Supporting Information Corrected February 16, 2016

Supporting information

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Materials and Methods

Stem-feeding nicotine to *N. attenuata* leaves

Completely expanded mature rosette stage *N. attenuata* leaves were detached from the plant along with their petioles. Through the perforated lid of a Teflon tube (15cc), the petiole was immersed in either a 1mM nicotine or water (control) solution, as shown in Fig. S1*D*. These tubes were incubated for 24h at 26°C/16h light, 24°C/ 8h dark and 60% humidity in a growth chamber (Snijders Scientific). After 24h of stem-feeding, leaves were harvested for the analysis of nicotine contents (Fig. S1*E* and *F*) using HPLC (Agilent 1100 series) or fed to *M. sexta* larvae.

RNA isolation and quantitative real time PCR

Persistence of CYP6B46 silencing during nicotine flux determination experiments.

In the nicotine flux determination experiments, to render the larvae nicotine-free or to feed them diets with the same nicotine contents, 4th instar *CYP*-silenced larvae were fed (for 6-12h) on ir*PMT* or EV plants, respectively. To evaluate if the *CYP* silencing persisted in such *CYP*-silenced larvae when they were feeding on other hostplants not expressing dsRNA of *MsCYP*6B46, *CYP*6B46 transcripts were profiled in their midguts after larvae had fed on ir*PMT* or EV plants for 24h (Fig. S5*B*).

Waldbauer assays for nicotine budgeting

Freshly hatched *M. sexta* neonates were placed on leaves of EV, ir*CYP* and ir*PMT* rosette-stage plants and larvae were allowed to feed until they had reached the 4th instar. The mass of each larva was recorded. All larvae were then starved for 4h to empty their guts. Again after starvation, the mass of each larva was measured. Each larva was then provided with a known mass of leaf material from the same genotype of plant that they had been fed previously. Larvae were allowed to feed for 24h in an incubator maintained at 26°C/ 16h light, 24°C/ 8h dark and 60% relative humidity. Blotting paper disks of known masses were placed at the bottom of the assay container to absorb any excreted liquids. After 24h of feeding, the mass of each larva and the mass of remaining leaf material were recorded. All larvae were again subjected to 4h of starvation, during which time they had emptied their guts. Frass excreted by each larva, during the 24h of feeding and the 4h of starvation were collected, weighed along with the paper disk and stored at -80°C until further use. Mass lost by the leaves of each line, due to evaporation in the incubator during the 24h assay was recorded and used to correct wet-to-dry mass conversion values. Nicotine levels of fresh and

weight-loss leaves of each line were measured by HPLC (Agilent 1100 series) (see 'Extraction and quantification of nicotine'). The mass lost by the leaves of each line in the 24h feeding trial was used to calculate the exact amount of leaf material not consumed by each larva after 24h; this allowed for the precise quantification of the amount of nicotine ingested by each larva. Nicotine levels in the collected frass samples (along with the blotting paper discs) were also measured and corrected considering the mass of the blotting paper disk. Percentage of nicotine excreted was determined by calculating the ratio of the amount of nicotine excreted/ amount of nicotine in the ingested food X 100.

Volatile nicotine trapping

In all the experiments, before measuring volatile nicotine, larvae were washed with water at least three times, in order to remove any potential background signal of exoskeleton-adsorbed nicotine arising from direct larval contact with the food. 1mL water was used for each wash of 2nd instar larvae and 20mL water was used for 4th instar larvae. Larvae were washed until the nicotine concentration of the wash reduced below the detectable limit of HPLC/ESI-Q3-MS (Varian 1200) (see 'Extraction and quantification of nicotine').

Measuring nicotine in larval headspace

Washed larvae were placed in a sealed glass vial (5cc) fitted with a PDMS tube suspended in the headspace from the seal with a solid needle; an injection needle (0.08X40mm, BRAUN, Germany) was inserted in the seal for ventilation (Fig. S6*B*). Each larva was incubated in this setup for 1h at 30^{o}C, before extracting nicotine from the PDMS tube (see 'Extraction and quantification of nicotine'). A standard curve based on the amounts of PDMS-adsorbed nicotine was analyzed to evaluate the linear response of PDMS to increasing nicotine concentrations. We incubated 3, 6, 12, 25, 50, 100 or 200 ng nicotine in the 5µL methanolic solution in the vial of the volatile trapping setup (4 replicates per nicotine concentration) for 1h. Nicotine adsorbed on the PDMS tube was extracted and measured by HPLC/ESI-Q3-MS (Varian 1200). A standard curve was used to evaluate the linearity of adsorption of volatile nicotine onto PDMS; the response was linear (R^2= 0.98) over a range of 0-200ng of volatilized nicotine (Fig. S6*C*). To evaluate how much of the nicotine present in the trapping vial had the potential to be volatilized and therefore could be adsorbed to the PDMS tube, 100ng nicotine (n= 4) was allowed to equilibrate with the headspace of a vial for 1h. After incubation, 60± 6% (mean± SE; wt/wt)) nicotine was found to be adsorbed on the PDMS tube and 34± 3% (mean± SE;wt/wt) remained in the vial, suggesting that almost all the volatilized nicotine was adsorbed on the PDMS tube. Therefore in the choice and no-choice

predation assays, the amount of nicotine recovered from the PDMS tubes proportion was considered to be the same as that emitted by the larvae.

To quantify the nicotine emitted by the spider's larval prey, we evaluated how much of the volatile nicotine (present in the vial) was adsorbed on the PDMS tube. PDMS tubes were individually exposed to 100ng nicotine (n= 4) for 1h. After incubation, nicotine adsorbed on the PDMS tube was extracted and measured by HPLC/ESI-Q3-MS (Varian 1200). Nicotine that did not volatilize and remained in the vial after the 1h assay was also collected and quantified; to measure the amount of nicotine lost during the collection of the non-volatilized nicotine remaining in the vial, we extracted vials that had been spiked with 100ng nicotine immediately after the nicotine was applied (allowing the least time for volatilization) and quantified the nicotine. Since all the applied nicotine could be recovered from this rapid collection, the efficiency of collection was considered 100%. The amount of nicotine missing from the collection after 1h incubation was considered to be volatilized; the proportion of this missing nicotine that was actually recovered from the PDMS tube was calculated and this proportion was used to calculate the quantity of nicotine emitted by the 2nd instar larva (used in the choice and no-choice predation assays).

Headspace-nicotine during the no-choice assays with perfuming

A PDMS tube was suspended in each no-choice assay container from the seal attached to a solid needle, immediately after placing the larva and the cotton swab used to perfume the chamber. Volatile nicotine in the headspace of larva used in water- or nicotine-perfumed no-choice assay was allowed to adsorb on the PDMS tube for 1h. Adsorbed nicotine was extracted and quantified by HPLC/ESI-Q3-MS (Varian 1200) (see 'Extraction and quantification of nicotine'); since these quantifications were relative, the values were used only for comparing the headspace nicotine content of the water- and nicotine-perfumed larvae.

Trapping nicotine emitted from spiracle and cuticle

Larvae that bled after injection were not used in the analysis. The injection site was carefully cleaned, the wipes were extracted and the nicotine concentration of these wipes was determined using HPLC/ESI-Q3-MS (Varian 1200) (see 'Extraction and quantification of nicotine'). Larvae which had leaked some of the injected nicotine onto the cuticle around the injection site were also not included in the analysis. Any potential differences in nicotine emission from the different larval spiracles were randomized amongst treatments by randomly

selecting a different sampling spiracle in every biological replicate. Sampling-location bias was likewise avoided by randomly selecting a different sampling body segment, in every biological replicate.

Not all the nicotine emitted by the sampled spiracle was adsorbed to the PDMS tube and nicotine lost to the environment could not be quantified. Therefore the results obtained from this analysis were only used to compare the relative emissions of control and *CYP*-silenced larvae.

Extraction and quantification of nicotine and other secondary metabolites

Leaf and larval frass

1mL nicotine extraction buffer A [60% methanol (vol/vol) containing 0.05% glacial acetic acid(vol/vol)] was added to 100mg crushed leaf or to 50mg crushed frass in a 2mL Eppendorf tube. Samples containing extraction buffer were homogenized using ceramic beads (0.9 g: Sili GmbH, Germany) on Geno/Grinder 2000 (Elvatech, Ukraine) for 2min with 600 strokes/min. Homogenized samples were centrifuged at 13.4 g for 20min, at RT. Supernatant was transferred to a fresh 1.5mL Eppendorf tube and was centrifuged again at 13.4g for 20min at 4°C. Clear supernatant was collected and analyzed on HPLC (Agilent 1100 series) as described by Keinaenen *et al* (49).

Chlorogenic acid, caffeoyl putrescine, rutin and diterpene glycosides were extracted from the EV and ir*CYP* developmentally-matched leaves of plants growing in the field-plot by the above procedure and analyzed by HPLC (Agilent 1100 series) as described by Keinaenen *et al* (49).

Analysis of cotinine, CNO and NNO

Cotinine was procured from Sigma-Aldrich (Germany). Cotinine N-oxide (CNO) was synthesized as described by Dagne and Castagnoli (1) and nicotine 1-N-oxide (NNO) was synthesized as described by Craig and Purushothaman (2). Retention times and molecular ions of these compounds were determined by loading 1ng of each of these compounds onto a Phenomenex Gemini NX 5 (5 x 2.0 mm) U(H)PLC column (particle size 3 µM) with solvent A [0.1% ammonium hydroxide (vol/vol) in ultrapure Millipore H_2O, pH 10] and solvent B (100% methanol). The gradient of 0 min/ 5% B, 0.5min/ 5% B, 2 min/80% B, 6.5min/80% B, 8.5min/5% B, 10min/ 5% B was used. Compounds were detected using a qToF-mass spectrometer (microTOF QII Bruker Daltonik, Bremen, Germany) equipped with an electrospray ionization (ESI) source in positive ion mode (instrument settings: capillary

voltage, 4500V; capillary exit, 130V; dry gas temperature, 200C; dry gas flow, 8L/min). Calibration was performed using sodium formate clusters [10 mM solution of NaOH in 50/50% (vol/vol) isopropanol/water containing 0.2% formic acid (vol/vol)]. 0.05, 0.25, 0.5, 1.0, 2.0 or 4.0ng of each above-mentioned compound (3 replicates/ each concentration of compound) were analyzed. For each compound, the lowest amount (among these injected quantities) that was detected by the microToF MS was considered to be the limit of detection.

The efficiency of extraction of each compound from frass was determined as follows. Crushed dried frass (50mg) of ir*PMT* fed larvae was spiked with 50, 250, 500, 1000, 2000 or 4000ng nicotine, cotinine, CNO or NNO (3 replicates/ each concentration of compound). Spiked frass samples were extracted in 1mL extraction buffer C [60% methanol (vol/vol) containing 0.05% glacial acetic acid (vol/vol) and 1µg each of d_3-nicotine, d_3-cotinine, d_3-CNO and d_3-NNO (Cambridge Isotope Laboratories Inc, USA) as internal standards]. 1µL of these extracts were chromatographed and detected with a microToF mass spectrometer, as mentioned above. The detected quantity of the spiked compound was calculated relative to its respective d_3-internal standards from which the efficiency of recovery of each compound from the frass was calculated. The efficiency of extraction from hemolymph was assumed to be 100% for all the compounds.

To detect and quantify nicotine, cotinine, CNO and NNO in the frass or hemolymph of control or *CYP*-silenced larvae, 50mg frass or 50µL hemolymph was extracted in 1mL extraction buffer C. In addition, to be able to detect these nicotine metabolites that might be present in the frass at lower concentrations than that of nicotine, a aliquot of each extract was concentrated 5-fold under vacuum. 1µL and 10µL of the original extracts and 10µL of the concentrated extracts were chromatographed, as described above.

Field predation assays and no-choice assays with *G. pallens* and antlions

To evaluate the effect of dietary nicotine on *M. sexta*'s survival, we exposed control and ir*PMT*-fed larvae to the diurnal predators in the native habitat of *N. attenuata* (Tab *S1*). In 2004, we exposed day-old, 1st instar larvae fed ir*PMT* or EV foliage in pairs on the same plants in the field plantation, in 3 field assays. In this field plantation in 2004, 0.22 ± 0.01 individuals (mean± SE) of this predator were observed per plant (monitored every alternate day for 14d; n=1982 plant observations). Survival of *M. sexta* larvae was recorded after 5h during the daytime and in the latter 2 assays, larvae were pre-fed on excised WT leaves that were stem-fed in a 1mM nicotine solution, whereas ir*PMT* leaves were only supplied with water to enhance the difference in nicotine levels. Additionally, 1st instar *M. sexta* larvae fed

for 24h on WT, ir*PMT* plants were placed on plants of the same genotype growing in the field plantation (2004, groups of three larvae were applied to a plant) or a native population (2005; dead or missing larvae were replaced during the first 3 days with new 1st instar larvae of the same age maintained on the same genotype in boxes). In 2012, survival of 2nd instar larvae fed on EV, ir*CYP* or ir*PMT* plants was assessed for 2d after placing on plants of the same genotypes in the field plantation. During all the diurnal survivorship assays, predation events by *G. pallens* were commonly observed. In 2013, diurnal and nocturnal predation rates on 2nd instar larvae fed on EV, ir*CYP* or ir*PMT* plants were determined separately.

In no-choice assays, survival of 2nd instar *M. sexta* larvae fed WT, ir*PMT*, or ir*CYP* plants was determined when larvae were exposed to predation by antlion larvae or *G. pallens* adults (Table *S4*). Survival of a single larva that was enclosed in soufflé cups (Solo 29.6mL, P100, Urbana, IL) with a moist paper tissue and a *G. pallens* individual was assessed after 1h (in 2012 and 2013). One larva was dropped in each antlion sand pit in the field (in 2004) as well as in the sand pits formed in soufflé cups (Solo 29.6mL, P100, Urbana, IL) by field collected antlion larvae (in 2012/13) and the immediate response (feeding or rejection) as described in Eisner *et al* (29) and larval survival after 1h were recorded.

Supporting Figures

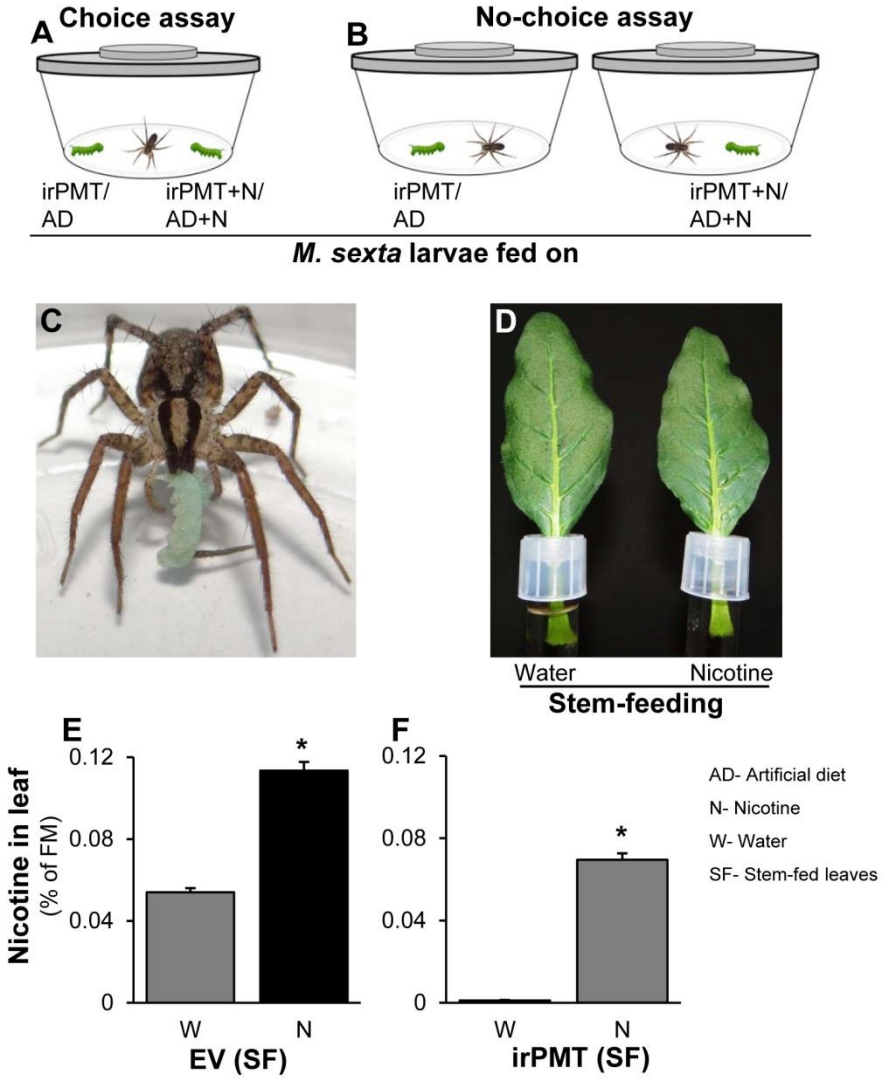

Fig S1. Setups for spider assays and stem-feeding nicotine into leaves and nicotine levels in control and stem-fed leaves

Schematic representation of the setup used for spider's **(*A*)** choice and **(*B*)** no-choice assays; both types of assays were conducted for 1h in 50mL polypropylene containers. **(*C*)** Spider feeding on 2nd instar *M. sexta* larva, during a no-choice assay. **(*D*)** Setup used to stem-feed leaves with water or 1mM nicotine. Nicotine levels [% (wt/wt) of FM] of **(*E*)** EV ($F_{1, 16}$= 57.14, $P \leq 0.0001$, n=5) and **(*F*)** ir*PMT* ($F_{1, 10}$= 444.45, $P \leq 0.0001$, n=6) leaves stem-fed water (W) or 1mM nicotine (N). Asterisks indicate significant differences determined by one way ANOVA. See Fig. 1 legend for the bar-shading codes.

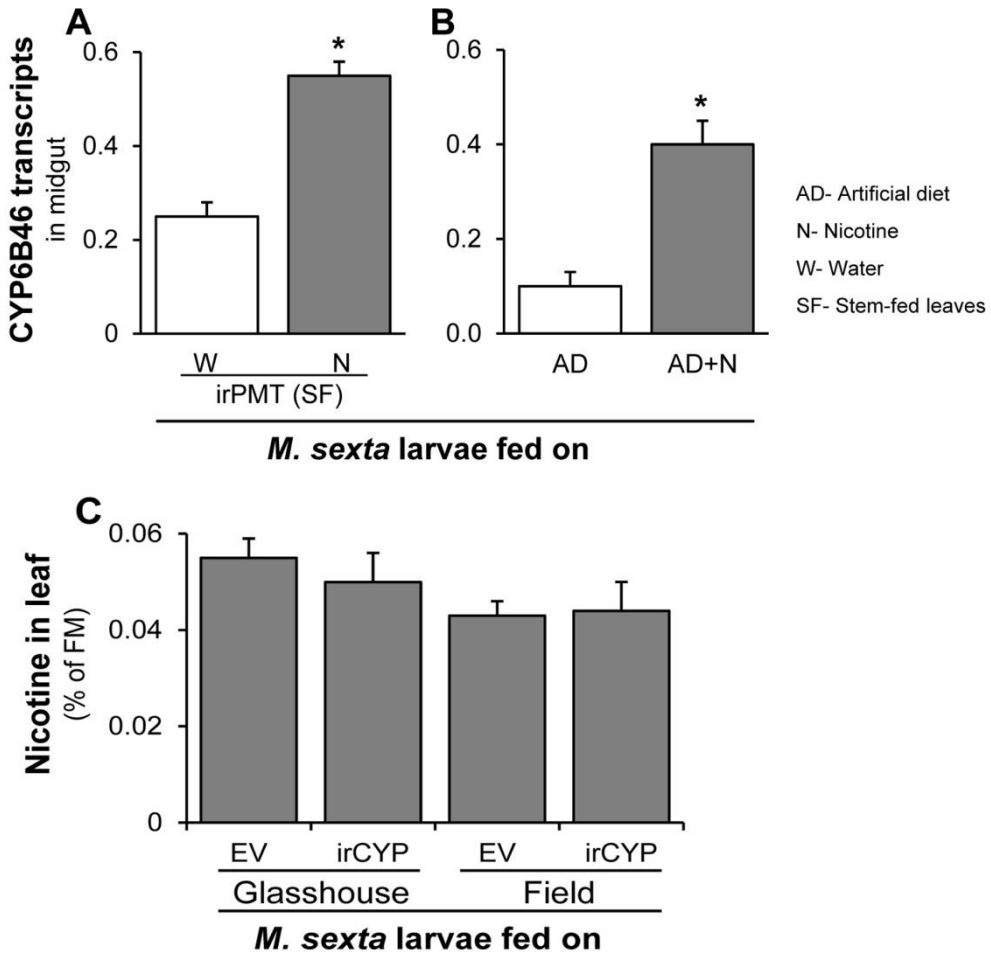

Fig S2. Midgut *MsCYP6B46* transcript levels in larvae feeding on *N. attenuata* leaves and artificial diets differing in nicotine contents

M. sexta CYP6B46 transcript levels (relative to ubiquitin) in midguts of 1st instar larvae fed (for 24h) on **(*A*)** W or N stem-fed ir*PMT* leaves ($F_{1, 8}$= 116.25, $P \leq 0.0001$, n=5) and **(*B*)** AD and AD containing 0.1% (wt/wt) of nicotine (AD+N) ($F_{1, 8}$= 8.10, $P \leq 0.05$, n=5). **(*C*)** Nicotine levels [% (wt/wt) of FM] of the leaves of EV and ir*CYP* plants grown in the glasshouse and the field (n=3). See Fig. 1 legend for the bar-shading codes.

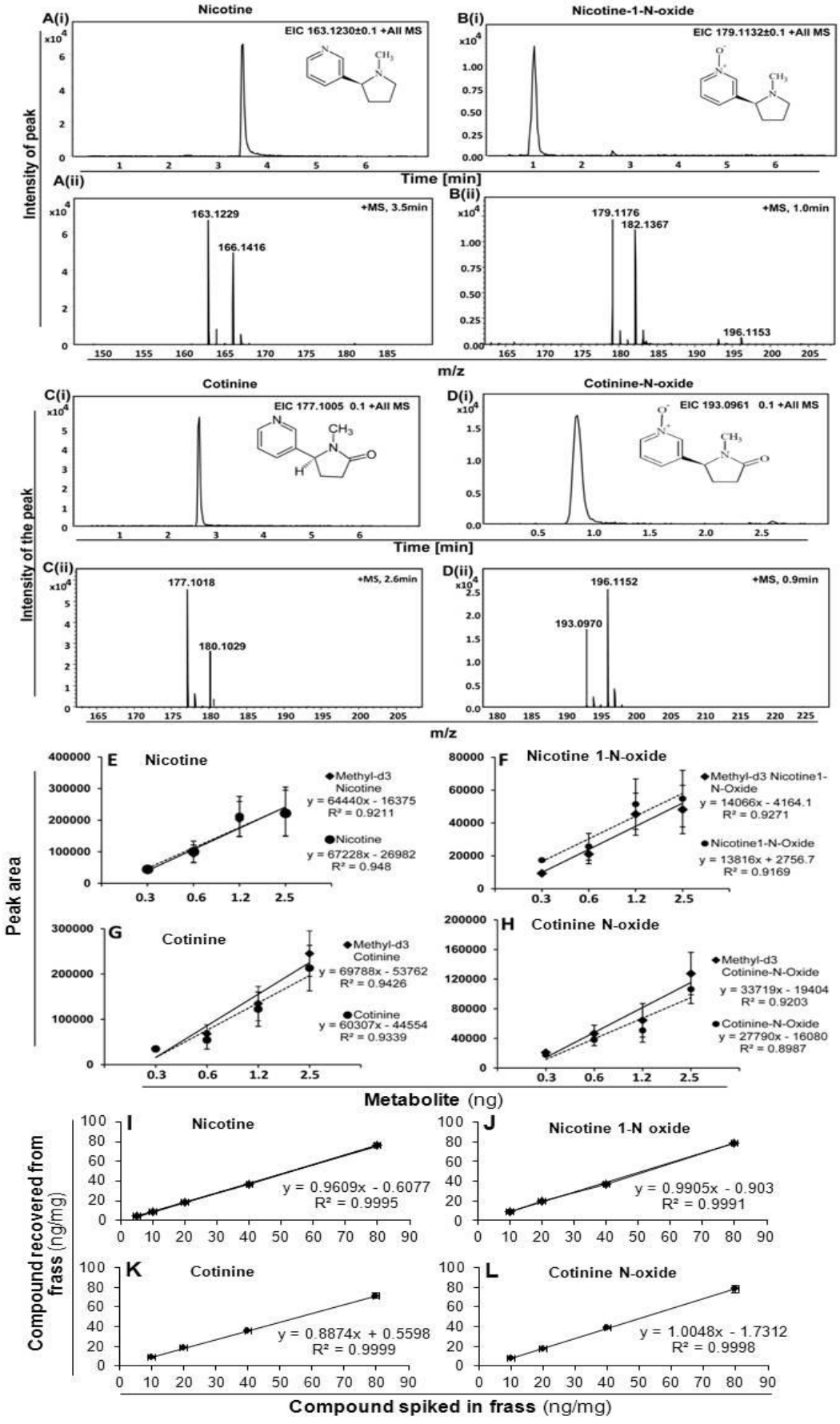

Fig S3. U(H)PLC/ ESI-QTOF-MS based analysis of nicotine and metabolites

U(H)PLC/ESI-QTOF MS based validation of **(*A*)** nicotine **(*B*)** NNO **(*C*)** cotinine and **(*D*)** CNO. **(i)** Extracted Ion Chromatograms (EICs) of metabolites along with their chemical structures. **(ii)** Mass Spectra (MS) of the extracted metabolites along with their deuterated forms, which co-elute with the target metabolites. Deuterated metabolites were used as internal standards for quantification. Standard curves of **(*E*)** nicotine **(*F*)** NNO **(*G*)** cotinine and **(*H*)** CNO along with equal amounts of their respective deuterated standards revealed linear responses of U(H)PLC/ESI-QTOF MS to all metabolites (n= 3 for each concentration of a compound). For all compounds, the peak area of a given concentration did not differ significantly from that of the same concentration of the respective deuterated standard. Plots showing linear response of U(H)PLC/ ESI-QTOF-MS for **(*I*)** nicotine, **(*J*)** NNO, **(*K*)** cotinine and **(*L*)** CNO and the efficiency of their extraction (>90% for all the compounds) from standard addition experiments with frass; 50, 250, 500, 1000, 2000 or 4000ng of each compound was spiked to 50mg frass before extraction (n= 3 for each spiking concentration).

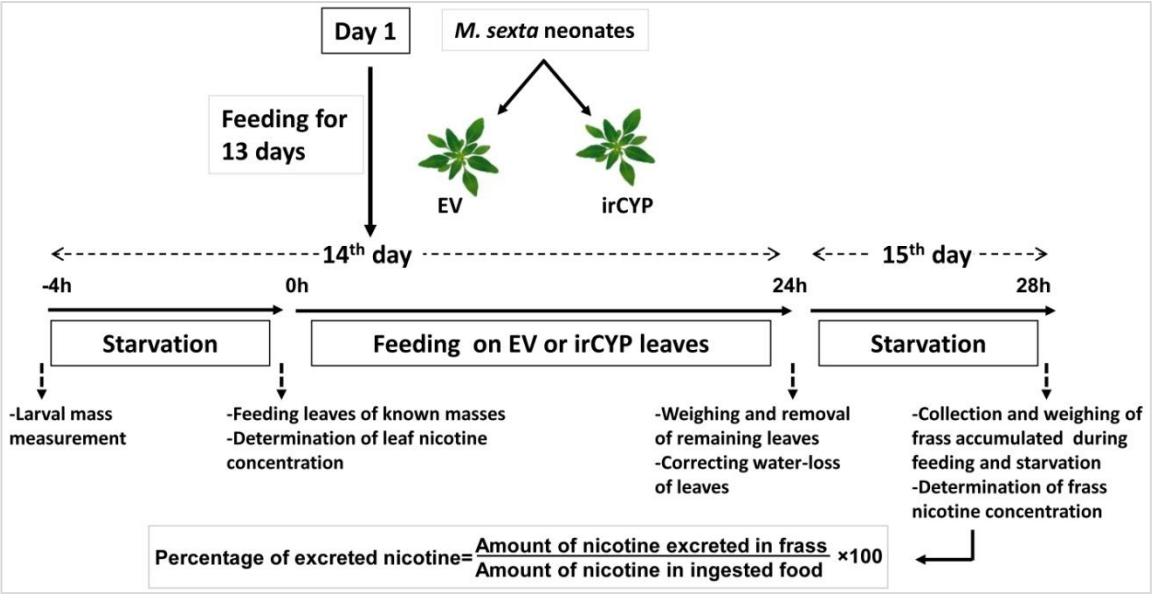

Fig S4. The Waldbauer assay procedure

Schematic of the Waldbauer assay used to quantify ingested and excreted nicotine and its metabolites in control and *CYP*-silenced *M. sexta* larvae.

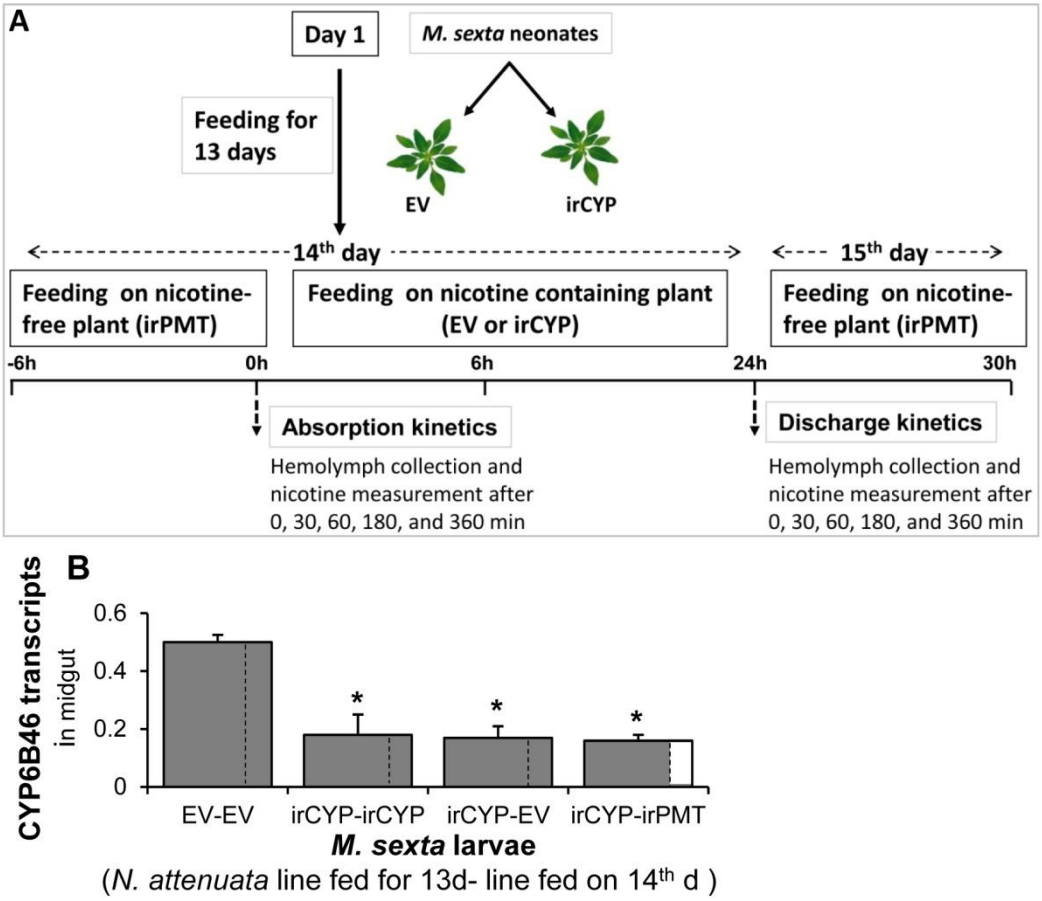

Fig S5. Schematic of the experiments used to determine the kinetics of nicotine flux in larvae and the persistence of *CYP*-silencing during this procedure

(*A*) Schematic detailing the experimental protocol used to measure the kinetics of nicotine absorption in and discharge from the hemolymph of control and *CYP*-silenced larvae. **(*B*)** *CYP*6B46 transcript levels (relative to ubiquitin) in midguts of 4th instar larvae that fed on EV or ir*CYP* plants for 13d and then fed for 24h on EV, EV, ir*CYP* or ir*PMT* plants, respectively ($F_{3, 20}$= 164.28.2, $P \leq 0.0001$, n=5, 5, 8 and 6, respectively). Dashed line dividing each bar indicates the transfer on 13th day and the change in bar color indicates the relative nicotine concentration of the diet. See Fig. 1 legend for the bar-shading codes.

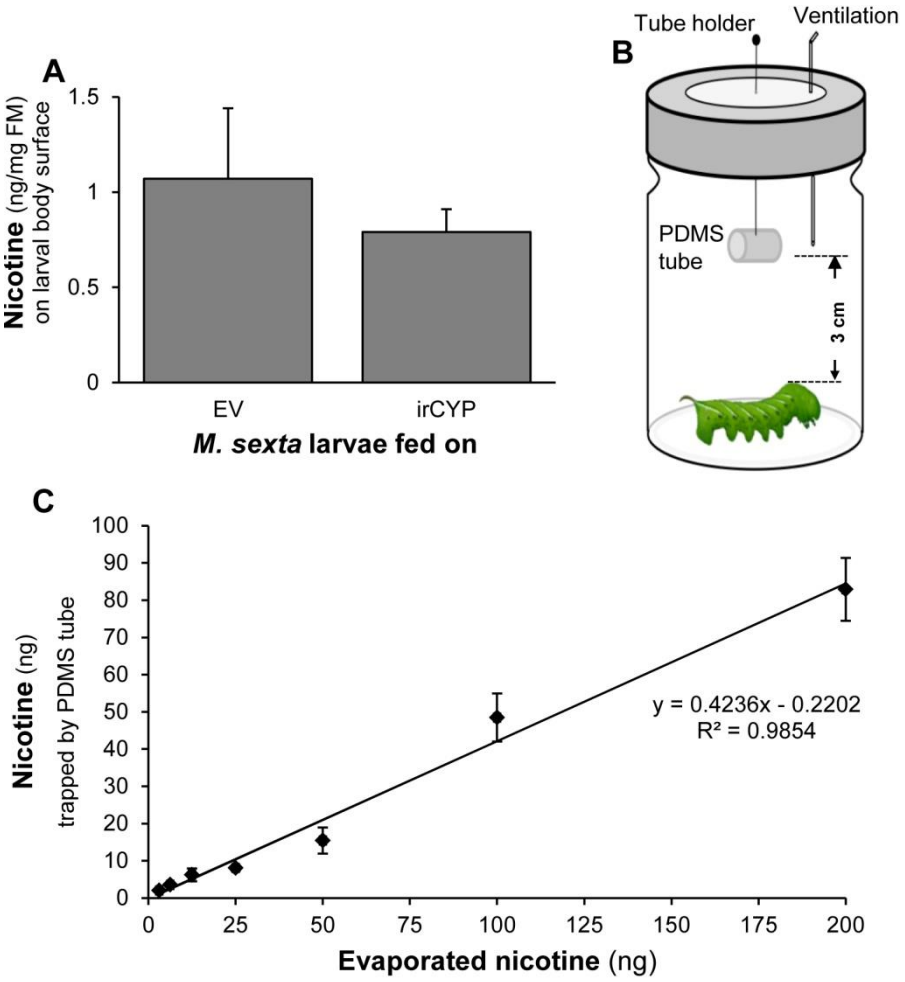

Fig S6. Trapping and quantification of nicotine from larval surface and in larval headspace

(*A*) Amount of nicotine (mean± SE) adsorbed to the body surface of control (n= 11) and *CYP*-silenced (n= 12) larvae; nicotine was recovered from the body-wash of intact larvae. **(*B*)** Schematic of the collection of larval nicotine headspace. **(*C*)** A standard curve based on the amounts of PDMS-adsorbed nicotine to evaluate the linear response of PDMS to the increasing headspace nicotine concentrations (n=4 for each nicotine concentration). See Fig. 1 legend for the bar-shading codes.

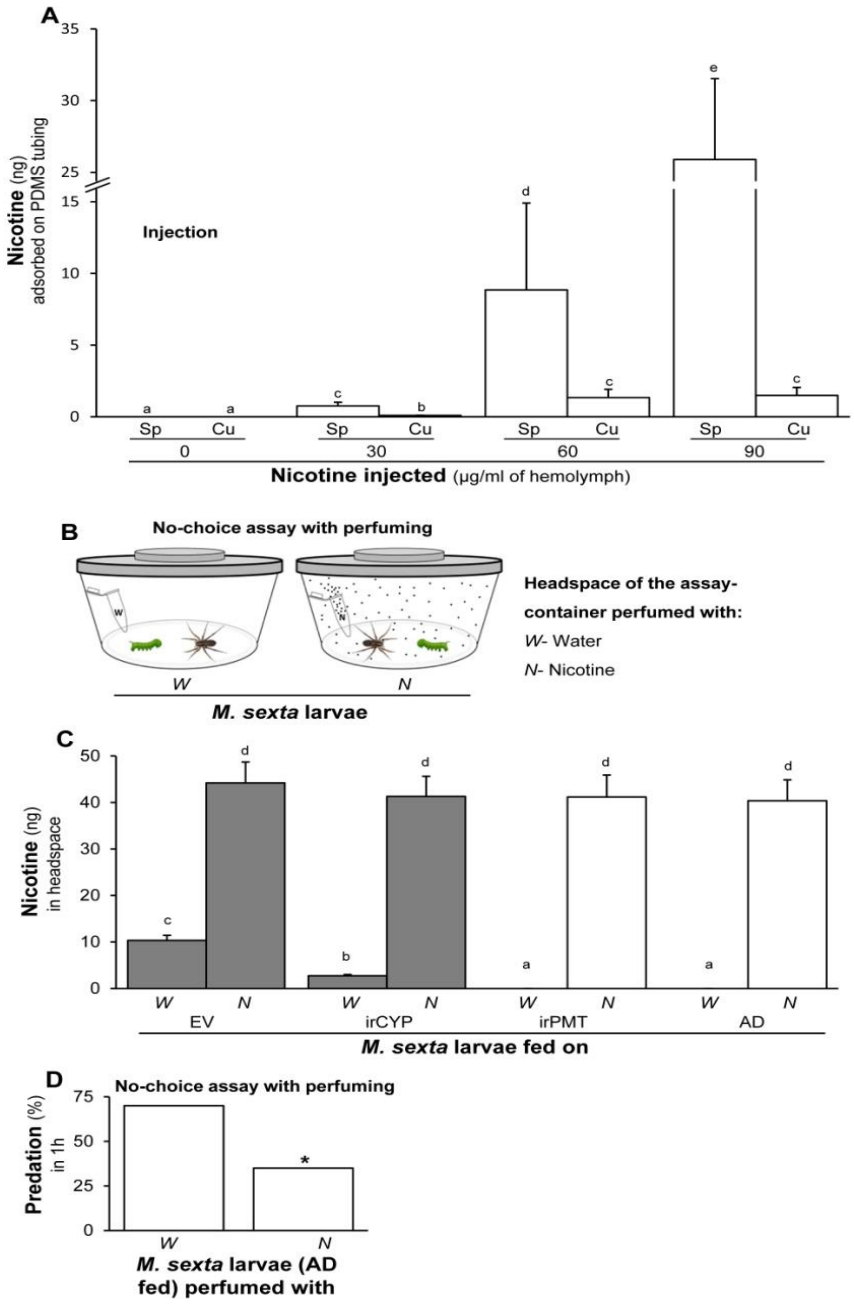

Fig S7. The release of nicotine through spiracles in larvae differing in hemolymph nicotine concentrations, schematic of no-choice assays with nicotine perfuming, nicotine in headspace of perfuming assays and consequences of perfuming on spider predation
(*A*) Relative amounts of nicotine adsorbed by the PDMS tubes attached to spiracles (Sp) and cuticle (Cu), after injecting differing amounts of nicotine into the hemolymph of artificial diet fed 4th instar larvae [(mean± SE) $F_{7, 40}$= 10.45, P≤0.0001, n=6]. **(*B*)** Schematic of a no-choice assay; assay environment was perfumed using 500µL of 1mM nicotine on a cotton swab (500µL water on a cotton swab was used as the control). **(*C*)** Relative amounts (ng) of nicotine (adsorbed by the PDMS tubes suspended) in the headspace of assay-containers of water- and nicotine- perfumed larvae, during no-choice assays [(mean± SE) $F_{7, 16}$= 42.01, P≤0.0001, n=3]; small letters indicate significant differences determined by one-way ANOVA. **(*D*)** Spider predation (%) on the larvae fed on AD after perfuming the (no-choice) assay environment with water (n=20) or nicotine (n=20); asterisk indicates significant difference (P≤0.05) by Fisher's exact test. See Fig. 1 legend for the bar-shading codes.

Tables

Table S1. Field survival of *M. sexta* larvae fed WT/EV, ir*PMT*, or ir*CYP* plants. Either pairs of 1st instar larvae that were pre-fed for 1d were exposed to native predators in a field plantation for 5h during daytime (in 2004) or larvae were placed individually on the respective genotype growing in a field plantation (in 2004 & 2012) or a native *N. attenuata* population (in 2005) and survival was recorded for 1, 2, or 5 days respectively (2004). No significant differences in survival rates were detected in all assays but the trend of a higher survival of WT/EV fed larvae when exposed over days (including night times) motivated the examination of diurnal and nocturnal predation rates separately in 2013. During the daytime, survival rates of WT and ir*PMT* fed larvae did not differ, however, the survival of ir*PMT* and ir*CYP* fed larvae was significantly lower than those of WT/EV fed larvae during night times. *P*-values refer to Fishers exact test.

Year	**Assay no.**	**N**	**Assay duration**	**% Survival**			***P***
				WT/EV	**ir*PMT***	**ir*CYP***	
2004	Diurnal	15	5h	66.6	66.6	-	0.300
		23	5h	56.5	43.5	-	0.159
		18	5h	44.4	50.0	-	0.247
		42	1d	66.3	38.1	-	0.163
2005	Diurnal	27/30	5d	51.9	40.0	-	0.142
2012	Diurnal	13	2d	46.2	15.4	23.1	0.15 (ir*PMT*) 0.21 (ir*CYP*)
2013	Diurnal	50	14h	76	72	74	0.12 (ir*PMT* and ir*CYP*)
	Nocturnal	50	10h	80	50	50	0.045 (ir*PMT* and ir*CYP*)

Table S2. Secondary metabolite concentrations in EV and ir*CYP* leaves. (n= 6; n.s.- no significant difference).

Metabolite	**EV**	**ir*CYP***
Chlorogenic acid (µg/ g leaf FM)	157.37± 10.72	141.66± 5.6 (n.s)
Caffeoyl putrescine (µg/ g leaf FM)	178.35± 1.86	176.62± 4.81(n.s)
Rutin (µg/ g leaf FM)	1436.81± 39.62	1250.9± 113.16 (n.s)
Diterpene glycosides (collective peak area/ mg leaf FM)	15.20± 3.21	10.49± 2.38 (n.s)

Table S3. Amounts of ingested and excreted leaf mass and nicotine during the 24h Waldbauer assays. For a detailed description of the procedure of these assays, refer to Fig. S4 (n= 8; n.s.-no significant difference).

Parameter	**EV fed larvae**	**ir*CYP* fed larvae**
Food (leaf) ingested (g)	3.5± 0.17	3.3± 0.38 (n.s.)
Food excreted (g)	2.86± 0.67	2.64± 0.31 (n.s.)
Nicotine ingested (mg)	1.43± 0.22	1.80± 0.10 (n.s.)
Nicotine excreted (mg)	1.23± 0.20	1.51±0.06 (n.s.)
% nicotine excreted (of ingested)	86.99± 7.2	84.16± 2.10 (n.s.)

Table S4. Predation of with *G. pallens* and antlions in no-choice assays over *M. sexta* larvae fed WT/EV, ir*PMT*, or ir*CYP* plants. 1st instar larvae were dropped into native antlion pits (in 2004) and 2nd instar larvae were used in predation assays in cups with both predators (in 2012 and 2013).

Predator	**Year**	**N**	**% Larvae preyed upon**		
			EV	**ir*PMT***	**ir*CYP***
Antlion	2004	19/14	52.6	57.1	-
	2012	26	70.0	70.0	70.0
	2013	15	73.3	73.3	66.6
G. pallens	2012	10	70.0	60.0	70.0
	2013	15	66.6	66.6	60.0

Table S5. APHIS notification numbers under which transgenic *N. attenuata* seeds were imported and plants released at the field station in Utah.

Line	Import #	Year	Release #
EV	07-341-101n	2012	11-350-101r
		2013	12-333-101r
ir*PMT* (*NaPMT* NCBI accession no. AF280402)	04-020-07n	2004	04-020-08n
	04-344-06n	2005	04-344-07n
	07-341-101n	2012	11-350-101r
		2013	12-333-101r
ir*CYP* (*MsCYP* NCBI accession no. GU731529)	10-004-105m	2012	11-350-101r
		2013	12-333-101r

Videos

Video S1. Spider's attack behavior when presented with EV- (A), *CYP*- (B) and ir*PMT*-fed (C) *M. sexta* larvae.

A spider in the assay container (50cc) was offered *M. sexta* larvae (2nd instar) that had fed since hatching on (A) EV, (B) ir*CYP*, or (C) ir*PMT* plants. Video shows how the spider is rapidly repelled by larvae fed on EV plants after a first contact, but readily attack and consume larvae had fed on the nicotine-free ir*PMT* plants or the nicotine-replete ir*CYP* plants that had silenced their midgut expressed *MsCYP*6B46 transcripts.

Supporting references

1. Dagne E & Castagno N (1972) Cotinine N-oxide, a new metabolite of nicotine. *J Med Chem* 15(8):840-841.
2. Craig JC & Purushothaman KK (1970) An improved preparation of tertiary amine N-oxides. *J Org Chem* 35(5):1721-1722.