Pesticide Biochemistry and Physiology 102 (2012) 56–60

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Effects of mutations in *Drosophila* nicotinic acetylcholine receptor subunits on sensitivity to insecticides targeting nicotinic acetylcholine receptors

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ARTICLE INFO

Article history:
Received 15 July 2011
Accepted 26 October 2011
Available online 10 November 2011

Keywords:
Nicotinic acetylcholine receptor
Insecticide resistance
Neonicotinoids
Sulfoximines
Sulfoxaflor
Spinosad
Drosophila melanogaster

ABSTRACT

Several strains of *Drosophila melanogaster* possess mutant alleles in nicotinic acetylcholine receptor (nAChR) subunits, Dα1 and Dβ2 that confer resistance to neonicotinoids such as imidacloprid and nitenpyram, and Dα6, that confers resistance to spinosyns. These mutant strains were bioassayed with a selected set of nAChR active insecticides including neonicotinoids, spinosad, and sulfoxaflor, a new sulfoximine insecticide. All of the neonicotinoids examined, except dinotefuran showed reduced insecticidal efficacy on larvae of the Dα1 mutant, suggesting that this subunit may be important in the action of these insecticides. All of the neonicotinoids, including dinotefuran, showed reduced insecticidal efficacy on larvae possessing the Dβ2 mutation. A similar pattern of broad neonicotinoid resistance to that of Dβ2 alone was also observed for larvae with both the mutations (Dα1 + Dβ2). The Dβ2 mutation exhibited a lower level of cross-resistance to sulfoxaflor (<3-fold) than to any of the neonicotinoids (>13-fold). In contrast, there was no cross-resistance for any of the neonicotinoids or sulfoxaflor in adult flies with the Dα6 mutation, which confers high levels of resistance to spinosad. Thus in the *D. melanogaster* strains studied, target site resistance observed for the neonicotinoids and the spinosyns does not translate directly to resistance towards sulfoxaflor.

1. Introduction

Effective control of sap feeding insects such as *Bemisia tabaci* (whitefly), *Myzus persicae* (green peach aphid) and *Nilaparvata lugens* (brown planthopper) with organophosphates, carbamates and pyrethroids has deteriorated through the emergence of resistance [1]. The introduction of insect growth regulators [2,3] and neonicotinoids [4], with novel chemical structures and modes of action, helped restore control of resistant populations [5]. Strict insecticide resistance management (IRM) strategies are required for control of these sap feeding pests due to their propensity to evolve resistance to any compound used. Such strategies can assist in maintaining insecticidal efficacy for prolonged periods of time [1], but even with the neonicotinoids, their widespread use has led to increasing reports of resistance (Arthropod Pesticide Resistance Database (2011, http://www.pesticideresistance.org)). Although resistance to the neonicotinoids has been slow to develop, both field-isolated and laboratory-selected resistant insects have been characterized [6–10]. Neonicotinoid resistance has thus far been associated primarily with enhanced cytochrome P450-based metabolism [11]. Target-based mechanisms are known [7], with evidence obtained from a laboratory selected strain. These mechanisms can also co-exist in a single insect strain, as recently demonstrated for a field clone of *Myzus persicae* [6]. Thus, there is a continuing need for new classes of insecticides to control resistant sap-feeding insects.

Insect nicotinic acetylcholine receptors (nAChRs) are the target sites for several classes of insecticides including the nereistoxin analogs, spinosyns and neonicotinoids [12–16]. Recent evidence suggests that strains of *Drosophila melanogaster* with mutated Dα1 or Dβ2 nAChR subunits (see Section 2.1 $D\alpha1^{EMS1}$, $D\beta2^{EMS2}$) have reduced sensitivity to the neonicotinoids nitenpyram, imidacloprid (Fig. 1) and thiamethoxam [17]. In addition, other *D. melanogaster* strains possessing a mutated Dα6 nAChR subunit have been shown to be highly resistant to spinosyn insecticides [18,19]. A detailed characterization of one of the spinosyn resistant strains, DAS1, demonstrated a lack of resistance to a variety of other insecticide classes including the neonicotinoids [17]. Thus, it appears that distinct nAChR subunits may be involved in the action of the different nAChR acting insecticides.

The newly-discovered sulfoximine insecticide sulfoxaflor (Fig. 1) also interacts with nAChRs, but in a manner distinguishable from the neonicotinoids [14,20] and other insecticides targeting sap

Abbreviations: EMS, ethyl methanesulfonate; IPM, integrated pest management; IRM, insecticide resistance management; nAChR, nicotinic acetylcholine receptor.

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doi:10.1016/j.pestbp.2011.10.010

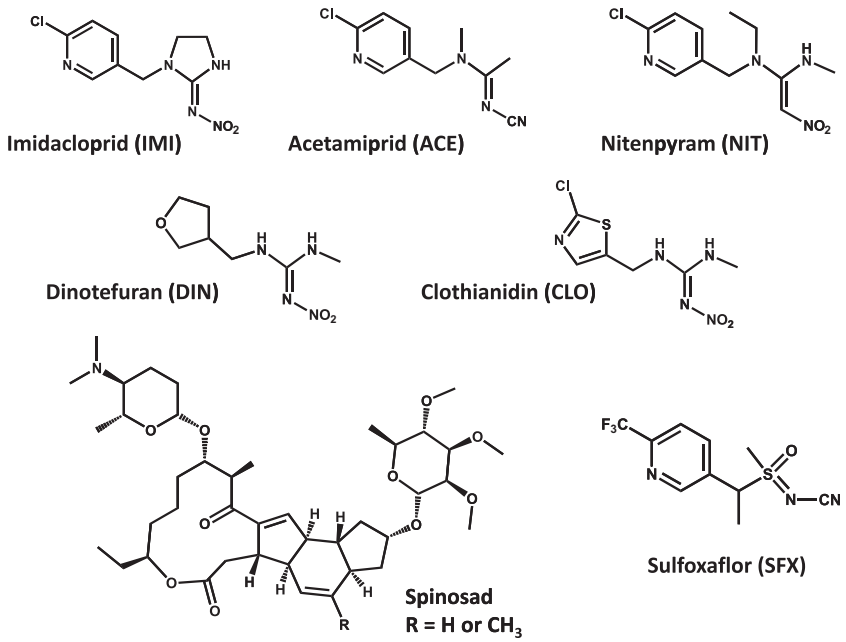

Fig. 1. Structures for the neonicotinoids, sulfoxaflor and spinosad.

feeding insects. Sulfoxaflor has potent insecticidal activity against a variety of sap-feeding insects including aphids, whiteflies, *Lygus* bugs and planthoppers [20]. Testing of sulfoxaflor against organophosphate and pyrethroid resistant Homoptera showed no evidence of cross-resistance [21]. Additionally, neonicotinoid-resistant strains of *M. persicae*, *B. tabaci* and *N. lugens* showed no cross-resistance to sulfoxaflor [20,21]. Further, preliminary evidence indicates that sulfoxaflor may not be a suitable substrate for some metabolic enzymes involved in detoxification of the neonicotinoids in resistant species [20].

While there is growing evidence suggesting that insects lack cross-resistance to sulfoxaflor in instances where insecticide resistance is metabolism based, little is known about the effect of nAChR target site based resistance on the potency of sulfoxaflor. Therefore, in the present study, we were interested in characterizing several nAChR-acting insecticides, including sulfoxaflor, spinosad, and a broad sampling of the neonicotinoids against four *D. melanogaster* strains possessing mutations in genes associated with Dα1, Dβ2, and Dα6 nAChR subunits. Relative to the novel insecticide sulfoxaflor, this characterization contributes to the understanding of the relative risk of cross-resistance in these models of nAChR target site-based resistance. Such studies are critical to ensuring the appropriate use of this new class of insecticide as it is incorporated into integrated pest management (IPM) strategies.

2. Material and methods

2.1. Strains

Six *D. melanogaster* strains were used for this study. The four strains used in larval screening were described previously [17]. These included Armenia (a susceptible parental strain) and three resistant strains derived from it by ethyl methanesulfonate (EMS) mutagenesis and selection with nitenpyram. $D\alpha1^{EMS1}$ affects the Dα1 nAChR subunit through the deletion of 11 nucleotides that leads to a frame-shift that eliminates the predicted TM4 domain structure and would extend the protein by 72 missense amino acids, $D\beta2^{EMS2}$ affects the Dβ2 nAChR subunit where a deletion of 53 bases causes a frameshift and introduces a stop codon within the cytoplasmic loop between TM3 and TM4. Both these mutations are severe and function is likely to be lost. They are not dominant negative mutations as the phenotype is recessive. The 4A4D strain is a homozygous strain of these two mutations generated through mating and recombination [17]. Resistance to nitenpyram, imidacloprid and thiamethoxam was previously detected but not quantified accurately [17]. The two other strains used for adult screening assays were the *Oregon-R* susceptible strain (OR) and a strain (*DAS1*) which was the result of EMS mutagenesis followed by selection with spinosyn A [19].

2.2. Compounds

Imidacloprid, clothianidin, acetamiprid, and dinotefuran (Fig. 1) from Chem Services (West Chester PA), were all >99% in purity, and were diluted in acetone for media preparation. Nitenpyram (99.9%) from Sigma Chemical (St. Louis, MO) was diluted in molecular grade water for media preparation. Spinosad (Fig. 1) and sulfoxaflor were from Dow AgroSciences and were diluted in 2:1 v/v acetone:10% sucrose solution [19].

2.3. Bioassays

Two bioassay methods were used. Bioassays on the neonicotinoid target site resistant strains [17] used embryos collected from 50 mm laying plates in mass-bred cages and spread onto 90 mm laying plates. Five replicates of 50 first instar larvae were used for each dose. These were placed onto screening media containing the compounds and kept in the dark at 25 °C until adult eclosion was recorded at 16–18 days. Assays on the spinosad resistant *DAS1* strain were performed on adult flies as described previously [19]. Briefly, test solutions (in 2:1 acetone:water/10% sucrose solution) were applied to an agarose substrate in 128-well assays trays. After drying, adult flies were placed in the wells, 3–5 flies per well, four wells per dose. Typically each test was replicated at least three times with each dose response line composed of four to six doses with an average (total) of 305 and 236 flies, respectively for the susceptible and resistant strains. Flies were held at 25 °C and examined for mortality (inability to translocate) at 48 h post-treatment. Controls for each assay included solvent-only and untreated wells.