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Genetics and mechanisms of imidacloprid resistance in house flies

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ABSTRACT

Imidacloprid is the most widely used neonicotinoid insecticide against house flies, which are major pests at animal production facilities worldwide. However, cases of both physiological and behavior resistance have been reported. Recently, physiological resistance to imidacloprid was found in the United States (California and Florida). However, no studies have been undertaken to characterize this resistance in house flies from the United States.

Three imidacloprid selections of a strain originally collected from Florida increased the level of resistance, ultimately resulting in a strain that had 2300-fold resistance in females and 130-fold in males. Imidacloprid resistance was not overcome with piperonyl butoxide (PBO) suggesting that resistance is not due to detoxification by cytochrome P450s. Resistance was mapped to autosomes 3 and 4. There was ⩾100-fold cross-resistance to acetamiprid and dinotefuran, but no cross-resistance to spinosad. The resistance in this imidacloprid selected population was unstable and declined over a period of several months. The significance of these results to management of imidacloprid resistance in the field, and potential mechanisms of resistance involved, are discussed.

1. Introduction

The house fly, *Musca domestica* is a significant pest affecting animal production facilities and public health. House flies are mechanical vectors of pathogens that cause >100 diseases in livestock, poultry and humans [1–3]. Control of house flies has been addressed in several ways, with one of the most widely used being insecticides. However, house flies have shown a remarkable speed in which they are able to evolve resistance to insecticides. Understanding the mechanisms of resistance is important for the development of successful resistance monitoring and management [4].

Neonicotinoids are the most recently registered class of insecticides for house fly control, and they exert their toxicity via interactions with insect nicotinic acetylcholine receptors (nAChR). Neonicotinoids such as imidacloprid are of enormous economic importance globally, especially in the control of pests that have previously developed resistance to other classes of insecticides [5].

Imidacloprid is the most widely used neonicotinoid for house fly control and was originally formulated as a bait. Both physiological resistance (observed using no choice feeding assays) and behavioral resistance (observed using choice feeding assays) to imidacloprid have been detected in house fly populations from California [6]. Two surveys of house fly populations across the USA have failed to detect physiological resistance [7,8], although resistance ratios of 2.1- to 12.8-fold were observed in Florida [9]. In Denmark, 20- to 22-fold imidacloprid resistance has been detected in a field collected strain [10].

Laboratory selections of field collected house flies from Denmark [10] and Florida (FDm strain) [11] have produced strains that are 75- to 150-fold and 330-fold resistant to imidacloprid, respectively. Cross-resistance to imidacloprid was suppressible by piperonyl butoxide (PBO) in the AVER strain [12] and imidacloprid resistance in house flies from Denmark was 1.9- to 6.3-fold suppressible with piperonyl butoxide (PBO) [10] suggesting that cytochrome P450s play a role in imidacloprid resistance in these strains. Imidacloprid resistance was 2.0-fold higher in females than males the 791imi (imidacloprid selected) strain [10]. Imidacloprid resistance in Danish house flies was also associated with a reduction in expression of the nAChR subunit *Mdα2* [13]. The genetics and mechanisms involved in the imidacloprid resistant strain from Florida (FDm) have not been investigated.

The aim of this study was to further characterize imidacloprid resistance in the house fly. We found that further selections (of the FDm strain) increased the level of resistance and that resistance levels were higher in females than males. Resistance was unstable and was not overcome with PBO. Resistance mapped to autosomes 3 and 4 and there was ⩾100-fold cross-resistance to acetamiprid and dinotefuran.

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2. Materials and methods

2.1. Chemicals

Imidacloprid (99.5%), spinosad (98.6%), thiamethoxam (99.5%), acetamiprid (99.2%), dinotefuran (98.2%), nitenpyram (99%) and cartap hydrochloride (99.5%) were obtained from Chem Service (Westchester, PA). Nithiazine (98.94%) was from Wellmark International (Dallas, TX). PBO (90%) was from Sigma–Aldrich (St. Louis, MO).

2.2. House fly strains

Two parental strains were used: aabys is a susceptible strain bearing recessive morphological markers ali-curve (*ac*), aristapedia (*ar*), brown body (*bwb*), yellow eyes (*ye*) and snipped wings (*snp*) on autosomes 1, 2, 3, 4 and 5, respectively; KS8 is an imidacloprid resistant strain obtained by consecutive selections of the FDm strain with 1%, imidacloprid-containing QuickBayt® for five generations [11] and then reared with no selection pressure. House flies were reared as previously described [14]. OCR is a cyclodiene resistant strain that is homozygous for the A302S *Rdl* mutation (the strain served as a control for *Rdl* genotyping) and was obtained from samples that were frozen in 2002 [15].

Strains carrying resistance factors from autosome 3 or 4 (from KS8S2) were isolated as follows. Unmated individuals of the appropriate phenotype (aa+ys for chromosome 3 and aab+s for chromosome 4) were isolated from backcrosses of aabys females and F_1 males (details in Section 2.4). Two colony cages of $\geqslant$350 females and $\geqslant$110 males were set up for each phenotype. Unmated progeny were sorted for appropriate phenotype (aa+ys or aab+s) and caged. Unmated progeny from the sorted flies were selected with 107 μg imidacloprid/g of sugar for 72 h (to kill heterozygous individuals). For the aa+ys phenotype, 1647 females (82% mortality) and 1,075 males (85% mortality) were treated, whereas for the aab+s phenotype, 1,462 females (83% mortality) and 1106 males (67% mortality) were treated. Surviving flies were caged and their unmated progeny were sorted for phenotype and caged. Progeny of these flies were sorted for appropriate phenotype and selected with imidacloprid as described above. Survivors of the imidacloprid selection were used to establish the IR3 (Imidacloprid Resistant chromosome 3) and IR4 (Imidacloprid Resistant chromosome 4) strains.

2.3. Bioassays and selections

Sugar cubes (2.33 g, Domino Dots, Domino Food Inc., Yonkers, NY), were treated with 0.25 mL of insecticide (or a solvent only control) and allowed to dry for $\geqslant$30 min. Imidacloprid, spinosad, thiamethoxam, acetamiprid, dinotefuran, nitenpyram and PBO were dissolved in acetone, whereas cartap hydrochloride and nithiazine were dissolved in water. For the highest concentrations of imidacloprid it was necessary to use multiple 0.25 mL applications (sugar cubes were allowed to dry between applications).

House flies (25, 3–5 d old) were placed in 180 mL waxed paper cups (Solo Cup Co., Lake Forest, IL) covered with nylon tulle (Jo Ann Fabric, Ithaca, NY), and fasted for 6 h. Then the treated sugar cube was introduced, flies were provided water via a piece of wet cotton on top of the cup (water was provided daily) and they were held at 25 °C with 12:12 h light:dark photoperiod. Percent mortality (defined as flies that were ataxic) was determined after 72 h. Three to six concentrations giving greater than 0% and less than 100% kill were used for each LC_{50} determination. A minimum of four replicates were run for each bioassay. To evaluate the role of P450 monooxygenases in resistance, the P450 inhibitor PBO was applied (1 μg/fly) in 0.5 μL of acetone to the thoracic notum 1 h before the introduction of the treated sugar cube to the bioassay cup. Bioassay data were pooled and analyzed via standard probit analysis [16], as adapted to personal computer use by Raymond [17] using Abbott's [18] correction for control mortality. The LC_{50} values in the imidacloprid and imidacloprid + PBO bioassays were used to determine the synergistic ratio (SR). In order to compare SRs between different strains it was necessary to generate an estimate of the variability in the SR. This was done using the 95% CIs of the LC_{50}s to generate a range for the SRs (i.e. SR range = [lower CI from the without PBO assay/upper CI from the with PBO assay–upper CI from the without PBO assay/lower CI from the with PBO assay]. The SR range of each strain was then compared to the SR range of the aabys strain to determine whether they overlapped or not. If they overlapped, then enhancement of toxicity was not significantly different from that of the susceptible strain. If the SR range of the resistant strain was higher and did not overlap with that of aabys, then the effect of PBO was judged to be more significant in the resistant strain. The degree of dominance (D) for imidacloprid resistance was calculated using Stone's equation [19].

Three generations of imidacloprid selection were carried out starting with the KS8 strain. Selections were done using unmated females (collected within 8 h of emergence) and males. Flies (1 d old) were fasted for 6 h and then provided with sugar cubes that had been treated with 1.07 mg imidacloprid/g of sugar as described above. Survivors were removed after 72 h, provided with untreated food and water and were used to generate the next generation.

2.4. Linkage analysis

Chromosomes involved in imidacloprid resistance in the KS8S2 strain were assessed by the F_1 male backcross method of Tsukamoto [20], using a total of 7,987 female and male flies. This method involved crossing aabys with KS8S2 (reciprocal crosses were done), backcrossing the F_1 males to unmated aabys females, separating flies by phenotype and then determining percent mortality at a diagnostic concentration of imidacloprid. Because crossing over is very rare in male house flies [21], the method allows the detection and measurement of the "dominant" effect of each chromosome. Two reciprocal crosses were set up for the linkage analyses: Cross A (aabys female × KS8S2 male.) and Cross B (KS8S2 female × aabys male). The F_1 males of these crosses (F_1A and F_1B) were backcrossed with unmated aabys females and the progeny of each cross separated by phenotypes and tested with a diagnostic concentration of 35.4 μg imidacloprid/g of sugar (as described above) to evaluate the effect of each chromosome [20].

2.5. Rdl genotyping

Genomic DNA was extracted from heads of individual females of the aabys, OCR, IR3 and IR4 strains or the whole bodies of males of the IR3 and IR4 using the quick fly genomic DNA prep method (www.fruitfly.org) as described previously [22]. The DNA pellet was dissolved in 30–50 μL of EB buffer (Qiagen, Valencia, CA) and stored at −20 °C. A 286-bp genomic fragment of *Rdl* containing the A302S mutation was obtained by PCR using primers MdRdlF2 (5′-TCTTACAGGAAATTATTCGCGTC-3′) and MdRdlR2 (5′-ACTGGCA AAGACCATCACGAAACAC-3′) [9] using GoTaq® Green Master Mix from Promega corporation (Madison, WI). The following thermal cycler conditions were used: 95 °C for 1 min followed by 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 30 s and a final extension at 72 °C for 10 min. An aliquot (5 μL) of the PCR product was treated with 1.1 μL ExoSap (Thermo Fisher Scientific, West Palm Beach, FL), held at 37 °C for 15 min and then 85 °C for 15 min. Samples were sequenced at Cornell's Biotechnology Resource Center. Individual sequences were manually inspected for the