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Adaptation to Nicotine Feeding in *Myzus persicae*

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Abstract

Lineages of the generalist hemipteran herbivore *Myzus persicae* (green peach aphid) that have expanded their host range to include tobacco often have elevated nicotine tolerance. The tobacco-adapted *M. persicae* lineage used in this study was able to reproduce on nicotine-containing artificial diets at concentrations that were 15-fold higher than those that were lethal to a non-adapted *M. persicae* lineage. Fecundity of the nicotine-tolerant *M. persicae* lineage was increased by 100 μM nicotine in artificial diet, suggesting that this otherwise toxic alkaloid can serve as a feeding stimulant at low concentrations. This lineage also was pre-adapted to growth on tobacco, exhibiting no drop in fecundity when it was moved onto tobacco from a different host plant. Although growth of the non-tobacco-adapted *M. persicae* lineage improved after three generations on tobacco, this higher reproductive rate was not associated with increased nicotine tolerance. *M. persicae* gene expression microarrays were used to identify transcripts that are up-regulated in response to nicotine in the tobacco-adapted lineage. Induced expression was found for *CYP6CY3*, which detoxifies nicotine in *M. persicae*, other genes encoding known classes of detoxifying enzymes, as well as genes encoding secreted *M. persicae* salivary proteins.

Keywords

Myzus persicae; nicotine; tobacco; cytochrome P450

INTRODUCTION

Myzus persicae (Hemiptera: Aphididae; green peach aphid) is a broad generalist phloem-feeding herbivore, consuming more than 400 plant species from over 40 families, including many economically significant crop plants (Blackman and Eastop, 2000). Lineage-specific adaptations conferring resistance to plant defenses associated with new host plants have been documented in *M. persicae* populations. For instance, artificial diet experiments show that Australian lineages of *M. persicae*, which have expanded their host range to include *Lupinus angustifoluis* (narrow-leafed lupine), are more resistant to the lupine-specific alkaloid lupanine than non-adapted lineages (Cardoza et al., 2006).

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Tobacco feeding represents another relatively recent host switch by *M. persicae.* Since the discovery of tobacco-adapted *M. persicae* in Japan in the 1900s, similar populations have been documented in several other tobacco-growing countries (Margaritopoulos et al., 2009; Zepeda-Paulo et al., 2010). Elevated resistance to both nicotine fumigation and treatment with neonicotinoid insecticides has been reported in these tobacco-adapted lineages (Devine et al., 1996). Whereas most *M. persicae* lineages are green or yellow, tobacco-adapted *M. persicae* lineages are generally dark red in color. There are, however, several examples of exceptions to this rule. More recent surveys in Japan showed an apparent increase in the number of green clones feeding on tobacco relative to previous studies (Shigehara and Takada, 2003).

Cytochrome P450 enzymes are frequently associated with the detoxification of plant secondary metabolites, including nicotine, in insects. Several tobacco-adapted lineages of *M. persicae* have genomic increases in the copy number of *CYP6CY3*, which encodes a nicotine-detoxifying cytochrome P450 enzyme (Bass et al., 2014; Puinean et al., 2010). Similarly, nicotine resistance in *Bemisia tabaci* (whitefly) and *Manduca sexta* (tobacco hornworm) is associated with specific cytochrome P450 detoxifying enzymes (Nauen and Denholm, 2005; Snyder and Feyereisen, 1993). Other resistance mechanisms in *M. sexta* include nicotine pumps that move nicotine away from the acetylcholine receptors of the nervous system (Murray et al., 1994) and excretion via the spiracles (Kumar et al., 2014).

The availability of large cDNA and genomic DNA sequence data sets for *M. persicae* has paved the way for functional genomics studies, which have the potential to identify aphid genes involved in resistance to plant defenses and insecticides (Leshkowitz et al., 2006; Ramsey et al., 2007). The goal of the current study is to examine the nicotine resistance, growth, and transcriptional responses of tobacco-adapted and non-adapted *M. persicae* lineages.

METHODS AND MATERIALS

Plant Growth and Insect Rearing

Seeds from the *N. tabacum* lines NC95 and LAFC53 were kindly provided by Dr. Ramsey Lewis (North Carolina State University). Seeds were sown in MetroMix 360 (SunGro). After two weeks, seedlings were transplanted in Cornell mix [by weight, 56% peat moss, 35% vermiculite, 4% lime, 4% Osmocoat slow-release fertilizer (Scotts), and 1% Unimix (Peters)]. Cabbage (*Brassica oleracea* var. Wisconsin Golden Acres; Seedway) was grown in Cornell mix. Plants were reared in Conviron growth chambers (60% relative humidity, 23 °C, 16:8 h light:dark cycle, 180 μmol m−2 s−1 photosynthetic photon flux density).

A tobacco-adapted red lineage of *M. persicae* was obtained from S. Gray (USDA Plant Soil and Nutrition Laboratory, Ithaca, NY, USA). A green lineage of *M. persicae*, not adapted to tobacco, was obtained in the Boyce Thompson Institute greenhouse, where it was feeding from cabbage. Aphids were raised in growth chambers on tobacco or cabbage (16:8 h light:dark cycle, 150 μmols m−2s−1, at 24°C ± 1 day, 19°C ± 1 night, 50% relative humidity).

Insect Feeding and Fecundity Assays

A stock solution of 100 mM nicotine (S-(−)-isomer, purity >99%, Fluka) in methanol was prepared and stored at −80°C. The stock solution was used to prepare aliquots of artificial diet containing varying concentrations of nicotine for use in toxicity assays. Nicotine was added to a previously described *M. persicae* diet containing water, amino acids, and sucrose (Kim and Jander, 2007). The diet was sterilized by passing through a 0.20 micron syringe filter (Corning) and stored as 10 mL aliquots at −80°C. Four adult *M. persicae* of the red or green lineages were transferred from *B. oleracea* into vented plastic cups (30 ml volume) for artificial diet experiments (Ramsey and Jander, 2008). Liquid diet was placed between two layers of Parafilm stretched over the rim of the cup. Surviving adults and offspring were counted after 3 days. Linear regression analysis was performed using R (R Development Core Team, 2005).

For tobacco fitness assays, single adult *M. persicae* of the green or red lineage were grown for at least three generations on either *N. tabacum* or *B. oleracea*, and were then transferred onto the center of a four-week old NC95 or LAFC53 *N. tabacum* plant. Individual plants were enclosed in cages and aphid progeny were counted after 6 days.

M. persicae Microarray Gene Expression Assays

M. persicae gene expression microarray slides (Ramsey et al., 2007) were obtained from Agilent (http://earray.chem.agilent.com). One slide consists of eight microarrays, each containing 15,744 60-mer oligonucleotide features, representing 10,295 unique *M. persicae* genes. Four arrays compared gene expression between heads of aphids feeding on control and 100 μM nicotine containing diets, and four arrays compared gene expression between heads of aphids feeding on control and 250 μM nicotine containing diets. For each comparison, three arrays represented biological replicates, and the fourth array was a technical replicate.

Heads were dissected from red aphids 24 hours after they were transferred from *B. oleracea* onto artificial diets containing 0, 100, or 250 μM nicotine. Aphids observed to be actively feeding from liquid diet were collected individually with a paint brush, immersed in 70% ethanol for 1–2 seconds, and placed in distilled water on a microscope slide. Aphid heads were sliced off with razor blades (Electron Microscopy Sciences) and immediately transferred by forceps into a microcentrifuge tube on dry ice. Each sample for subsequent DNA microarray experiments consisted of 15 aphid heads.

Aphid RNA was extracted using the Ribopure kit (Ambion). Tissue was homogenized using a 21 gauge needle attached to a 1 mL syringe, following the addition of 0.5 mL Tri Reagant (Ambion) to the sample. RNA quantitation was performed using the Nanodrop (Thermo Scientific). Agilent Bioanalyzer analysis was conducted by the Cornell Microarray Core Facility to confirm RNA quality.

The Amino Allyl MessageAmp II aRNA Amplification kit (Ambion) was used to prepare RNA samples for array hybridization. RNA spike-ins (Two-Color RNA Spike-In kit, Agilent) were added to each sample. Reverse transcription was performed using a T7 oligo dT primer. Following second strand synthesis and purification of cDNA, *in vitro*

transcription was carried out with amino-allyl modified UTP added to the reaction mix. The resulting amplified RNA (aRNA) including amino-allyl modified nucleotides was stored at −80°C until immediately prior to hybridization. At that time, a dye coupling reaction with the dyes Alexa Fluor 555 and 647, which conjugate with the modified UTP moieties on the RNA molecules, was performed. Purified aRNA was fragmented at 60°C following Agilent's Two-Color Microarray-Based Gene Expression Analysis protocol. Fragmentation was terminated by addition of 2x GEx Hybridization Buffer HI-RPM, and samples were placed on ice and immediately loaded onto the array.

Hybridization, wash, and scan of microarrays were performed according to the Agilent Two-Color Microarray-Based Gene Expression Analysis. Agilent scanner settings for 8×15K slide formats were used, and data were extracted using Agilent Feature Extraction software.

Microarray data analysis was carried out using the Limma package within R (Smyth, 2005; Smyth and Speed, 2003). Within-array normalization of was performed by the loess method without background subtraction. Linear modeling of arrays was performed with the lmFit function, and statistical analysis was performed using the eBayes function. Genes with a P-value ≤ 0.05 and a fold change ≥ 2 or ≤ 0.5 were considered to be differentially expressed.

Quantitative Gene Expression Analysis

Total RNA was extracted from aphid heads using the SV Total RNA Isolation Kit with on-column DNase treatment (Promega), 20 heads per sample. Transcript abundance was analyzed by quantitative real-time reverse transcription qRT-PCR, using ribosomal gene RpL7, ubiquitously expressed and likely a single copy gene, as an internal standard. After extraction and DNAse treatment, 1μg of RNA was reverse transcribed using SMART MMLV reverse transcriptase (Clonetech) and oligo-$dT_{12–18}$ as a primer. Gene specific primers were designed using Primer3 (iotools.umassmed.edu/bioapps/primer3_www.cgi). Reactions were performed with 5 μl of 2x Power SYBR Green (Applied Biosystems) and 800 nM primer in the 7900HT instrument (Applied Biosystems) with an initial incubation at 95°C for 10 min. The following cycle was repeated 40 times: 95°C for 15s, 55°C for 15s, and 72°C for 15s and C_T values were quantified and analyzed according to the standard curve method.

RESULTS

Nicotine Resistance in Field-Collected M. persicae

A red, tobacco-adapted *M. persicae* lineage and a green lineage that was not specifically adapted to tobacco were used to investigate nicotine resistance in this species. Fecundity of the two *M. persicae* strains is comparable when feeding on control diet. However, similar to what has been reported with other tobacco-adapted *M. persicae* (Bass et al., 2014), the red aphid strain was able to survive and reproduce on diets with ~15-fold higher nicotine levels those that are lethal to aphids of the green lineage (Figure 1A). The IC_{50} (nicotine concentration that inhibited aphid reproduction by 50%) was determined by linear regression analysis to be 20 μM for the green and 330 μM for the red *M. persicae* lineage. It appeared that red *M. persicae* were growing slightly better on diet with low amounts of nicotine that

on control diet (Fig. 1A). Therefore, this experiment was repeated at higher replication with only two nicotine concentrations, 0 and 100 μM, showing that nicotine can indeed increase reproduction of the red *M. persicae* strain on artificial diet (Fig. 1B).

Prior Host Effects on the Performance of Green and Red aphids on Tobacco

Tobacco lines NC95 and LAFC53, with high (~125 μg/mg) and low (~15 μg/mg) levels of nicotine, respectively (Kinnersley and Dougall, 1980; Kliot et al., 2014), were used to determine whether *in planta* nicotine concentrations affect *M. persicae* reproduction. Green *M. persicae* transferred from cabbage were able to survive and reproduce on *N. tabacum*, but their fecundity was significantly lower than that of nicotine-tolerant red aphids (Figure 2). However, when green *M. persicae* were on high-nicotine NC95 for at least three generations, they displayed significantly greater fecundity on both NC95 and LAFC53 than green aphids with cabbage as their prior host (Figure 2). Improved growth of green *M. persicae* after several generations on tobacco could result from the acquisition of increased nicotine tolerance. However, green aphids reared continuously on tobacco are not able to tolerate significantly higher levels of nicotine in artificial diets than those reared for several generations on cabbage (Figure 3). In contrast to the green strain, the red *M. persicae* strain did not show prior-host effects, appeared to be pre-adapted to grow on tobacco, and grew equally well on high- and low-nicotine tobacco (Figure 2).

Nicotine-Induced Gene Expression Changes in M. persicae

Previously described 60-mer DNA oligonucleotide microarrays representing ~10,000 unique *M. persicae* transcripts (Ramsey et al., 2007) were used to identify gene expression that is altered in red *M. persicae* by feeding from diets containing 100 or 250 μM nicotine. As salivary genes might be altered in response to nicotine feeding and the nervous system is the target of nicotine, these DNA microarray experiments were specifically focused on aphid heads.

Genes with a P-value ≤ 0.05 and a fold change ≥ 2 or ≤ 0.5 were considered to be differentially expressed. A total of 130 genes were identified as differentially expressed between tissue from control and 100 μM nicotine diets, all of which were up-regulated (Supplemental Table 1). A total of 746 genes were differentially expressed between tissue from control and 250 μM nicotine diets, of which 305 were down-regulated and 441 were up-regulated (Supplemental Table 2). Volcano plots were produced using the R Limma package depicting the log base 2 fold change (X axis) and the log odds differential expression (Y axis) for each spot on the array (Figure 4). Analysis in Limma incorporates use of a stringently adjusted P-value to control the false discovery rate. In Figure 4, spots with Y-values above zero are predicted to be differentially expressed according to the adjusted P-values. The volcano plots reveal a large number of spots from the 250 μM nicotine diet arrays to fall above 0 on the Y axis, while no spots from the 100 μM nicotine diet arrays meet this stringent standard for differential expression (Figure 4). For downstream analysis of candidate genes, the less stringent standard of the non-adjusted P-value was used to select putative differentially expressed genes. The abundance of genes in the 250 μM nicotine diet arrays predicted to be differentially expressed using the stringent adjusted P-value standard demonstrates that, as predicted from our artificial diet assays, the

aphid is mounting a much more dramatic transcriptional response to 250 μM than 100 μM dietary nicotine. However, when analyzing the data using non-adjusted P-values, it appears that the expression of many of the same well-characterized salivary (Table 1) and detoxification-associated (Table 2) genes changes in similar ways in response to both nicotine concentrations.

Eight genes with significantly altered gene expression at 100 μM and/or 250 μM nicotine in the diet (7 up-regulated genes and 1 down-regulated gene; Supplemental Tables 1 and 2) were selected for confirmation of expression changes by quantitative RT-PCR (qRT-PCR). Probes chosen with gene expression changes in both the 100 μM and 250 μM treatments were 933a, a sucrase, and 3987a, an alpha-amylase. For the 100 μM treatment 356a, a transaminase, 3298b, an aminopeptidase, and 254a, a cathepsin gene, were chosen. 3435a, 479a and 9835a, an alpha-glucosidase, a cytochrome P450 *CYP6CY3*, and a protein with no predicted function, respectively, were analyzed from aphids feeding on 250 μM nicotine. Samples of aphids feeding from diet with and without 100 μM or 250 μM nicotine were generated independently from those used for microarray experiments. In each case, the expression changes observed in the DNA microarray experiments were independently verified by qRT-PCR (Figure 5).

Enzymatic activity detected in aphid saliva includes α-amylase, α-glucosidase, glucose dehydrogenase, sucrase, and trehalase (Miles, 1999). Genes predicted to encode such enzymes were up-regulated in response to both 100 and 250 μM nicotine (Table 1). Out of all differentially expressed genes, predicted sucrases and α-amylases are among those most highly induced by nicotine, with 12- to 14-fold increases in gene expression. Several proteomic studies have identified secreted aphid salivary proteins (Carolan et al., 2011; Carolan et al., 2009; Cooper et al., 2010; Cooper et al., 2011; Cui et al., 2012; Harmel et al., 2008b; Nicholson et al., 2012; Rao et al., 2013; Will et al., 2012). Expression of some of secreted aphid salivary genes is regulated by the host plants on which *M. persicae* are feeding (Elzinga et al., 2014). Similarly, eleven *M. persicae* genes that had been previously identified by proteomics of aphid saliva (Carolan et al., 2009; Harmel et al., 2008a) are differentially regulated in response to nicotine in the aphid diet (Table 1).

Enzymes implicated in the detoxification of xenobiotics by *M persicae* and other insects include carboxylesterases, glutathione-*S*-transferases (GSTs), and cytochrome P450 monooxygenases (P450s) (Ramsey et al., 2010). *M. persicae* contigs annotated as FE4 carboxylesterases, represented by five unique microarray probes, were induced by 250 μM nicotine, yet displayed no significant change in response to 100 μM nicotine (Table 2). Among 24 *M. persicae* contigs that are annotated as GSTs, three genes were up-regulated by 100 μM nicotine and seven genes, represented by ten microarray robes, were up-regulated by 250 μM nicotine. Among 56 contigs that are annotated as P450s. Three P450s were induced in aphid heads by100 μM nicotine, and a different set of eight P450s was induced by 250 μM nicotine (Table 2).

An additional nicotine-induced gene potentially associated with aphid salivation is a 1,4-beta-xylanase (contig 3525, orthologous to *A. pisum* gene ACYPI005168), which is predicted to be involved in the degradation of hemicellulose, a major component of plant

cell walls. This enzyme activity is typically associated with microbes from insect guts, where it plays an essential role in hydrolyzing degradation-resistant cell wall polysaccharides (Brennan et al., 2004). Aphid xylanase may play a role in clearing a path for the stylet through cell wall hemicellulose as the insects probe intercellularly to initiate feeding from the plant phloem.

DISCUSSION

Nicotine is synthesized in roots and transported via the xylem to foliar tissues in tobacco. To the best of our knowledge, nicotine levels in tobacco phloem sap have not been quantified. However, tobacco-feeding *M. persicae* are likely to also come into contact with nicotine through periodic ingestion of xylem sap and through exposure to alkaloid secretions by glandular trichomes (Thurston et al., 1966). Given that the green *M. persicae* strain used in this study can only tolerate 10 μM nicotine in artificial diet (Figure 1), the concentration that the insects encounter when reproducing on tobacco is likely lower than this.

There is significant variation in the nicotine tolerance among natural isolates of *M. persicae* (Figure 1; Bass et al., 2014). This suggests that the development of nicotine resistance has accompanied host range expansion to include tobacco. Nevertheless, higher-level nicotine resistance is not essential for *M. persicae* and *B. tabaci* feeding from tobacco in a laboratory setting. After being reared on tobacco in the lab for several generations, the green, non-adapted *M. persicae* lineage showed improved fecundity on the high-nicotine NC95 genotype (Figure 2). However, this tobacco exposure did not significantly enhance nicotine tolerance in artificial diets (Figure 3). Therefore, it is likely that additional defenses, other than nicotine, limit the reproduction of non-adapted *M. persicae* on tobacco.

Growth of the red *M. persicae* strain is improved by addition of 100 μM nicotine to artificial diet (Figure 1). Although nicotine is a possible source of nitrogen and could lead to improved growth, this hypothesis is unlikely because the artificial diet contains each of the 20 protein amino acids at much higher (millimolar) levels. An alternate hypothesis, that nicotine is a feeding stimulant, is consistent with prior results showing that a tobacco-adapted *M. persicae* strain used both olfactory cues and contact-mediated cues to identify tobacco as a preferred host plant (Vargas et al, 2005). Our observation of a lag in growth of the green aphid strain upon transfer to tobacco (Figure 2) is similar to published results (Olivares-Donoso et al., 2007). In contrast, our red strain seems to be pre-adapted for tobacco feeding and does not suffer a growth lag when transferred from cabbage to tobacco.

Gene expression changes in tobacco-adapted *M. persicae* in response to nicotine include representatives of the three main classes of insect detoxification genes, cytochrome P450s, carboxylesterases, and glutathione S-transferases (Table 2). Unlike the salivary enzymes, which were generally induced at both nicotine concentrations (Table 1), the P450 and GST genes induced by the lower dose of nicotine are not among the genes induced by the higher, growth-inhibiting dose, suggesting that the detoxification response to different levels of nicotine may be fundamentally different.

Among the cytochrome P450s that were induced by nicotine (Table 2), seven belong to the CYP6 class, which has been implicated in insect responses to allelochemicals (Scott et al., 1998; Yang et al., 2005), suggesting that these genes are induced as a detoxification response. Three elements on the microarray (497a, 497b, and 5173a) correspond to *CYP6CY3*, a gene that is amplified in the genomes of tobacco-adapted *M. persicae* strains (Bass et al., 2014). Our results show that this gene is not only expressed at a constitutively higher level in nicotine-tolerant *M. persicae*, but is also induced by the presence of nicotine (Table 2 and Figure 2B). Therefore, induced gene expression may facilitate tobacco feeding by this *M. persicae* isolate.

Together, the results presented here show differences in the responses to plant defenses among tobacco-adapted and non-adapted *M. persicae*. DNA microarray expression studies point to genes that may contribute to induced nicotine resistance and improved growth on tobacco. One of the identified genes, *CYP6CY3*, has a known function in nicotine detoxification, and future analysis of other nicotine-induced genes form this study may identify other mechanisms of nicotine tolerance and detoxification in *M. persicae*. A better understanding of the physiological, behavioral and molecular processes underlying nicotine resistance in *M. persicae* will shed light on the host range expansion of these polyphagous herbivores, and may inform future crop protection strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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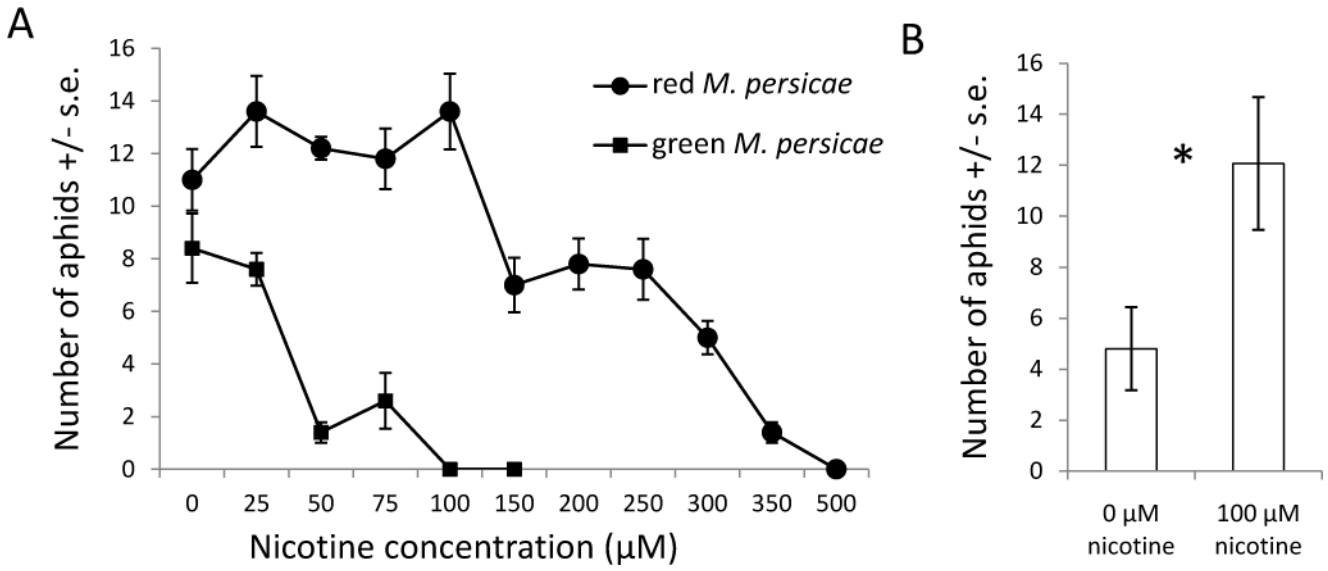

Figure 1.

(A) Fecundity of red and green *M. persicae* strains on nicotine-containing artificial diets. Progeny from four adult aphids were counted after three days. Mean +/− s.e. of n = 5. (B) When 100 µM nicotine was added to artificial diet, the number of offspring produced by tobacco-adapted red *M. persicae* increased. Progeny from four adult aphids were counted after three days. Mean +/− s.e. of N = 15, *P < 0.05 by 2-tailed Student's t-test.

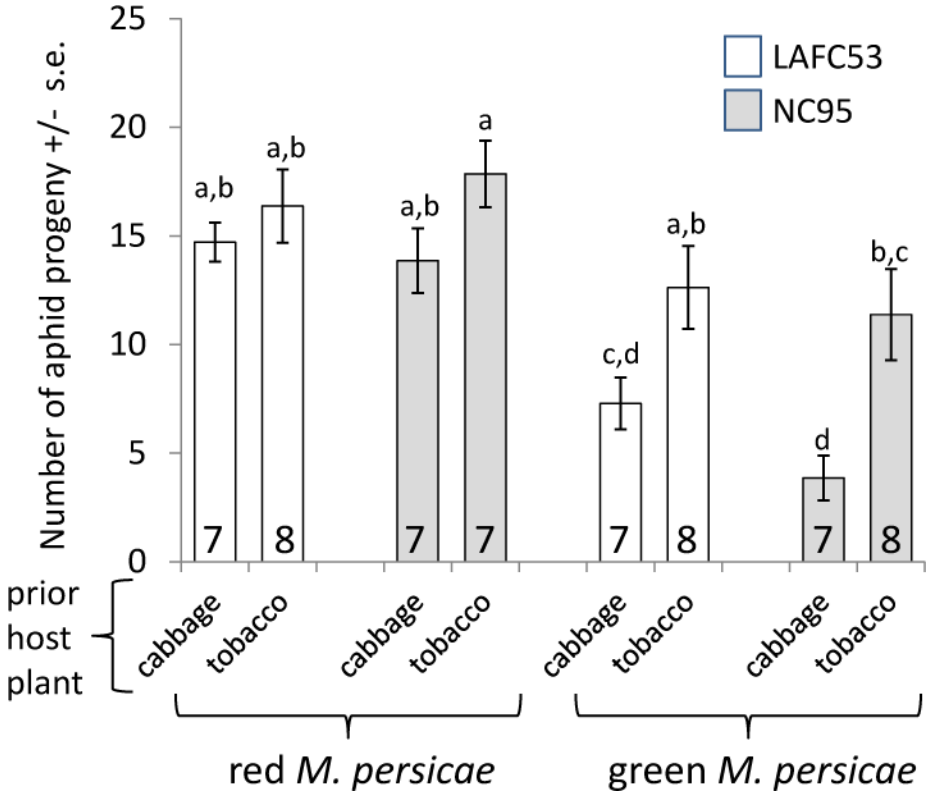

Figure 2.

Fecundity of green but not red *M. persicae* on tobacco is improved by prior growth on NC95 tobacco rather than cabbage. Aphids were raised for at least three generations on cabbage or tobacco (NC95). Single adult aphids were caged on four-week old tobacco plants with high (NC95) or low (LAFC53) nicotine, and progeny were counted after six days. Mean +/− s.e.; numbers in bars indicate sample sizes; different letters show significant differences, $P < 0.05$, ANOVA followed by Tukey's HSD.

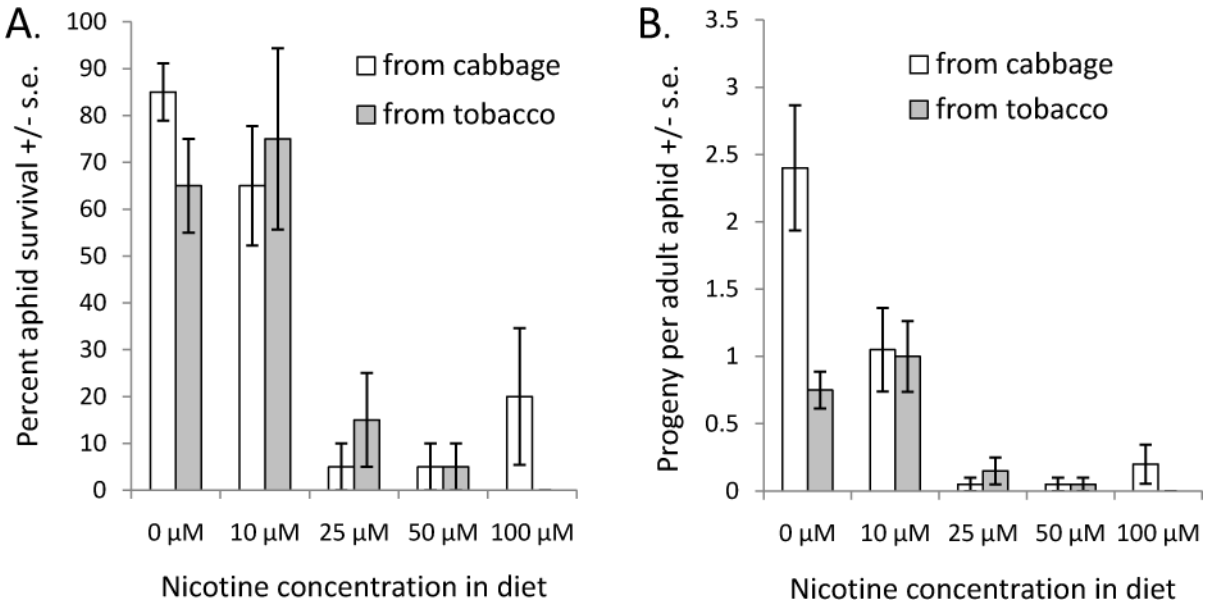

Figure 3.

Prior feeding from tobacco does not increase nicotine tolerance of green *M. persicae*. Aphids were raised for at least three generations on cabbage or tobacco (NC95) before being moved to artificial diet with differing amounts of nicotine. Adult aphid survival (A) and progeny production (B) were assessed after three days. No significant differences were observed between cabbage and tobacco-derived aphids, with the exception of progeny production on diet without nicotine ($P < 0.05$, *t*-test). All data represent mean +/− s.e. of n = 5.

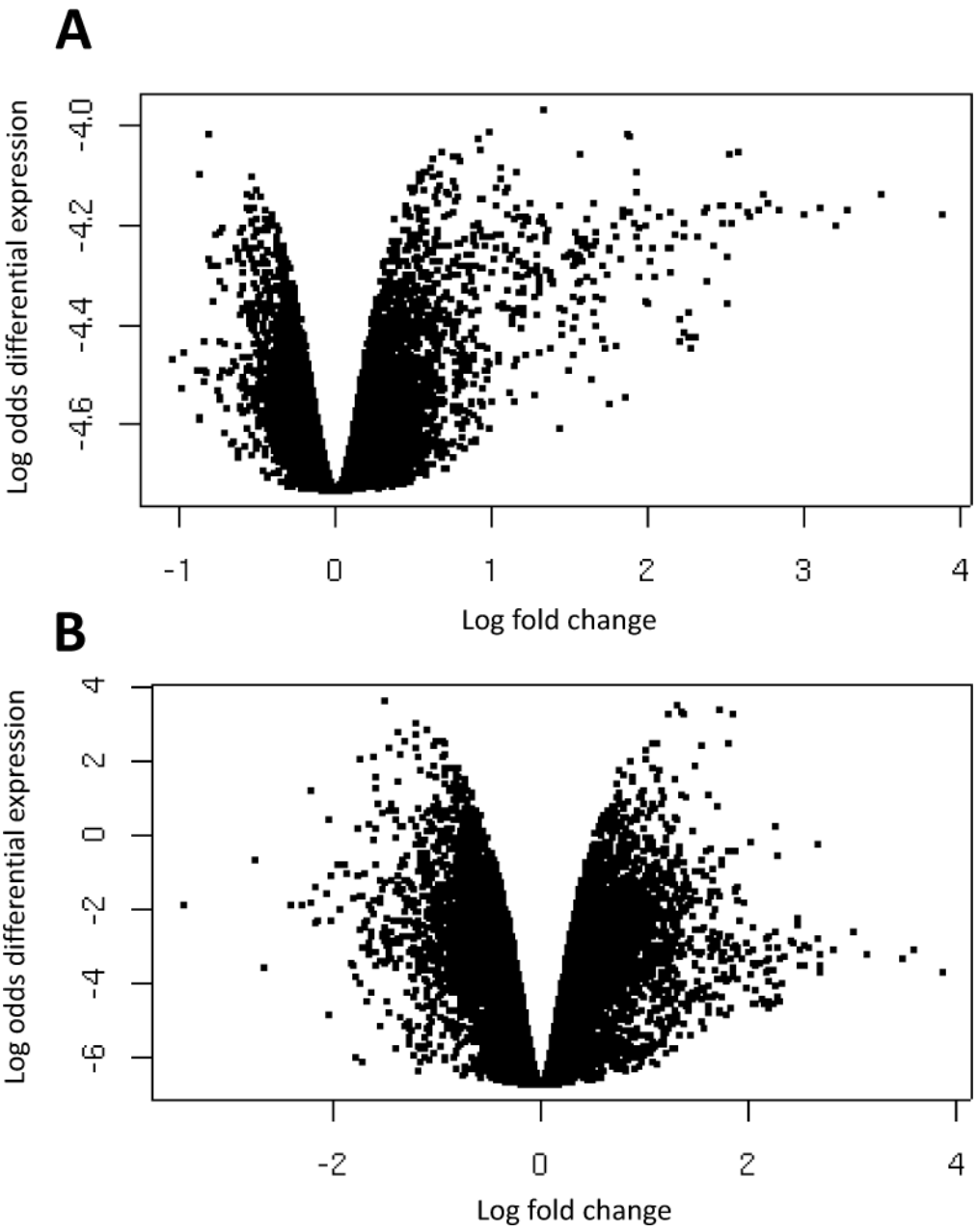

Figure 4.

Volcano plots depicting Log fold change (X axis) vs Log odds differential expression (Y axis) for each spot on the *M. persicae* gene expression microarrays. (A) Effect of 100 μM nicotine diet on gene expression in red aphid heads; (B) Effect of 250 μM nicotine diet on gene expression in red aphid heads.

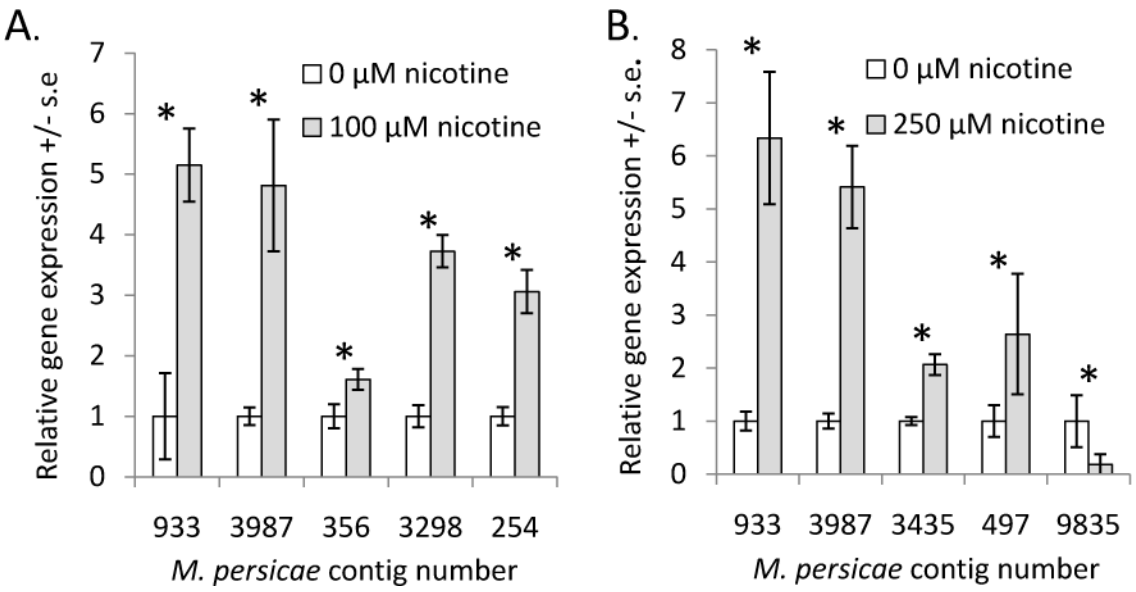

Figure 5.

Confirmation of DNA microarray gene expression data by quantitative reverse transcriptase-PCR (qRT-PCR). Five genes with significantly altered expression at 100 µM and/or 250 µM nicotine (Supplemental Tables 1 and 2) were chosen for confirmation by qRT-PCR using independently generated aphid samples. All data are normalized so that expression of the respective genes on diet without nicotine is set to one. Mean +/− s.e. of n = 5. *P < 0.05, two-tailed *t*-test.

Table 1

Aphid genes associated with salivary enzymes induced by nicotine in artificial diet.

ProbeID	Nicotine Concentration	Predicted enzyme activity	Gene ID of closest *A. pisum* ortholog	Fold Change
3682a	100 μM	alpha-amylase	ACYPI007122	11.23
3682b	100 μM	alpha-amylase	ACYPI007122	5.50
3682a	250 μM	alpha-amylase	ACYPI007122	11.31
3682b	250 μM	alpha-amylase	ACYPI007122	6.40
3987a	100 μM	alpha-amylase	ACYPI007122	9.71
3987b	100 μM	alpha-amylase	ACYPI007122	8.57
3987a	250 μM	alpha-amylase	ACYPI007122	12.12
3987b	250 μM	alpha-amylase	ACYPI007122	8.81
2868a*	100 μM	alpha-amylase	ACYPI009042	3.45
2868b*	100 μM	alpha-amylase	ACYPI009042	3.55
2868a*	250 μM	alpha-amylase	ACYPI009042	4.53
2868b*	250 μM	alpha-amylase	ACYPI009042	4.56
3812a	100 μM	alpha-amylase	ACYPI009042	3.38
3812a	250 μM	alpha-amylase	ACYPI009042	4.62
3812b	250 μM	alpha-amylase	ACYPI009042	5.85
8554a	100 μM	alpha-glucosidase	ACYPI001718	3.73
3806b	100 μM	alpha-glucosidase	ACYPI001718	3.13
3435b	100 μM	alpha-glucosidase	ACYPI005549	4.05
3435a	250 μM	alpha-glucosidase	ACYPI005549	8.11
3435b	250 μM	alpha-glucosidase	ACYPI005549	5.09
6126a	250 μM	alpha-glucosidase	ACYPI008059	2.42
4022a	250 μM	S1sucrase	ACYPI000002	3.45
4022b	250 μM	S1sucrase	ACYPI000002	5.65
7717a	250 μM	S1sucrase	ACYPI000002	3.13
933a	100 μM	sucrase	ACYPI001436	14.72
933b	100 μM	sucrase	ACYPI001436	6.19
933a	250 μM	sucrase	ACYPI001436	14.6
933b	250 μM	sucrase	ACYPI001436	5.81
10020a	250 μM	sucrase	ACYPI001436	3.63
10020a	100 μM	sucrase	ACYPI001436	2.11
688a◆	250 μM	glucose dehydrogenase	ACYPI000113	2.60
688b◆	250 μM	glucose dehydrogenase	ACYPI000113	2.82
1461a	250 μM	trehalase	ACYPI002298	2.34
1461b	250 μM	trehalase	ACYPI002298	2.29
3014a*	250 μM	glucuronyltransferase I	ACYPI000921	−2.16

ProbeID	Nicotine Concentration	Predicted enzyme activity	Gene ID of closest *A. pisum* ortholog	Fold Change
3014b*	250 μM	glucuronyltransferase I	ACYPI000921	−2.06
4b*	250 μM	membrane protein	ACYPI008945	3.94
3414a*	250 μM	C002; unknown function	ACYPI008617	4.49
5790a◆	250 μM	M1 zinc metalloprotease	ACYPI009427	−2.73

*proteomic identification by Harmel et al 2008

◆proteomic identification by Carolan et al 2009

Table 2

M. persicae detoxification genes induced by nicotine in artificial diet experiments.

ProbeID	Nicotine Concentration	Predicted enzyme activity	Gene ID of closest *A. pisum* ortholog	Fold Change
1501a	100μM	CYP6AX1	ACYPI000639	2.11
1504b	100μM	cytochromeP450	ACYPI002079	2.07
8290a	100μM	cytochromeP450	ACYPI010012	2.37
9053a	250μM	cytochromeP450	ACYPI001913	2.31
970a	250μM	CYP6AX1	ACYPI008473	2.11
970b	250μM	CYP6AX1	ACYPI008473	2.54
497a	250μM	CYP6CY3	ACYPI008843	2.58
497b	250μM	CYP6CY3	ACYPI008843	3.29
5173a	250μM	CYP6CY3	ACYPI008843	2.92
5223a	250μM	CYP6AX1	ACYPI003528	2.12
8290a	250μM	cytochromeP450	ACYPI010012	3.60
9095a	250μM	CYP6AX1	ACYPI003528	2.23
3931b	250μM	cytochromeP450	ACYPI005477	2.47
720a	250μM	carboxylesterase	ACYPI000631	3.03
720b	250μM	carboxylesterase	ACYPI000631	3.16
3118a	250μM	carboxylesterase	ACYPI000631	2.26
3118b	250μM	carboxylesterase	ACYPI000631	2.29
9215a	250μM	carboxylesterase	ACYPI000631	3.73
1196b	100μM	glutathione S-transferase	ACYPI009519	2.56
4744a	100μM	glutathione S-transferase	ACYPI009519	3.78
1196a	100μM	glutathione S-transferase	ACYPI009519	3.68
7666a	250μM	glutathione S-transferase	ACYPI009519	2.18
3031a	250μM	glutathione S-transferase	ACYPI002679	2.32
3031b	250μM	glutathione S-transferase	ACYPI002679	2.23
3676b	250μM	glutathione S-transferase	ACYPI000794	2.11
3677a	250μM	glutathione S-transferase	ACYPI000794	3.48
3677b	250μM	glutathione S-transferase	ACYPI000794	2.82
3678a	250μM	glutathione S-transferase	ACYPI000794	2.00
3678b	250μM	glutathione S-transferase	ACYPI000794	2.15
4715a	250μM	glutathione S-transferase	ACYPI000794	2.18
8694a	250μM	glutathione S-transferase	ACYPI000794	2.31