

# Monitoring Susceptibility of House Flies (*Musca domestica* L.) in the United States to Imidacloprid<sup>1</sup>

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**ABSTRACT** Neonicotinoids are currently the fastest-growing class of insecticides. In 2004, imidacloprid (a neonicotinoid) was registered for house fly control in the United States. This study was undertaken to examine the relative susceptibility of laboratory strains of house flies to imidacloprid using a feeding assay, to determine the relative susceptibility of field collected house flies before imidacloprid use, and to determine the susceptibility 1 and 2 years after imidacloprid use. We found substantial variation in imidacloprid susceptibility in laboratory strains and in populations collected from the field before imidacloprid use. We observed an increase in susceptibility at one site from 2004 to 2005 and a decrease at one site from 2005 to 2006, although all populations in 2005 and 2006 were within the range of responses observed in 2004. The implications of these results to the future use of imidacloprid for house fly control are discussed.

**KEY WORDS** Neonicotinoid resistance, *Musca domestica*, Insecta, cross-resistance, imidacloprid

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Neonicotinoids currently are the fastest-growing class of insecticides and exert their toxic effects via interactions with the nicotinic acetylcholine receptor (nAChR) (Jeschke & Nauen 2005). The most widely used neonicotinoid insecticide, imidacloprid, is highly effective against many important pests, including the house fly (Jeschke & Nauen 2005). Baits containing imidacloprid (QuickBayt®) were registered for house fly control in the United States in 2004. This represents the first granular insecticide bait for fly control that has been registered in the United States in nearly three decades. A potential limitation to the long-term usefulness of imidacloprid is the development of resistance, and early detection of resistance is considered critical for the implementation of successful resistance management strategies (Leeper et al. 1986). In addition to being an important pest, house flies are often a reliable indicator of the resistance mechanisms that develop in other pests.

Although cross-resistance to imidacloprid has been investigated in house flies

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by topical application (Wen & Scott 1997), it is unknown whether cross-resistance would be conferred to imidacloprid in a feeding assay (representative of exposure to a bait). Despite the importance of imidacloprid for house fly control, it is unknown what level of variation in susceptibility exists in populations that have not been exposed to imidacloprid or whether there is any indication of resistance evolving in house fly populations since the introduction of imidacloprid.

We investigated baseline susceptibility to imidacloprid using laboratory strains of house flies that were susceptible or resistant to various other insecticides to evaluate the potential for cross-resistance to imidacloprid. We then monitored the susceptibility to imidacloprid in house fly populations from poultry, dairy, and hog facilities collected before the first use of imidacloprid in 2004, and after 1 (2005) or 2 (2005, 2006) years of use.

### Materials and Methods

**House flies.** Nine strains of laboratory reared house flies were used. CS (Scott et al. 1996) is an insecticide-susceptible strain. SRS is a standard reference susceptible (WHO-SRS) strain created in 1961 (Keiding 1999), which was obtained from M. Kristensen (Lyngby, Denmark). OCR is a cyclodiene-resistant (*Rdl*, Kozaki et al. unpublished) strain that is susceptible to pyrethroids (Scott & Wen 1997), spinosad (Scott 1998), and indoxacarb (Shono et al. 2004). OCR was provided by Dr F.W. Plapp Jr. in 1996 and maintained under biannual selection with dieldrin. Cornell-R is an organophosphate-resistant strain with insensitive AChE. This strain was selected by tetrachlorvinphos (an organophosphate) from a colony collected in Tompkins Co., New York, in the 1970s (Tripathi & O'Brien 1973) and is maintained under biannual selection with tetrachlorvinphos. NYINDR is an indoxacarb-resistant strain derived from flies collected at seven different locations in New York in 1999 (Shono et al. 2004). LPR is a multiresistant strain with high levels of pyrethroid resistance as the result of increased oxidative metabolism by cytochrome P450 CYP6D1 (Scott & Georghiou 1986, Wheelock & Scott 1992), *kdr*, and decreased cuticular penetration (Scott & Georghiou 1986, Shono et al. 2002). Its parental colony was collected from the Learn Dairy (New York) in 1980 (Scott & Georghiou 1985). NG98 is a multiresistant strain with pyrethroid resistance resulting from monooxygenase-mediated detoxification (CYP6D1) and *kdr*. This strain was established from a colony collected in Georgia in 1998 (Kasai & Scott 2000). NYSPINR is a multiresistant strain with high levels of resistance to spinosad (Shono & Scott 2003), established from collections made in New York in 1999 (Kaufman et al. 2001). YPER is a multiresistant strain established by the selection of permethrin from a Yumenoshima population from Japan in 1997 (pyrethroid resistance attributable to *super-kdr* and monooxygenase-mediated detoxification; Shono et al. 2002).

In 2004, house flies were collected from dairy (Alachua Co., Florida, Schuyler Co., New York, and Tompkins Co., New York), poultry (Sullivan Co., New York, and Wayne Co., New York) and hog farms (Wake Co., North Carolina). Adult flies were captured with a sweep net from inside barns and around calf hutches, except for North Carolina where pupae were collected from around calf hutches. Field-collected animals were used to establish laboratory colonies. In 2005, house flies were collected from two dairies in Tompkins Co., New York, one poultry house in Sullivan Co., New York, and from two dairies (#1 and #2) in San Bernardino Co.,

California. The 2004 and 2005 collections were made before the start of insecticide applications for the season. In 2006, house flies were collected midseason from dairies in San Bernardino (#1) and Riverside Co., California. The three dairies in California all used QuickBayt intensively throughout the year starting in 2004. There was little or no use of QuickBayt at any of the other sites. House flies were reared using standard conditions as described previously (Scott et al. 2000). All bioassays on the field collected house flies were completed within two generations' rearing in the laboratory.

**Bioassays.** Susceptibility to imidacloprid was assessed using a feeding bioassay. Twenty female flies (3–5 d old) were placed in glass jars (230 mL, internal surface area is 180 cm<sup>2</sup>) and given two 2-cm pieces of cotton dental wick that were soaked in 20% sugar water containing different concentrations of imidacloprid (97.4%, Miles Inc. Kansas City, Missouri). Mortality (flies that were ataxic were considered dead) was recorded at 72 h (dental wicks were hydrated at 24 and 48 h). At least five concentrations were used for each bioassay. Bioassays were held at 25°C (approx 60% relative humidity). Bioassay data from a minimum of three replications were pooled and analyzed by standard probit analysis (Finney 1971), as adapted to personal computer use by Raymond (Raymond 1985) using Abbott's correction (Abbott 1925) for control mortality. LC<sub>50</sub> values were judged as significantly different if there was no overlap in 95% confidence intervals.

To evaluate the susceptibility of house fly populations after the introduction of imidacloprid, house flies collected in 2005 and 2006 were tested using a diagnostic concentration ( $2 \times \text{LC}_{99}$  of the susceptible CS strain) of imidacloprid. At this concentration there was no survival in any of the four "imidacloprid susceptible" (CS, SRS, OCR and Cornell-R) strains. All other conditions were as described above. A minimum of 350 flies of each strain were tested. Data were arc sine transformed and means were compared by Tukey's *t*-test using inerSTAT-a software.

## Results and Discussion

Susceptibility of the laboratory strains of house fly to imidacloprid varied considerably, with LC<sub>50</sub> values ranging from 9.0 µg/mL to 170 µg/mL (Table 1). There was a 2-fold difference in the imidacloprid LC<sub>50</sub> between the two susceptible strains (CS and SRS). The Cornell-R and OCR strains have been reared in the laboratory for decades, have never been selected with neonicotinoid insecticides, and have resistance mechanisms (altered acetylcholinesterase and *Rdl*, respectively) that can reasonably be expected to offer no protection to imidacloprid. Therefore, these strains could also be considered "imidacloprid susceptible." Comparison of SRS and CS with these "imidacloprid-susceptible" strains shows that the LC<sub>50</sub> values range from 9 µg/mL to 30 µg/mL, indicating more than a 3-fold variation in susceptibility to imidacloprid in "susceptible" strains. This is similar to the 2-fold difference in susceptibility noted between susceptible strains by topical application for imidacloprid (Wen & Scott 1997), fipronil (Scott & Wen 1997) and spinosad (Scott 1998). Variation in susceptibility to imidacloprid between susceptible strains makes the precise detection of resistance in field populations more difficult.

Two of the five insecticide-resistant strains (YPER and LPR) were significantly cross-resistant to imidacloprid. There was a 5.6-fold cross-resistance in the

**Table 1. Toxicity of imidacloprid to nine laboratory and six field collected (2004) strains of house flies exposed using a no-choice feeding assay.**

Strain	LC <sub>50</sub> <sup>a</sup> (95% CI)	n	Slope (SE)	RR <sup>b</sup>
CS	30 (28–33)	1320	3.3 (0.2)	—
SRS	17 (14–20)	2760	3.4 (0.4)	0.6*
OCR	9.0 (7.0–11)	1160	3.1 (0.4)	0.3*
Cornell-R	11 (9.1–14)	600	2.2 (0.3)	0.4*
NYINDR	30 (26–34)	1420	1.7 (0.1)	1.0
NYSPINR	40 (33–47)	720	2.2 (0.2)	1.3
YPER	170 (130–240)	1240	2.9 (0.6)	5.6*
NG98	48 (31–53)	1120	3.5 (0.7)	1.6
LPR	58 (50–65)	560	2.9 (0.3)	1.9*
Schuyler, NY	210 (180–240)	560	2.9 (0.4)	7.0*
Sullivan, NY	240 (220–280)	1000	2.5 (0.3)	8.0*
Tompkins, NY	170 (130–230)	760	2.5 (0.5)	5.7*
Wayne, NY	92 (82–110)	2080	1.8 (0.1)	3.1*
Wake, NC	170 (150–190)	1100	2.7 (0.2)	5.7*
Alachua, FL	94 (78–110)	2060	2.1 (0.2)	3.1*

<sup>a</sup>In units of µg/ml.

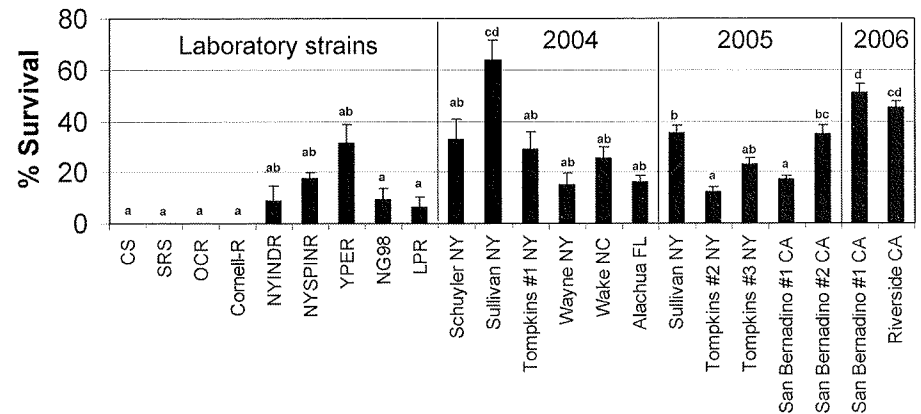
<sup>b</sup>Resistance ratio = LC<sub>50</sub> resistant strain / LC<sub>50</sub> susceptible (CS) strain.

\*Significantly different from CS based on nonoverlap of 95% confidence intervals.

multiresistant YPER strain, which is highly resistant to numerous insecticides (Shono et al. 2002). The mechanism(s) responsible for this low level of cross-resistance in YPER is unknown. The multiresistant LPR strain was 1.9-fold cross-resistant to imidacloprid (relative to CS; Table 1), which is lower than the >4.2-fold cross-resistance to imidacloprid by topical application (Wen & Scott 1997). Spinosad resistance in NYSPINR is the result of an altered target site (Shono & Scott 2003), which may be a nicotinic acetylcholine receptor (Salgado & Sparks 2005). It is therefore interesting that this putative change in a nAChR did not confer cross-resistance to imidacloprid (Table 1).

All six colonies of the 2004 field collected house flies showed limited cross-resistance to imidacloprid, ranging from 3.1- to 8.0-fold (Table 1), even though neonicotinoid insecticides had not been used at these facilities. Results from the YPER and LPR strains (above), and from the Sullivan Co. site indicate that low levels (<10-fold) of cross-resistance exist in some house fly populations. Given that 64% of the house flies from the Sullivan Co., New York site survived the diagnostic concentration of imidacloprid (Fig. 1) suggests that there may be occasional facilities where imidacloprid is less effective than others.

House flies collected in 2005 showed similar susceptibility to imidacloprid, compared with 2004, with survival ranging from 12% to 35% at the diagnostic concentration (Fig. 1). One exception was the Sullivan Co., New York population, which had a significantly lower percent survival (35%) in 2005, relative to 2004 (64%).



**Fig. 1.** Percent survival at a diagnostic concentration ( $2 \times LC_{99}$  of susceptible CS strain) of imidacloprid (feeding assay) to laboratory and field collected house flies. Field collected flies came from dairies, except for Sullivan Co., New York, Wayne Co., New York, (poultry) and Wake Co. North Carolina (hog farm). Values represent the average of at least five replications. Error bars represent the standard deviation of the mean and values with different letters are significantly different ( $P < 0.05$ ).

Overall, house flies collected in 2006 showed relatively greater percent survival, relative to 2004 and 2005 (Fig. 1). The San Bernardino Co., California #1 population had a significant increase in percent survival (51%) relative to 2005 (17%). This is consistent with the intensive use of QuickBayt at this dairy starting in 2004 and may be an indication that imidacloprid resistance is evolving at this dairy. Monitoring imidacloprid resistance at this site should be given a priority. Unfortunately, because of financial limitations, the number of sites we were able to monitor in 2006 was limited to two.

Results of this study provide important baseline data for susceptibility of field collected house flies to imidacloprid, as well as understanding the potential for cross-resistance to imidacloprid in house flies resistant to other insecticides. Further work is needed to determine why there is a 3-fold variation in toxicity between imidacloprid susceptible strains and to understand why some populations are cross-resistant to imidacloprid. In addition, it will be critically important to continue monitoring efforts, so that appropriate steps can be taken if resistance levels start to increase.

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