant ($p < 0.001$) tolerance of 4-fold and 2-fold respectively, to nicotine when compared to the control line. *CYP6DP3* of *B. tabaci* also exhibited significant nicotine tolerance (2-fold, $p < 0.001$). However, the line expressing *B. tabaci CYP6DP5* did not

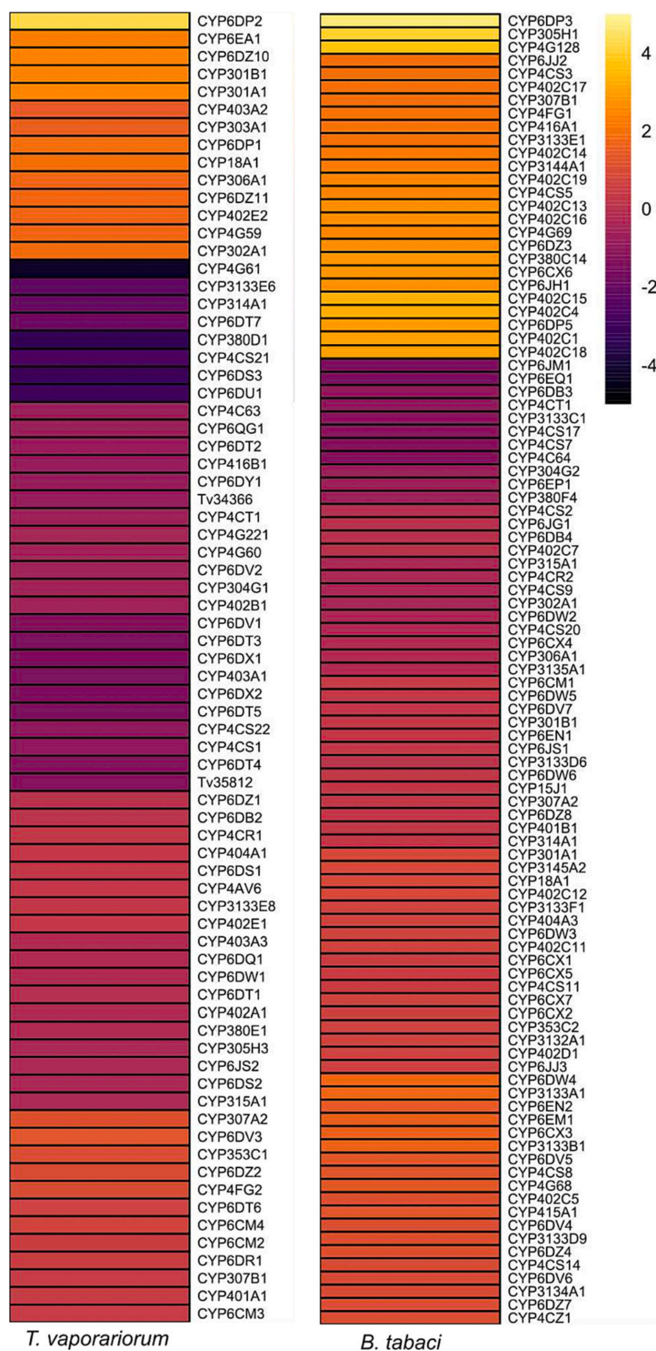

Fig. 3. Relative expression of P450 genes in *T. vaporariorum* and *B. tabaci* lines feeding on tobacco compared to lines feeding on French bean. Lighter colours denote over-expression and darker colours, under-expression. Enzymes with expression $-1 > x > 1$ were included.

display significant tolerance to this compound compared to the control ($p = 0.26$) suggesting that this enzyme may lack the capacity to metabolise nicotine.

To investigate whether these P450s also have the capacity to confer tolerance to imidacloprid, the sensitivity of *CYP6DP*-expressing *D. melanogaster* lines to this neonicotinoid was examined in full-dose response bioassays. As observed for nicotine, the lines expressing *CYP6DP1* and *CYP6DP2* of *T. vaporariorum* displayed a significantly ($p < 0.001$) increased tolerance to the compound (2.69 and 2.07-fold respectively) compared to the control line. In the case of lines expressing *B. tabaci* P450s, the *CYP6DP3*-expessing line showed, as for nicotine, a significant ($p < 0.001$) tolerance to imidacloprid (1.99-fold). More surprisingly, the *CYP6DP5*-expressing line also exhibited a significant tolerance to imidacloprid (2.78-fold, $p < 0.001$) in contrast to its lack of resistance to nicotine. Together these results demonstrate the causal role of *CYP6DP1, CYP6DP2* and *CYP6DP3* in tolerance to the alkaloid nicotine and also demonstrate that these P450s are preadapted to confer a degree of tolerance to chemically-related synthetic insecticides.

4. Discussion

Whiteflies are among the world's most damaging group of insect crop pests and have evolved resistance to a remarkable array of synthetic and natural toxins (Prabhaker et al., 1985; Horowitz et al., 2020; Wardlow et al., 1972). Relatively few insect species can utilise tobacco as a host, due to its potent chemical defences, but the whiteflies, *B. tabaci* and *T. vaporariorum,* are among those that have evolved means to overcome this (Chowański et al., 2016). However, to date, the mechanisms by which the two species detoxify nicotine, the most abundant volatile alkaloid in tobacco leaves (Tayoub et al., 2015), and the differences in the sensitivity of the two species to this compound (Gorman et al., 2002), has remained unresolved. Furthermore, while over-expression of the cytochrome P450 CYP6CM1 in *B. tabaci* has been shown to confer resistance to the structurally related synthetic neonicotinoid insecticides (Jones et al., 2011; Karunker et al., 2008), the relationship between the mechanisms underpinning resistance to nicotine and neonicotinoids is unclear. A previous study linked *B. tabaci* fitness on tobacco to an increase in muscle content (Xia et al., 2017) but this mechanism would not cause a higher tolerance to nicotine. Our data therefore provides new insight into these long-standing knowledge gaps.

Investigation of the impact of host plant on nicotine sensitivity in the two whitefly species in this study revealed that tobacco-reared lines of *B. tabaci* and *T. vaporariorum* exhibit much greater tolerance to nicotine compared to those reared on French bean. This finding is consistent with previous research (Kliot et al., 2014; Pym et al., 2019), and is likely the result of adaptation to nicotine exposure when feeding on tobacco, for example, by the upregulation of genes encoding enzymes that can detoxify this compound. In such a scenario, tobacco-reared lines exposed to nicotine in insecticide bioassay, already overexpress enzymes capable of metabolising the compound, resulting in the reduced sensitivity of these lines relative to tobacco-unadapted lines. In chemistry, this effect is known as 'mithridatism', where resistance to poisoning is acquired by enzymatic activation or metabolic functional changes due to prior exposure to sub-lethal doses of the same, or a related, poison (Tsatsakis et al., 2018). Intriguingly, our data revealed a much larger difference in induced tolerance in *B. tabaci* than *T. vaporariorum* suggesting a greater adaptive response in the former than the latter. Despite this, insecticide bioassays confirmed that both the intrinsic and induced tolerance was higher in *T. vaporariorum* than *B. tabaci*, despite its common name of the 'tobacco whitefly'. The same finding has been reported in a previous study (Gorman et al., 2002) using different strains of whiteflies, suggesting that this pattern is consistent across different genetic backgrounds of the two species. *B. tabaci* is generally considered to have a higher propensity to develop resistance to synthetic insecticides than *T. vaporariorum* and is able to detoxify over 50 insecticides with 555 documented cases of resistance (Sparks and Nauen, 2015). Additionally, multi-resistant populations are not uncommon, especially in the MED biotype (IRAC, 2023). In this case, however, the greater tolerance of *T. vaporariorum* to nicotine suggests the underling mechanisms in this species confer greater protection against this compound than those present in *B. tabaci*. This differential sensitivity of the two species could be caused by multiple factors, including more efficient detoxification by nicotine metabolising enzymes in *T. vaporariorum*, reduced affinity of nicotine for the nicotinic acetylcholine receptor (nAChR) of *T. vaporariorum* than that of *B. tabaci*, or other physiological difference between the two species. However, the marked phenotypic plasticity associated with tobacco feeding, *i.e.* enhanced nicotine tolerance following tobacco feeding, observed in this study, is consistent with the

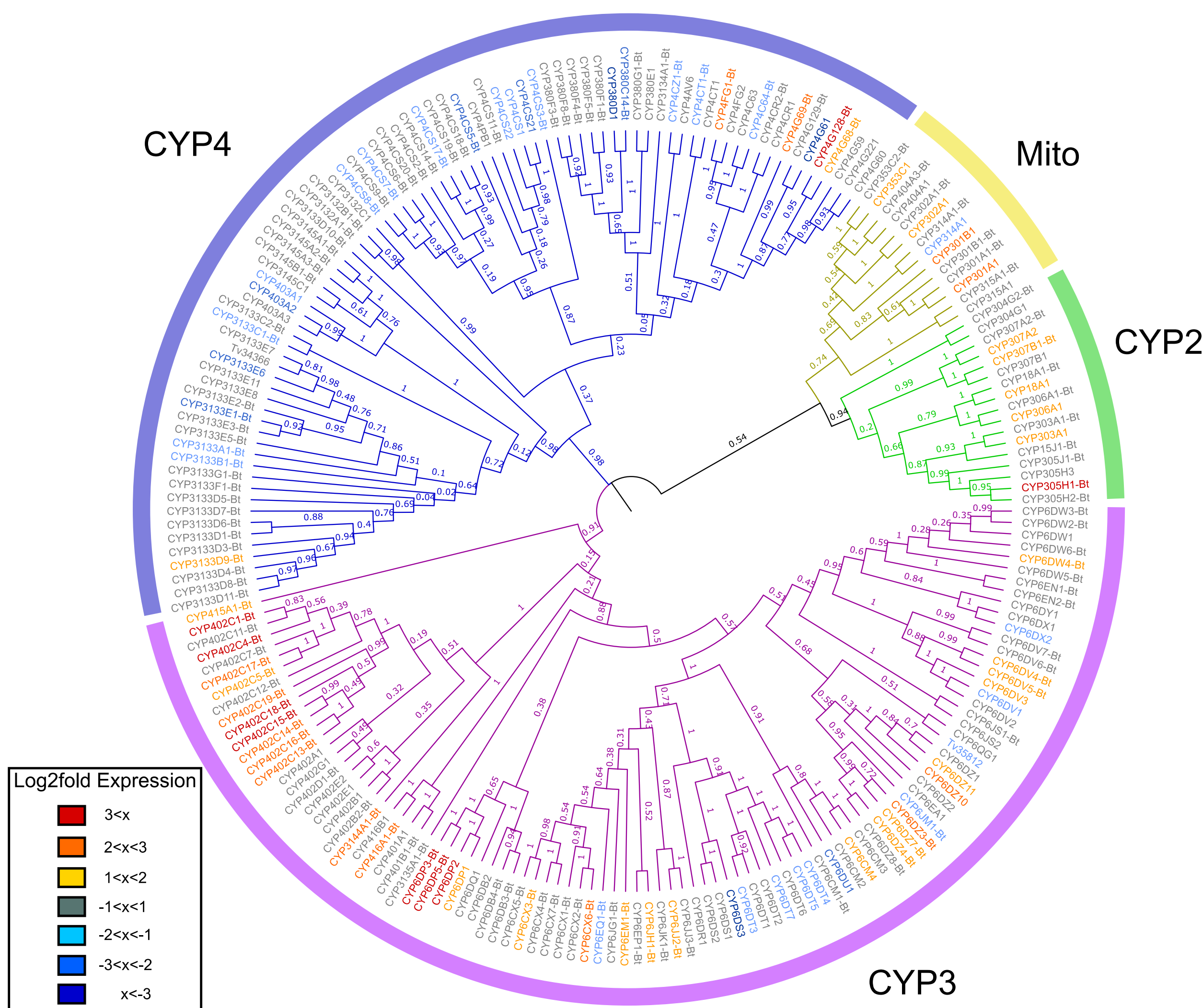

Fig. 4. Phylogenetic tree of *T. vaporariorum* and *B. tabaci* P450s. Differentially expressed P450s in tobacco reared lines compared to French bean reared lines are coloured according to expression with under-expressed genes in blue and over-expressed genes in red. Node numbers represent bootstrap support values (1000 replicates). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

upregulation of genes that confer resistance to nicotine.

Transcriptome profiling of *T. vaporariorum* and *B. tabaci* reared on tobacco *versus* French bean revealed profound differences in gene expression associated with feeding and reproducing on these different host plants. This observed transcriptional plasticity aligns with the results of studies on host adaptation in whiteflies and several other insect species (Pym et al., 2019; Yu et al., 2016; Dermauw et al., 2013), which together demonstrate the capacity of generalist species to rapidly modulate gene expression in a host-dependent manner. Recent work on *T. vaporariorum* has revealed that these profound changes in gene expression during host adaptation are associated with marked changes in tolerance to both natural and synthetic insecticides (Pym et al., 2019), as observed in our study.

Previous research has implicated members of the P450 gene superfamily in nicotine resistance in both whiteflies and other insect species (Pym et al., 2019; Bass et al., 2013; Snyder et al., 1994; Rand et al., 2015). The transcriptome profiling conducted in our study revealed marked changes in the expression of numerous individual P450 genes in comparisons of the lines of the two whitefly species reared on different host plants. Surprisingly, in the case of P450s belonging to the CYP6CM subfamilu, neither gene was upregulated in *B. tabaci* and only one showed upregulation in *T. vaporariorum* in response to tobacco-feeding. This gene, *CYP6CM4* of *T. vaporariorum*, has been previously shown to confer low (1.5-fold) levels of nicotine tolerance when ectopically expressed in transgenic *D. melanogaster* (Pym et al., 2019). Thus, while P450s belonging to the CYP6CM subfamily may play some role in nicotine tolerance, they are unlikely to fully explain the much greater levels of nicotine tolerance observed in tobacco-reared whitefly lines compared to those reared on bean. In contrast to these findings, the pattern of expression of P450 genes belonging to the CYP6DP subfamily makes them much stronger candidates for a role in induced nicotine resistance, with *CYP6DP1* and *CYP6DP2* of *T. vaporariorum,* and *CYP6DP3* and *CYP6DP5* of *B. tabaci* all exhibiting much stronger and more consistent upregulation upon tobacco feeding.

Functional characterisation of these genes by ectopic expression in *D. melanogaster* provided causal evidence that three of the four P450s confer resistance to nicotine *in vivo.* More specifically, both CYP6DP subfamily P450s from *T. vaporariorum* conferred significant resistance to nicotine, with flies expressing *CYP6DP1* and *CYP6DP2* significantly more resistant to nicotine than flies of the same genetic background lacking these P450s (4- and 2-fold respectively). In the case of *B. tabaci*, only *CYP6DP3* conferred a significant resistance to nicotine (2-fold). Whereas, flies expressing *CYP6DP5* exhibited no significant resistance to the same compound. These results correlate with the phenotypic levels of nicotine tolerance exhibited by the two whitefly species. Specifically, the higher levels of intrinsic tolerance to this compound observed in

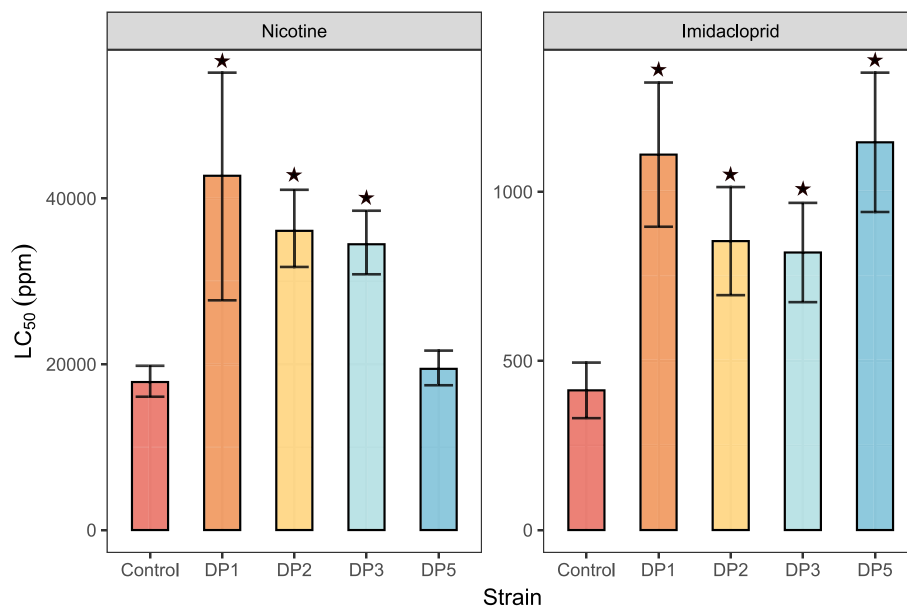

Fig. 5. Sensitivity of transgenic strains of *Drosophila melanogaster* expressing *CYP6DP* genes to the insecticides nicotine and imidacloprid. Lethal concentration 50% (LC_{50}) values for the transgenic strains of *D. melanogaster* expressing each of the *CYP6DP* genes to (A) nicotine and (B) imidacloprid. The control strain is a line of *D. melanogaster* of the same genetic background as the transgenic strains but lacking a transgene. Error bars are 95% confidence intervals and stars denote significance ($p < 0.05$).

T. vaporariorum are consistent with the fact that this species has two CYP6DP subfamily P450s which confer resistance, of which one, CYP6DP1 appears to be particularly active against nicotine. In contrast, *B. tabaci* appears to have a single nicotine metabolising CYP6DP P450 which confers modest levels of resistance.

Links between insect and mite host plant adaption and increased tolerance to synthetic insecticides have been previously identified in several species (Dermauw et al., 2013; Bass et al., 2013). For example, work on *M. persicae*, revealed that tobacco-derived clones exhibit increased tolerance to both nicotine and neonicotinoid insecticides, despite the fact that they had never been exposed to the latter (Bass et al., 2013). This was linked to the over-expression of the P450 *CYP6CY3*, which confers resistance to nicotine and pre-adapts tobacco-derived clones to resist neonicotinoid insecticides (Bass et al., 2013; Puinean et al., 2010). Here we demonstrate that whitefly P450s of the CYP6DP subfamily have a similar capacity to detoxify both natural (nicotine) and synthetic (imidacloprid) insecticides. However, our findings, in combination with previous work (Bass et al., 2013; Dermauw et al., 2013), also illustrate that this does not necessarily mean that enzymes that are preadapted to detoxify synthetic insecticides will be co-opted in the evolution of insecticide resistance. More specifically, in *B. tabaci* (Hamada et al., 2019; Jones et al., 2011; Karunker et al., 2008), neonicotinoid resistance has been most frequently linked to the over-expression of *CYP6CM1*, not members of the CYP6DP subfamily of P450s. The reasons for this are unclear and might simply reflect the stochastic process of evolution, the fact that upregulation of *CYP6CM1* results in greater resistance to neonicotinoids, or lower fitness costs in the absence of insecticide, than upregulation of *CYP6DP* P450 genes. Future research is required to investigate this further. Regardless, our findings that CYP6DP P450s have the capacity to confer tolerance to imidacloprid once again illustrates the inherent risk of developing insecticidal compounds that share chemical similarity with natural compounds encountered by herbivorous insects.

In conclusion, our data provide new insight into the molecular basis of nicotine resistance in whiteflies and reveal the role of CYP6DP subfamily P450s in both constitutive and induced resistance to this compound. Furthermore, they illustrate how divergence in the evolution of this subfamily of P450 genes in whiteflies may have impacted the extent to which different species can tolerate a potent natural insecticide. Finally, our findings inform research on the link between host-plant adaptation and insecticide resistance by providing an example of how insect detoxication enzymes that detoxify plant secondary metabolites can be pre-adapted to resist synthetic insecticides.

CRediT authorship contribution statement

Adam Pym: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Bartlomiej J. Troczka:** Investigation, Methodology, Visualization, Writing – review & editing. **Angela Hayward:** Investigation, Methodology, Writing – review & editing. **Bin Zeng:** Investigation, Methodology, Writing – review & editing. **Cong-Fen Gao:** Supervision, Writing – review & editing. **Jan Elias:** Funding acquisition, Supervision, Writing – review & editing. **Russell Slater:** Funding acquisition, Supervision, Writing – review & editing. **Christoph T. Zimmer:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Chris Bass:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Data availability

Sequence data has been deposited with the NCBI Short Read Archive as BioProject PRJNA548670.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2023.105743.

References

Allaire, J., 2012. RStudio: Integrated Development Environment for R. Boston, MA, 770, pp. 165–171.

Alyokhin, A., Chen, Y.H., 2017. Adaptation to toxic hosts as a factor in the evolution of insecticide resistance. Curr. Opin. Insect Sci. 21, 33–38.

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. In: Babraham Bioinformatics. Babraham Institute, Cambridge, United Kingdom.

Bass, C., Zimmer, C.T., Riveron, J.M., Wilding, C.S., Wondji, C.S., Kaussmann, M., Field, L.M., Williamson, M.S., Nauen, R., 2013. Gene amplification and microsatellite polymorphism underlie a recent insect host shift. Proc. Natl. Acad. Sci. 110, 19460–19465.

Bernays, E.A., 1998. Evolution of feeding behavior in insect herbivores. Bioscience 48, 35–44.

Biorender, B., 2022. BioRender. Com.

Birnbaum, S.S.L., Abbot, P., 2020. Gene expression and diet breadth in plant-feeding insects: summarizing trends. Trends Ecol. Evol. 35, 259–277.

Byrne, D.N., Bellows, J.R., 1991. Whitefly biology. Annu. Rev. Entomol. 36, 431–457.

Chen, W., Hasegawa, D.K., Kaur, N., Kliot, A., Pinheiro, P.V., Luan, J., Stensmyr, M.C., Zheng, Y., Liu, W., Sun, H., 2016. The draft genome of whitefly Bemisia tabaci MEAM1, a global crop pest, provides novel insights into virus transmission, host adaptation, and insecticide resistance. BMC Biol. 14, 1–15.

Choi, W.-I., Lee, E.-H., Choi, B.-R., Park, H.-M., Ahn, Y.-J., 2003. Toxicity of plant essential oils to Trialeurodes vaporariorum (Homoptera: Aleyrodidae). J. Econ. Entomol. 96, 1479–1484.

Chowański, S., Adamski, Z., Marciniak, P., Rosiński, G., Büyükgüzel, E., Büyükgüzel, K., Falabella, P., Scrano, L., Ventrella, E., Lelario, F., 2016. A review of bioinsecticidal activity of Solanaceae alkaloids. Toxins 8, 60.

Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., Grbić, M., Clark, R.M., Feyereisen, R., Van Leeuwen, T., 2013. A link between host plant adaptation and pesticide resistance in the polyphagous spider mite Tetranychus urticae. Proc. Natl. Acad. Sci. 110, E113–E122.

Feyereisen, R., 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–533.

Gloss, A.D., Abbot, P., Whiteman, N.K., 2019. How interactions with plant chemicals shape insect genomes. Curr. Opin. Insect Sci. 36, 149–156.

Gordon, H., 1961. Nutritional factors in insect resistance to chemicals. Annu. Rev. Entomol. 6, 27–54.

Gorman, K., Hewitt, F., Denholm, I., Devine, G.J., 2002. New developments in insecticide resistance in the glasshouse whitefly (Trialeurodes vaporariorum) and the two-spotted spider mite (Tetranychus urticae) in the UK. Pest Manag. Sci. 58, 123–130.

Gould, F., Carroll, C., Futuyma, D., 1982. Cross-resistance to pesticides and plant defenses: A study of the two-spotted spider mite. Entomol. Exp. Appl. 31, 175–180.

Hamada, A., Wahl, G.D., Nesterov, A., Nakao, T., Kawashima, M., Banba, S., 2019. Differential metabolism of imidacloprid and dinotefuran by Bemisia tabaci CYP6CM1 variants. Pestic. Biochem. Physiol. 159, 27–33.

Heidel-Fischer, H.M., Vogel, H., 2015. Molecular mechanisms of insect adaptation to plant secondary compounds. Curr. Opin. Insect Sci. 8, 8–14.

Horowitz, A.R., Ghanim, M., Roditakis, E., Nauen, R., Ishaaya, I., 2020. Insecticide resistance and its management in Bemisia tabaci species. J. Pest. Sci. 93, 893–910.

Inbar, M., Gerling, D., 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. Annu. Rev. Entomol. 53, 431–448.

IRAC, 2023. Bemisia tabaci. *https://irac-online.org/pests/bemisia-tabaci/*.

Jones, C.M., Daniels, M., Andrews, M., Slater, R., Lind, R.J., Gorman, K., Williamson, M. S., Denholm, I., 2011. Age-specific expression of a P450 monooxygenase (CYP6CM1) correlates with neonicotinoid resistance in Bemisia tabaci. Pestic. Biochem. Physiol. 101, 53–58.

Karunker, I., Benting, J., Lueke, B., Ponge, T., Nauen, R., Roditakis, E., Vontas, J., Gorman, K., Denholm, I., Morin, S., 2008. Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of Bemisia tabaci (Hemiptera: Aleyrodidae). Insect Biochem. Mol. Biol. 38, 634–644.

Karunker, I., Morou, E., Nikou, D., Nauen, R., Sertchook, R., Stevenson, B.J., Paine, M.J., Morin, S., Vontas, J., 2009. Structural model and functional characterization of the Bemisia tabaci CYP6CM1vQ, a cytochrome P450 associated with high levels of imidacloprid resistance. Insect Biochem. Mol. Biol. 39, 697–706.

Kim, D., Paggi, J.M., Park, C., Bennett, C., Salzberg, S.L., 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 37, 907–915.

Kliot, A., Kontsedalov, S., Ramsey, J.S., Jander, G., Ghanim, M., 2014. Adaptation to nicotine in the facultative tobacco-feeding hemipteran Bemisia tabaci. Pest Manag. Sci. 70, 1595–1603.

Kolde, R., Kolde, M.R., 2015. Package 'pheatmap'. R package, 1, p. 790.

Krueger, F., 2012. Trim Galore: A Wrapper Tool around Cutadapt and FastQC. Trim Galore.

Kumar, S., Stecher, G., LI, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547.

Li, X., Schuler, M.A., Berenbaum, M.R., 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52, 231–253.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.

Li, J., Li, X., Bai, R., Shi, Y., Tang, Q., An, S., Song, Q., Yan, F., 2015. RNA interference of the P450 CYP6CM1 gene has different efficacy in B and Q biotypes of Bemisia tabaci. Pest Manag. Sci. 71, 1175–1181.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Lloyd, L., 1922. The control of the greenhouse white fly (Asterochiton vaporariorum) with notes on its biology 1. Ann. Appl. Biol. 9, 1–32.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the world's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. Invasive Species Specialist Group Auckland.

Manjon, C., Troczka, B.J., Zaworra, M., Beadle, K., Randall, E., Hertlein, G., Singh, K.S., Zimmer, C.T., Homem, R.A., Lueke, B., 2018. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. Curr. Biol. 28 (1137–1143), e5.

Mao, W., Berhow, M.A., Zangerl, A.R., Mcgovern, J., Berenbaum, M.R., 2006. Cytochrome P450-mediated metabolism of xanthotoxin by Papilio multicaudatus. J. Chem. Ecol. 32, 523–536.

Markstein, M., Pitsouli, C., Villalta, C., Celniker, S.E., Perrimon, N., 2008. Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. Nat. Genet. 40, 476–483.

Mithöfer, A., Boland, W., 2012. Plant defense against herbivores: chemical aspects. Annu. Rev. Plant Biol. 63, 431–450.

Mitter, C., Farrell, B., Wiegmann, B., 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? Am. Nat. 132, 107–128.

Nauen, R., Vontas, J., Kaussmann, M., Wölfel, K., 2013. Pymetrozine is hydroxylated by CYP6CM1, a cytochrome P450 conferring neonicotinoid resistance in Bemisia tabaci. Pest Manag. Sci. 69, 457–461.

Nauen, R., Bass, C., Feyereisen, R., Vontas, J., 2022. The role of cytochrome P450s in insect toxicology and resistance. Annu. Rev. Entomol. 67, 105–124.

Paré, P.W., Tumlinson, J.H., 1999. Plant volatiles as a defense against insect herbivores. Plant Physiol. 121, 325–332.

Prabhaker, N., Coudriet, D., Meyerdirk, D., 1985. Insecticide resistance in the sweetpotato whitefly, Bemisia tabaci (Homoptera: Aleyrodidae). J. Econ. Entomol. 78, 748–752.

Puinean, A.M., Foster, S.P., Oliphant, L., Denholm, I., Field, L.M., Millar, N.S., Williamson, M.S., Bass, C., 2010. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. PLoS Genet. 6, e1000999.

Pym, A., Singh, K.S., Nordgren, Å., Davies, T., Zimmer, C.T., Elias, J., Slater, R., Bass, C., 2019. Host plant adaptation in the polyphagous whitefly, Trialeurodes vaporariorum, is associated with transcriptional plasticity and altered sensitivity to insecticides. BMC Genomics 20, 1–19.

Ramos, S.E., Schiestl, F.P., 2019. Rapid plant evolution driven by the interaction of pollination and herbivory. Science 364, 193–196.

Rand, E.E.D., Smit, S., Beukes, M., Apostolides, Z., Pirk, C.W., Nicolson, S.W., 2015. Detoxification mechanisms of honey bees (Apis mellifera) resulting in tolerance of dietary nicotine. Sci. Rep. 5, 1–11.

Rauch, N., Nauen, R., 2003. Identification of biochemical markers linked to neonicotinoid cross resistance in Bemisia tabaci (Hemiptera: Aleyrodidae). Arch. Insect Biochem. Physiol. 54, 165–176.

Ritz, C., Strebig, J.C., Ritz, M.C., 2016. Package 'drc'. Mountain View, CA, USA, Creative Commons.

Robinson, M.D., Mccarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.

Snyder, M., Glendinning, J., 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. J. Comp. Physiol. A. 179, 255–261.

Snyder, M.J., Walding, J.K., Feyereisen, R., 1994. Metabolic fate of the allelochemical nicotine in the tobacco hornworm Manduca sexta. Insect Biochem. Mol. Biol. 24, 837–846.

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

Tayoub, G., Sulaiman, H., Alorfi, M., 2015. Determination of nicotine levels in the leaves of some Nicotiana tabacum varieties cultivated in Syria. Herba Polonica 61.

Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45, 247–268.

Troczka, B.J., Singh, K.S., Zimmer, C.T., Vontas, J., Nauen, R., Hayward, A., Bass, C., 2021. Molecular innovations underlying resistance to nicotine and neonicotinoids in the aphid Myzus persicae. Pest Manag. Sci. 77, 5311–5320.

Tsatsakis, A.M., Vassilopoulou, L., Kovatsi, L., Tsitsimpikou, C., Karamanou, M., Leon, G., Liesivuori, J., Hayes, A.W., Spandidos, D.A., 2018. The dose response principle from philosophy to modern toxicology: the impact of ancient philosophy and medicine in modern toxicology science. Toxicol. Rep. 5, 1107–1113.

Van Lenteren, J.C.V., Noldus, L., 1990. Whitefly-plant relationships: behavioural and ecological aspects. In: Whiteflies: Their Bionomics, Pest Status and Management, 47, p. 49.

War, A.R., Taggar, G.K., Hussain, B., Taggar, M.S., Nair, R.M., Sharma, H.C., 2018. Plant defence against herbivory and insect adaptations. AoB Plants 10, ply037.

Wardlow, L., Ludlam, F., French, N., 1972. Insecticide resistance in glasshouse whitefly. Nature 239, 164–165.

Wickham, H., 2016. ggplot2: elegant graphics for data analysis. Springer.

Xia, W.-Q., Wang, X.-R., Liang, Y., Liu, S.-S., Wang, X.-W., 2017. Transcriptome analyses suggest a novel hypothesis for whitefly adaptation to tobacco. Sci. Rep. 7, 12102.

Xiao, N., Li, M., Xiao, M.N., Lazydata, T., 2018. Package 'ggsci'.

Xue, J., Zhou, X., Zhang, C.-X., Yu, L.-L., Fan, H.-W., Wang, Z., Xu, H.-J., Xi, Y., Zhu, Z.-R., Zhou, W.-W., 2014. Genomes of the rice pest brown planthopper and its

endosymbionts reveal complex complementary contributions for host adaptation. Genome Biol. 15, 1–20.

Yu, S., 1986. Consequences of Induction of Foreign Compound-Metabolizing Enzymes in Insects. Springer, Molecular aspects of insect-plant associations.

Yu, Q.Y., Fang, S.M., Zhang, Z., Jiggins, C.D., 2016. The transcriptome response of Heliconius melpomene larvae to a novel host plant. Mol. Ecol. 25, 4850–4865.