Susceptibility of Field Collected House Flies to Spinosad Before and After a Season of Use

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ABSTRACT Spinosad is a new and highly promising insecticide with a novel mode of action, and it was first available for house fly control in the United States in 2005. To maintain the effectiveness of this new insecticide, it will be important to monitor populations for the evolution of resistance. We compared three bioassay methods (topical application, residual exposure, and feeding) using laboratory strains of spinoead susceptible and resistant adult house flies. Topical application was the most reliable and efficient method for detection of spinoead-resistant individuals. Using this bioassay we evaluated house flies that were collected before spinoead use (in 2004 and 2005). We found significant differences in survival between dairies, suggesting there was inherent variability between house fly populations prior to spinoead use. We also evaluated field collected house flies from before and after one season of spinoead use (2005). There was no evidence spinoead resistance was evolving after one season of spraying. Uses and limitations of monitoring spinoead resistance by this method are discussed.

KEY WORDS insecticide resistance monitoring, Musca domestica, dairy, poultry

House flies, Musca domestica L. (Diptera: Muscidae), are major pests in and around dairy, poultry, and hog facilities. Given that resistance to organophosphate and pyrethroid insecticides in house flies in the United States is widespread (Scott et al. 1989, Scott et al. 2000, Kaufman & Rutz 2001, Kaufman et al. 2001, Darbro & Mullens 2004), there is an urgent need for new insecticides that are effective against this pest.

Spinosad is a new and highly promising insecticide, derived from the soil actinomycete Saccharopolyspora spinosa. In 2005, spinosad was made available for control of house flies in the United States. Spinosad acts at the nicotinic acetylcholine receptor and has efficacy against a wide range of insects, including house flies (Bret et al. 1997, Scott 1998, Salgado & Sparks 2005). Recently, a strain of house flies (NYSPINR) that had high levels of resistance to spinosad was

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selected (in the laboratory) from field collected house flies (Shono & Scott 2003), indicating that there was potential for the evolution of resistance after repeated use of spinosad in the field. Isolation of the NYSPINR strain required only 10 generations of selection, suggesting resistance might be able to evolve in as little as one season. However, there is no information about the baseline susceptibility of field populations of house flies to spinosad, and no method in place for resistance monitoring.

The goals of this study were to identify an effective bioassay method for detection of spinosad resistant house flies, survey for baseline susceptibility to spinosad in field collected house flies (i.e. determine variability between populations) in 2004 and 2005, and to determine if we could detect any increase in the frequency of resistant individuals at three dairies in California and three dairies in New York after a season of spinosad use (2005).

**Methods and Materials**

**House flies.** Two reference (laboratory) strains of house flies were used for comparison of topical, residual, and feeding bioassays. CS (Hamm et al. 2005) is an insecticide susceptible strain and NYSPINR is a spinosad-resistant strain (Shono & Scott 2003). To produce flies that were heterozygous for spinosad resistance (for bioassays) we crossed NYSPINR females and susceptible aabys (Hamm et al. 2005) males and *en masse*.

In 2004 (prior to spinosad use), six different strains of house flies (*Musca domestica*) were collected at various dairy (Alachua County, Florida, Schuyler and Tompkins Counties, New York), poultry (Sullivan and Wayne Counties, New York), and hog (Wake County, North Carolina) facilities in the Eastern United States. The levels of resistance to permethrin, cyfluthrin, pyrethrins, dimethoate, tetrachlorvinphos, and methomyl in house flies from the Schuyler County dairy and Wayne County poultry facility have been previously reported (Scott et al. 2000, Kaufman et al. 2001).

In 2005, house flies were collected from four dairies (P, M, H, and C) in Tompkins County New York and from four dairies in San Diego (DV), San Bernardino (AM and BJ), and Riverside (BS) Counties, California. These facilities were chosen because they were willing to participate in this study, they were within collecting distance, and because they represented two geographically distant regions (California and New York). Two collections were taken from each dairy. The first collection was made before spinosad was used. A second collection was taken at the end of the season ("postseason"), but while flies were still abundant. In New York, the dairies applied up to six applications of spinosad, except for the H dairy that served as our no spinosad control. In California, dairies applied spinosad four to five times, except for the DV dairy, which served as our no spinosad control.

House fly larvae were reared on medium containing 2.3 L of water, 0.5 kg of calf manna (Manna Pro Corp, St. Louis, Missouri), 90 g of bird and reptile little wood chips (Northeastern Products Corp, Warnersburg, New York), 0.8 kg of wheat bran (Agway; Ithaca, New York), and 50 g of dried active baker's yeast (ICN Biomedicals, Costa Mesa, California). Adult flies were raised on powdered milk + white granulated sugar (1:1 ratio by volume) and water *ad libitum*.

**Bioassays.** Three bioassay methods were evaluated in this study: topical application to the thoracic notum in 0.5 µL of acetone (Shono & Scott 2003),
residual exposure in glass jars (Hamm et al. 2005), and via feeding. For feeding assays, spinosad (spinosyns A and D (88.5% purity) from Dow AgroSciences, Indianapolis, Indiana) was applied (0.25 mL in acetone solution) to individual cubes of sugar (Domino Dots, Tate and Lyle, London, United Kingdom). Treated cubes were allowed to dry for at least 3 h. One cube of sugar was placed into a 180-mL Sweetheart waxed paper cup with 20 flies and a 2.5-cm dental wick soaked in water. Cups were covered with nylon tulle and secured with rubber bands. All bioassays were conducted with 3- to 5-d-old female flies held at 25°C. Mortality was assessed after 48 h with flies that were ataxic being scored as dead. For determination of LD_{50} or LC_{50} values using the laboratory strains, a minimum of four doses (or concentrations), giving >0% and <100% mortality, were used for each replication and the entire bioassay was replicated a minimum of three times. Bioassay data were pooled and analyzed by standard probit analysis (Finney 1971), as adapted to personal computer use by Raymond (Raymond 1985) using Abbott’s (Abbott 1925) correction for control mortality. Field collected flies were tested within four generations of being collected. Field collected flies were bioassayed by topical application at the LD_{99}, 3 \times LD_{99} and 10 \times LD_{99} of the susceptible strain (acetone only was used for the controls). CS flies were periodically tested side-by-side with the field collected flies. Percent mortality was arcsine transformed and differences were evaluated using Student’s t-test.

Results and Discussion

Spinosad was toxic to house flies by feeding, exposure to a residue, and by topical application (Table 1). The NYSPINR strain was resistant to spinosad by all of these methods (Table 1), which is consistent with target site insensitivity being the mechanism of resistance (Scott 1990), as was previously suggested (Shono & Scott 2003). Considering the cost (supplies, etc.), efficiency (time per assay), and heterogeneity of response (i.e., slope) between the three bioassay methods we chose to use topical application (low cost, time efficient, and a low heterogeneity of response) at three diagnostic doses to monitor resistance in field populations of house flies.

To generate baseline data for the effectiveness of spinosad against field collected house flies, we determined the percent survival at three diagnostic doses (susceptible strain LD_{99}, 3 \times LD_{99} and 10 \times LD_{99}) by topical application. Susceptibility of the different house fly strains to spinosad varied between collection sites (Fig. 1). At the susceptible strain LD_{99}, survival ranged from 1% (Alachua Co., Florida) to 61% (Wayne Co., New York), with all except one facility having <30% survival. At 3 \times LD_{99}, survival ranged from 0% (three sites) to 2% (Wayne Co., New York). There were no survivors from any strain at 10 \times LD_{99}. The higher percent survival at the Wayne Co. New York site (at the LD_{99} and 3 \times LD_{99}) suggests that there may be populations of house flies against which spinosad is less effective. Flies from the Florida dairy were highly susceptible, with few survivors at any dose. Although the highest percent survival was seen at two dairies, there was no correlation between type of facility and percent survival.

Evaluation of the New York dairies in 2005, before the use of spinosad, showed variability in response to spinosad similar to what was seen in 2004 with percent survival ranging from 16% to 21% at the susceptible strain LD_{99} (Fig. 2). However, survival of flies from the four California dairies was lower, ranging from
Table 1. Comparison of spinosad toxicity to susceptible (CS) and resistant (NYSPINR) strains of house fly by three bioassay methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>LC$<em>{50}$ or LD$</em>{50}$ (CI)</th>
<th>Slope (SE)</th>
<th>LC$<em>{50}$ or LD$</em>{50}$</th>
<th>RR</th>
</tr>
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<tbody>
<tr>
<td>Topical</td>
<td>0.054$^{a}$ (0.049–0.058)</td>
<td>5.8 (0.9)</td>
<td>&gt;10$^{b}$</td>
<td>&gt;150</td>
</tr>
<tr>
<td>Feeding</td>
<td>2.85$^{c}$ (2.53–3.30)</td>
<td>3.0 (0.3)</td>
<td>&gt;1000$^{d}$</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Residue</td>
<td>0.064$^{c}$ (0.038–0.108)</td>
<td>3.1 (1.6)</td>
<td>&gt;60$^{f}$</td>
<td>&gt;900</td>
</tr>
</tbody>
</table>

$^{a}$LD$_{50}$ in units of µg/fly at 48 h (Shono & Scott 2003).
$^{b}$Less than 50% mortality at 10 µg/fly.
$^{c}$LD$_{50}$ in units of µg/g at 48 h.
$^{d}$Less than 50% mortality at 1,000 µg/g.
$^{e}$LD$_{50}$ in units of µg/cm$^2$ at 48 h.
$^{f}$Less than 50% mortality at 60 µg/cm$^2$.

0.5% to 3.0%. After the use of spinosad for fly control during 2005, there was no indication that resistance was evolving (Fig. 2). To the contrary, the survival after a season of spinosad use was significantly lower at two of the collection sites. It is unclear why the flies collected in California were more sensitive (in most cases) than flies collected in New York.

To evaluate the sensitivity of our resistance monitoring bioassay for detection of homozygous- and heterozygous-resistant individuals, we evaluated survival of NYSPINR and F$_1$ (NYSPINR females × aabys males) house flies. The NYSPINR strain had nearly 100% survival at the LD$_{99}$ and 3 x LD$_{99}$ doses, whereas the F$_1$

![Graph](image)

**Fig. 1.** Mean percent survival of house flies collected in 2004 from six sites in the Eastern United States at the susceptible strain LD$_{99}$ and 3 x LD$_{99}$. All flies were from dairies, except for Wake (hog), Sullivan, and Wayne (poultry) counties. The spinosad-resistant NYSPINR and F$_1$ (NYSPINR x susceptible aabys strain) are shown for comparison. Bars represent the standard deviation from the mean. CS house flies treated at these doses had 0% survival.
Fig. 2. Mean percent survival of house flies collected in 2005 before or after a season using spinosad for fly control, except for the H and DV dairies, which served as negative controls (no spinosad was used at these dairies). Flies were tested at the susceptible strain LD$_{99}$. Bars represent the standard deviation from the mean.

had 19% and 0% survival at these doses, respectively (Fig. 1). Thus, the homozygous-resistant house flies (NYSPINR) are readily detected, but the heterozygous-resistant house flies (NYSPINR × aabys F$_1$) are indistinguishable from field collected house flies that have never been exposed to spinosad (Fig. 1). The highly recessive nature of this resistance (combined with inherent variability in the bioassay with field collected flies) will make it very difficult to detect heterozygous resistant individuals (at least when they are present at low frequencies) in field populations.

Our results indicate that there is variation in susceptibility to spinosad in flies collected from different sites. Whereas selection of field collected house flies produced a highly resistant strain of house fly after eight generations of selection (Shono & Scott 2003), we did not detect a decrease in percent survival at the diagnostic dose at any site after one season of use. Spinosead works at a novel target site (Salgado & Sparks 2005) and resistance in the house fly is highly recessive (Shono & Scott 2003), which would be expected to slow the rate of evolution of resistance in field populations (Georgiou 1983). However, spinosad must be used judiciously and periodic monitoring of resistance should continue. Spinosad resistance is highly recessive and heterozygous individuals can not be readily detected (especially against the normal variation that exists in populations) using insecticide bioassays. Given this limitation it will be important to identify the gene (and allele) responsible for spinosad resistance, so that a more sensitive detection method can be developed.

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