Title

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Publication Date

2023-11-02

DOI

10.1002/ps.7866

Research Article

Received: 9 May 2023 Revised: 18 October 2023 Accepted article published: 2 November 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7866

Evaluation of the stability of physiological and behavioral resistance to imidacloprid in the house fly (*Musca domestica* L.) (Diptera: Muscidae)

Caleb B. Hubbard,* Alec C. Gerry and Amy C. Murillo

Abstract

BACKGROUND: The house fly (*Musca domestica* L.) is a synanthropic fly species commonly associated with confined animal facilities. House fly control relies heavily on insecticide use. Neonicotinoids are currently the most widely used class of insecticide and have been formulated into granular fly baits since 2002. Physiological resistance to imidacloprid in house flies has been observed to be unstable and decline over time without continual selection pressure, indicating that resistance has a fitness cost to individuals in the absence of exposure to insecticides. The stability of behavioral resistance to imidacloprid in the house fly has not been evaluated. In the current study, we assess the stability of physiological and behavioral resistance in house flies to imidacloprid over time.

RESULTS: Physiological susceptibility to imidacloprid varied significantly among three house fly strains examined, with WT-15 exhibiting the greatest susceptibility to imidacloprid with an LC_{50} and LC_{95} of 109.29 (95.96–124.49) μg g^{-1} and 1486.95 (1097.15–2015.23) μg g^{-1}, respectively. No significant differences in survival were observed across 30 generations of a house fly strain (BRS-1) previously selected for behavioral resistance to imidacloprid with percentage survival ranging from 93.20% at F_0 in 2020 to 96.20% survival at F_{30} in 2022.

CONCLUSION: These results have significant implications for the management of house flies exhibiting behavioral resistance in field settings. It appears that standard resistance management tactics deployed to reduce the prevalence of physiological resistance, such as rotating or temporarily discontinuing the use of specific insecticides, may not lead to reduced behavioral resistance to imidacloprid.

Keywords: neonicotinoid; insecticide; aversion; IPM

1 INTRODUCTION

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a cosmopolitan and synanthropic pest fly species commonly associated with confined animal and urban waste facilities.[1,2] These facilities produce large amounts of microbe-rich materials, which are ideal for larval fly development.[3,4] The house fly has adapted to living in almost every environment, is known worldwide as a serious nuisance pest species, and has been implicated in the transmission of >200 different pathogens.[4,5] Under optimal environmental conditions large numbers of flies can be produced in a short period of time, with flies developing from egg to adult in as little as 7 days.[6] Although most house flies will remain on or near animal production facilities from which they developed,[7] house flies also are known to readily disperse from development sites. The production of large numbers of house flies can result in litigation against animal producers or urban waste facilities resulting in economic loss or forfeiture of operation.[8] House fly control relies heavily on insecticide use,[2] and toxic fly baits (granular/scatter baits) are commonly applied for control of adult house flies on California animal operations.[9]

Neonicotinoids are currently the most widely used class of insecticide and have been formulated into granular fly baits since 2002.[10] Neonicotinoids bind completely to the nicotinic acetylcholine receptor in insects, leading to paralysis.[11] Imidacloprid is one chemical in the neonicotinoid class of insecticides, and within a few years of the commercial release of imidacloprid fly bait both physiological[12] and behavioral[13] resistance were documented to occur in house flies. Recent studies have shown that imidacloprid behavioral resistance in the house fly is genetically inherited, with resistance factors located on autosomes 1 and 4.[14] This behavioral resistance is expressed as a contact-dependent avoidance behavior that reduces the length of time a fly is in contact with, and feeds on, imidacloprid-treated sucrose.[15] The reduction in feeding is concentration-dependent, as laboratory-selected behaviorally

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resistant flies fed on low concentrations (≤100 μg g^{-1}) of imidacloprid, but reduced feeding when concentrations were >100 μg g^{-1}.[16] To date, the stability of behavioral resistance to imidacloprid and the interaction between physiological and behavioral resistance in field or laboratory populations has not been investigated. Understanding the stability of insecticide resistance over time is imperative to understanding the underlying mechanisms of inherited resistance and can inform strategies for managing insecticide resistance. The present study was conducted to evaluate the stability of both physiological and behavioral resistance to imidacloprid over time in field-collected and laboratory-selected house fly strains.

2 MATERIALS AND METHODS

2.1 Reference Fly colonies

Eight house fly strains were used in this study (Table 1). These strains included a wild-type (WT) fly strain (WT-15) originally collected from a southern California dairy in 2015 and reared without insecticide exposure. Five additional fly strains (BRS 1–5) were derived from the WT-15 strain but were selected over 20 selection cycles and ~65 generations to exhibit a strong behavioral resistance phenotype that manifests as reduced feeding on sucrose containing imidacloprid.[15] Once selected for behavioral resistance to imidacloprid, these five behaviorally resistant fly strains were maintained without further exposure to imidacloprid or other insecticides for an additional 10 generations (~ 1 year). The BRS-1 fly strain was divided to form a new fly strain (BRS-1 SEL) that was further selected for behavioral resistance to imidacloprid following methods used for the initial selection of behavioral resistance.[15] The original BRS-1 fly strain continued to be maintained for an additional 20 generations (~ 2.5 years) without exposure to insecticides. Finally, a new wild-type fly strain (WT-21) was collected in 2021 from the same southern California dairy that WT-15 was collected from and also reared without exposure to insecticides. All fly strains were maintained in insectary rooms at 27 °C and 35% relative humidity (RH), under a 14 h:10 h, light:dark photoperiod, following standard practices.[17]

Table 1. Fly strains used to evaluate the stability of behavioral resistance

Reference fly strain	Origination	Resistance selection during experiment
WT-15	Southern California Dairy (2015)	No selection after colonization
WT-21	Southern California Dairy (2021)	No selection after colonization
BRS-1 SEL	Split from BRS-1 following 10 generations of no behavioral resistance selection.	Selected for behavioral resistance every three filial generations
BRS-1	Split from WT-15 in 2015 and selected for behavioral resistance.	Unselected for 30 generations (~2.5 year)
BRS-2 BRS-3 BRS-4 BRS-5	Flies were selected for a total of 20 selection cycles.	Unselected for 10 generations (~1 year)

2.2 Evaluation of physiological susceptibility to imidacloprid

Physiological resistance to imidacloprid was evaluated in the WT-15, BRS-1 SEL and WT-21 fly strains using no-choice bioassays as described in Hubbard and Gerry.[15] BRS-1 SEL flies were evaluated one behavioral resistance selection cycle after being split from the BRS-1 fly strain, and WT-21 flies were evaluated for behavioral resistance two generations after colonization. Briefly, adult house flies (3–5 days old, mixed sex) were starved overnight for 14–18 h, aspirated from a colony cage, sorted into groups of 25 on an electronic chill plate (catalog no. 1431; BioQuip Products Inc., Compton, CA, USA), and placed into assay chambers (inverted 947-mL polypropylene deli containers with a removable plastic lid and a bottom modified by adding a fiberglass screen) (n = 5 chambers, n = 125 total flies per trial). Flies were provided with water and one cup (37-mL soufflé cup) containing 1 g granular sucrose formulated with technical grade imidacloprid (CAS: 138261–41-3; Chem Service Inc., West Chester, PA, USA). Sucrose treated with imidacloprid was created by dissolving the desired test concentration of imidacloprid per gram of sucrose into acetone. The acetone-imidacloprid solution was then applied to the granular sucrose, mixed thoroughly to ensure even dispersal of the insecticide through the sucrose and placed in the fume hood for 24 h to allow the acetone to evaporate. An additional set of five assay chambers was set up where flies were provisioned with water and one cup containing 1 g granular sucrose treated with acetone only as described above (negative control). A minimum of five concentrations of imidacloprid was used to evaluate resistance in each population. Bioassays were performed under standard rearing conditions (27 °C, 35% RH, 14 h:10 h, light:dark photoperiod) and mortality was recorded at 72 h, with individual flies considered dead if they were unable to right themselves. As control mortality was <5%, no mortality correction was used. Probit analysis was used to estimate lethal concentration (LC_{50} and LC_{95}) values for the fly strains in R v3.6.3[18] following methods described by Burgess *et al.*,[19] and significant differences between LC_{50} and LC_{95} values for each fly strain was determined by nonoverlapping 95% confidence intervals.

2.3 Evaluation of the stability of behavioral resistance

Behavioral resistance was evaluated in the newly collected field strain (WT-21), and the behaviorally resistant (laboratory-selected) fly strains (BRS1-5). WT-21 flies were evaluated for behavioral resistance two and 10 generations after being colonized in the laboratory without exposure to imidacloprid. BRS 1–5 strains were tested for behavioral resistance to imidacloprid following the 20th selection for behavioral resistance (F_0) and again after 10 generations without additional exposure to imidacloprid. BRS-1 behavioral resistance was then further examined after 23 and 30 generations without additional exposure to imidacloprid. Behavioral resistance to imidacloprid was evaluated using choice bioassays as described in Hubbard and Gerry,[15] which were prepared following the methods described above for no-choice bioassays with the following modifications: each experimental chamber was provisioned with water and two cups, one containing 1 g f granular sucrose treated with acetone only, the other containing 1 g sucrose treated with 4000 μg g^{-1} imidacloprid (prepared as described above).[14–16] Negative control bioassay chambers were prepared by providing flies with water and two cups each containing 1 g granular sucrose treated with acetone only. Two complete experimental replicates were completed, with 10 replicates tested for each population and generation per

replicate. Fly survival was documented and converted to a proportion of flies surviving per assay chamber, then transformed using the arcsine of the square root of the proportion surviving before statistical analysis. Paired Student's *t*-tests were performed within fly strain and generation to determine if differences existed between experimental replicates. Differences in survival over time were evaluated utilizing a repeated measures ANOVA for BRS-1 with Tukey's *post hoc* test for separation of means, while a paired Student's *t*-test was used to examine survival differences for both WT-21 and BRS 2–5. Statistical analyses were performed in PRISM v9.3.1 for macOS (GraphPad Software, La Jolla, CA, USA; www.graphpad.com).

3 RESULTS

3.1 Physiological susceptibility to imidacloprid

Physiological susceptibility to imidacloprid, slopes of probit regression, LC_{50} and LC_{95} values, χ^2 goodness-of-fit values, and resistance ratios to imidacloprid are shown in Table 2. Physiological susceptibility to imidacloprid varied significantly among the three fly strains examined; with WT-15 exhibiting the greatest susceptibility to imidacloprid with an LC_{50} and LC_{95} of 109.29 (95.96–124.49) and 1486.95 (1097.15–2015.23) μg g^{-1} imidacloprid, respectively. LC_{50} values differed significantly (lack of overlapping confidence intervals) among each fly strain examined, but significant differences between LC_{95} values were observed only for the WT-15 fly strain (Table 1) as LC_{95} values overlapped for WT-21 and BRS-1 SEL.

3.2 Evaluation of the stability of behavioral resistance

No significant survival differences were found among replicates within fly strain and generation (all $t \leq 0.8558$; df = 4; $P > 0.4418$), so replicates within strain and generation were combined.

No significant survival differences were observed across 30 generations of no insecticidal selection in the BRS-1 fly strain ($F_{3,27} = 0.4616$; df = 3, 27; $P = 0.7114$) with percentage survival ranging from 93.20% at F_0 in 2020 to 96.20% survival at F_{30} in 2022 [Fig. 1(a)].

Additionally, no significant survival differences were observed for BRS 2–5 after 10 generations of no insecticidal selection (all $t < 1.796$; df = 9; $P > 0.1061$). Percentage survival varied little between generations, with <2% survival differences observed for any of the paired comparisons [Fig. 1(b)].

Newly colonized (F_2) fly strain WT-21 exhibited a high level of behavioral resistance to imidacloprid with 90.40 ± 3.73% survival in choice assays [Fig. 1(c)]. After 10 total generations of laboratory selection without exposure to imidacloprid, WT-21 exhibited a 70.00 ± 7.21% survival rate, and no significant difference in survival was observed between WT-21 F_2 and WT-21 F_{10} ($t = 1.707$; df = 9; $P = 0.1220$).

4 DISCUSSION/CONCLUSION

4.1 Physiological susceptibility assays

House fly physiological susceptibility to imidacloprid differed among the three populations of flies tested, with the WT-15 exhibiting the greatest susceptibility to imidacloprid. The WT-15 colony has been maintained under standard conditions without exposure to imidacloprid since its collection from the field in 2015. The WT-15 LC_{50} has decreased approximately six-fold since it was originally colonized nearly 7 years ago (>150 generations) as the 2015 LC_{50} was 619 (586–651) μg g^{-1}.[15] A decline in physiological resistance was expected as previous studies examining house fly physiological resistance to imidacloprid document a sharp decline in resistance following rearing without imidacloprid exposure [9–23-fold decrease in resistance over 12–14 months (approximately 26 generations) depending on the strain tested].[20] The difference in how quickly resistance waned in the Kavi *et al.*[20] study compared to the current study is likely to have resulted from the high levels of physiological resistance to imidacloprid exhibited by those flies (LC_{50} values of 2900–28000 μg g^{-1} imidacloprid depending on the strain examined), and the assumed high fitness cost associated with resistance.

The physiological susceptibility of the newly collected WT-21 field fly strain is of particular interest as we can temporally evaluate the resistance profile of this fly population over a 14-year period owing to the fact that fly collections and physiological susceptibility to imidacloprid screening had been completed from this dairy in 2008 and 2015 (WT-15). The WT-21 house flies exhibited a moderate level of physiological resistance to imidacloprid, although the LC_{50} value was approximately half of what it was in 2015 (619 *versus* 335 μg g^{-1}) and double that of 2008 (155.9 *versus* 335 μg g^{-1}).[13,15] Interestingly, the LC_{95} value for the WT-21 flies was higher, but not significantly different than the LC_{95} value for flies examined in 2015 (Hubbard, unpublished data). The increase in physiological resistance to imidacloprid from 2008 to 2015 was hypothesized to be a consequence of the continued use of fly baits containing neonicotinoids at the dairy and the surrounding region where flies were collected. The decrease in the LC_{50} value for flies collected in 2021 may indicate that there has been a reduction in the use

Table 2. Physiological susceptibility to imidacloprid in tested house fly strains

Fly Strain	*n*	Slope (SE)	LC_{50} (95% CI) (μg g^{-1})	LC_{95} (95% CI) (μg g^{-1})	χ^2 goodness-of-fit (*P*-value)	RR† (LC_{50})	RR‡ (LC_{95})
WT-15	1250	1.45 (0.29)	109.29 (95.96–124.49) **A**	1486.95 (1097.15–2015.23) **a**	120.16* (<0.0001)	–	–
WT-21	1375	1.62 (0.18)	334.73 (294.04–381.04) **B**	3468.35 (2674.07–4498.57) **b**	48.27* (<0.0001)	3.1	2.3
BRS-1 SEL	875	3.27 (1.43)	779.06 (709.43–855.52) **C**	2477.5 (2052.27–2990.84) **b**	123.4* (<0.0001)	7.1	1.7

Note: Significant differences in the lethal concentration (LC) value among fly strains were determined by nonoverlapping 95% confidence intervals (CIs) of the LC values and are indicated within columns by bold capital letters for LC_{50} and bold lowercase letters for LC_{95}.
**P*-value from χ^2 goodness-of-fit test were < 0.05, so a heterogeneity factor was incorporated into the computation of the CIs.
† RR = LC_{50} of fly strain (WT-21 or BRS-1 SEL)/LC_{50} of WT-15.
‡ RR = LC_{95} of fly strain (WT-21 or BRS-1 SEL)/LC_{95} of WT-15.

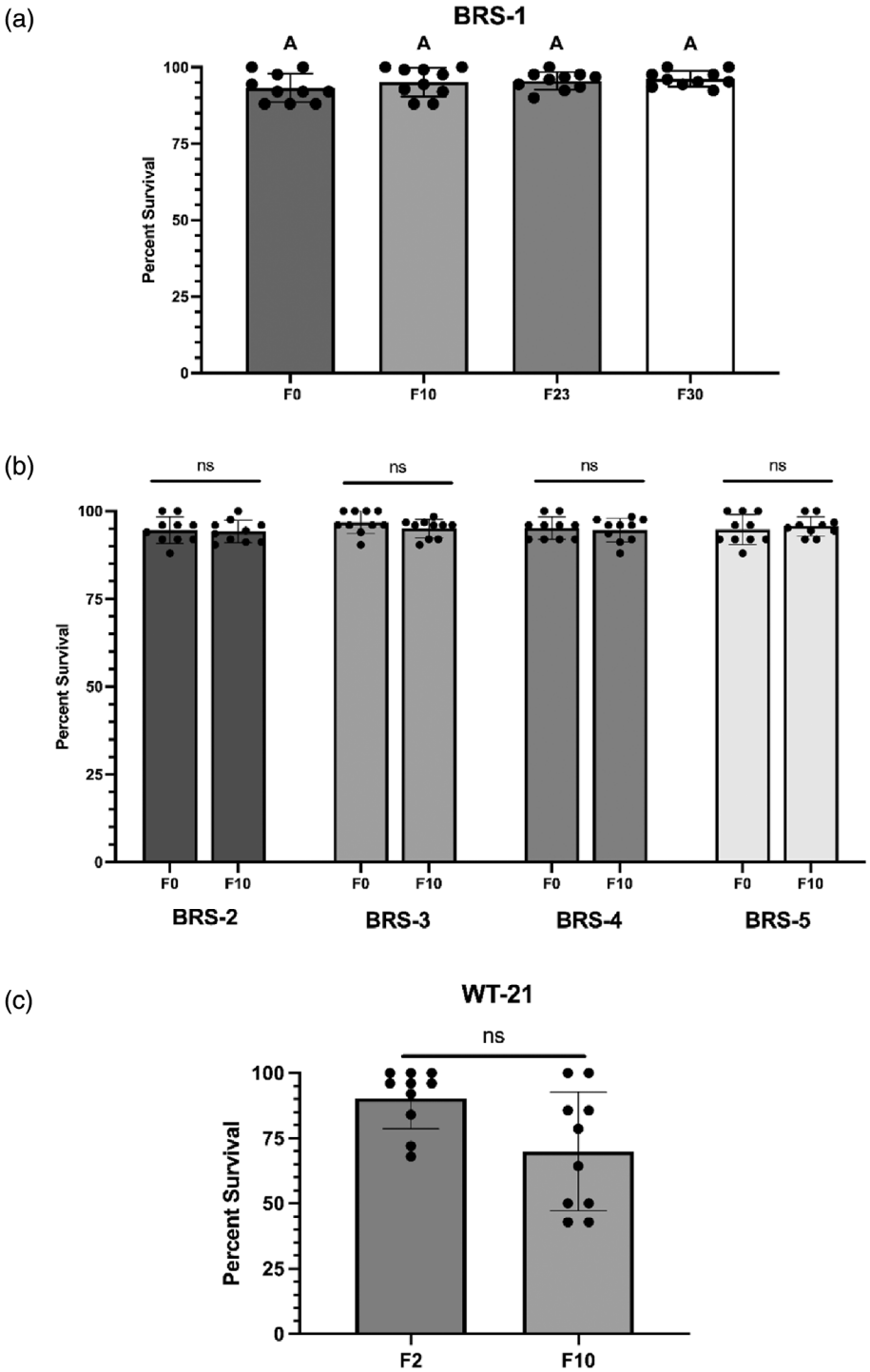

Figure 1. Evaluation of the stability of relative behavioral resistance to imidacloprid by fly strain over filial generations reared without imidacloprid exposure. Percentage fly survival ± SD is shown following 72-h exposure to a choice feeding assay with paired food dishes containing either sucrose alone or sucrose treated with imidacloprid (4000 μg g^{-1} sucrose). (a) BRS-1 fly survival differences were evaluated over 30 generations without imidacloprid exposure at filial generations 0, 10, 23 and 30; bars with the same letter indicate that no significant survival differences were observed ($P > 0.05$). (b) BRS 2–5 flies survival differences were evaluated over 10 generations without imidacloprid exposure at filial generations 0 and 10; 'ns' indicates that no significant survival differences were observed ($P > 0.05$) between generations 0 and 10 within fly strain. (c) WT-21 fly survival differences were evaluated over eight generations without imidacloprid exposure at filial generations 2 and 10, 'ns' indicates no significant survival differences were observed ($P > 0.05$).

of neonicotinoid-containing fly baits at this dairy or region, although records of insecticide use are not available. The stability or slight increase in the LC_{95} value for flies collected in 2021 compared to 2015 may indicate that physiological resistance to imidacloprid is stable and fixed in some individuals in the population. An alternative hypothesis is that resistance is maintained even in the absence of neonicotinoid fly baits on the dairy through the introgression of highly resistant house flies

from nearby dairies that do use neonicotinoids, such as the dinotefuran-based fly bait QuikStrike®, although further research is needed.

The highly selected and continually maintained behaviorally resistant fly strain, BRS-1 SEL, exhibited the highest level of physiological resistance among the three strains examined. However, the LC_{95} values did not differ significantly between the WT-21 and the BRS-1 SEL fly strain, which may provide evidence that resistance is somewhat fixed in these populations. The LC_{50} value of the BRS-1 SEL fly strain increased (from 539 to 779 μg g^{-1}) between the original evaluation of physiological resistance (after behavioral resistance selection) and the current study.[15] Although this increase is statistically significant, if behavioral resistance selection was increasing the physiological resistance profile of the flies, we would expect to observe a greater increase in resistance after 5 years of selection of this strain, as is observed in studies purposefully increasing the physiological resistance profile of house flies to imidacloprid.[20,21] Additionally, the LC_{95} values for the BRS-1 SEL fly strain did not increase significantly from the original evaluation to now (Hubbard, unpublished data). The stability or slight increase in physiological resistance observed in the BRS-1 SEL fly strain may be a result of the strain's constant exposure to imidacloprid when maintaining behavioral resistance through choice assays. This moderate level of physiological resistance may provide behaviorally resistant flies with a protective survival effect as the flies may not receive a lethal dose of imidacloprid during their brief contact with imidacloprid-treated sucrose in choice assays. An alternative hypothesis is that the genetic mechanisms for behavioral and physiological resistance to imidacloprid may be linked, and some factors contributing to physiological resistance may be inherited with behavioral resistance, although future research is needed to confirm this hypothesis.

4.2 Stability of behavioral resistance to imidacloprid

It is widely known and documented in the insecticide resistance literature that resistance alleles often have deleterious fitness costs and will disappear from field populations in the absence of insecticidal pressure.[22–32] However, the relative fitness of resistant populations may increase or decrease depending on circumstances.[33,34] Little has been documented regarding the stability of behavioral resistance or aversion to insecticides or components of toxic food baits with the exception of the current study and a study conducted by Jensen *et al.*[29] in glucose-averse German cockroaches. Jensen *et al.*[29] reported that following 12 months of rearing glucose-averse (GA) and WT German cockroaches together on different dietary substances, all cages contained significantly more of the WT than the GA cockroaches, suggesting that WT cockroaches outperform GA cockroaches even when reared in the absence of glucose-containing baits. These results indicate that glucose aversion in German cockroaches has a negative fitness cost and implies that in environments where GA and WT cockroaches exist, the WT genotype may predominate over time, potentially allowing for the successful use of glucose-containing insecticide baits once again.

In the examination of the behaviorally selected fly strains (BRS 1–5), long-lasting and stable behavioral resistance to imidacloprid in the absence of exposure to imidacloprid was observed. No reduction in resistance was observed for BRS 2–5 during 10 generations (≈1 year) without exposure and impressively no reduction in resistance was observed for 30 generations (≈2.5 years) in the BRS-1 fly strain. This lack of reduction of resistance indicates that behavioral resistance is stable and fixed in our highly selected behaviorally resistant fly populations. This may indicate that behavioral resistance to imidacloprid does not have deleterious fitness costs that would cause resistance to decline in the absence of imidacloprid. Alternatively, these fitness costs may not be realized under laboratory rearing settings. These results potentially have significant implications for the management of house fly populations exhibiting behavioral resistance to imidacloprid, although future research is needed to examine ways to overcome behavioral resistance. It appears that the standard integrated resistance management approaches commonly used to combat physiological resistance to insecticides, such as insecticide rotation or reduced use, will not work to recover populations from behavioral resistance to imidacloprid.[37,38]

The newly collected field population of house flies, WT-21, exhibited a high level of behavioral resistance to imidacloprid, with a drastically higher average survival than the WT-15 F_3 fly strain (28.67% *versus* 90.40%) when it was screened before behavioral resistance selection.[15]

This high level of behavioral resistance was shown to wane slightly over eight generations of laboratory colonization without exposure to imidacloprid, with survival decreasing by ≈20%, although the survival differences between generations 2 and 10 were not significantly different. The reduction in survival observed could be caused by genetic bottlenecking in the fly population resulting from colonization effects or it could be attributed to the absence of potential imidacloprid exposure.

While there is a potential for behavioral resistance levels in the field to be maintained through continual exposure to imidacloprid-containing baits still in use on this dairy or in the surrounding region, it would be surprising because these baits saw failure soon after the introduction in 2002 in Southern California and are likely not to be currently in use.[10,12,35,36] If imidacloprid-containing baits are not in use, this may indicate that behavioral resistance is fixed in a percentage of the population, as the behavioral resistance phenotype exhibited by flies is specific to the compound imidacloprid, and therefore would not be maintained via continual exposure to another neonicotinoid (cross-resistance).[15] The current data from the field collected and recently colonized WT-21 fly stain indicates that under ideal laboratory conditions, <10% of adult flies exposed to imidacloprid in a choice bioassay choose to feed on it and die. In field conditions, where many alternative food sources exist, the percentage of adult flies controlled by these baits is likely to be far less. While it is unknown what genetic resistance factors confer behavioral resistance to the WT-21 fly strain, we have confidence that behavioral resistance is present in this population as these flies are generally susceptible to imidacloprid at 4000 μg g^{-1} when exposed to no-choice bioassays but exhibit a high survival when exposed to a choice bioassay. These flies therefore can avoid the lethal effect of a toxicant (imidacloprid) through a behavioral modification, which is different than the typical behavior of susceptible house flies.[13]

In southern California we have evidence that behavioral resistance is a major factor contributing to the failure of imidacloprid-containing fly baits for house fly control, as baits failed to control house flies even though the physiological resistance profile of flies was significantly lower than what was formulated into imidacloprid-containing fly baits.[15] It is essential to better understand how behavioral resistance may be contributing to the failure of imidacloprid baits at regional or multistate levels, especially as we observe behavioral resistance to imidacloprid to be stable in the absence of imidacloprid exposure. A comprehensive physiological and behavioral resistance survey of house fly populations over larger geographical areas should be

conducted to determine the presence of behavioral resistance to imidacloprid.

ACKNOWLEDGEMENTS

We thank UCR students Jacqueline Holquinn, and Hannah Chu for valuable comments on this manuscript and assistance with fly colony care.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1 Keiding J, *Vector Control Series: The House Fly: Biology and Control*. Training and information guide. *World Health Organization*, Geneva, Switzerland (1986).
2 Geden CJ, Nayduch D, Scott JG, Burgess ER, Gerry AC, Kaufman PE *et al*., House Fly (Diptera: Muscidae): biology, Pest status, current management prospects, and research needs. *J Integr Pest Manag* **12**:1-38 (2021).
3 Schmidtmann ET and Martin PAW, Relationship between selected bacteria and the growth of immature house flies, Musca domestica, in an Axenic Test System. *J Med Entomol* **29**:232–235 (1992).
4 Nayduch D, Neupane S, Pickens V, Purvis T and Olds C, House Flies Are Underappreciated Yet Important Reservoirs and Vectors of Microbial Threats to Animal and Human Health. *Microorganisms* **11**:583 (2023).
5 Nayduch D and Burrus RG, Flourishing in filth: house Fly–microbe interactions across life history. *Ann Entomol Soc Am* **110**:6–18 (2017).
6 Larsen EB and Thomsen M, The influence of temperature on the development of some species of Diptera. *Vidensk Medd fra Dansk naturh Foren* **104**:1–75 (1940).
7 Lysyk T and Axtell R, Movement and distribution of house flies (Diptera: Muscidae) between habitats in two livestock farms. *J Econ Entomol* **79**:993–998 (1986).
8 Thomas G and Skoda S, Rural flies in the urban environment? *Res Bull* **317**:1–66 (1993).
9 Darbro JM and Mullens BA, Assessing insecticide resistance and aversion to methomyl-treated toxic baits in Musca domestica L (Diptera: Muscidae) populations in southern California. *Pest Manag Sci* **60**:901–908 (2004).
10 2002. U.S. Environmental Protection Agency, QuickBayt notice of pesticide registration. EPA Reg. No. 11556-137 *Office of Pesticide Programs Registration Division* (2002).
11 Jeschke P and Nauen R, Comprehensive molecular insect science, in *Neonicotinoid Insecticides*, ed. by Gilbert L I. Elsevier, Amsterdam pp.53–105 (2005).
12 Kaufman P, Gerry A, Rutz D and Scott J, Monitoring susceptibility of house flies (Musca domestica L.) in the United States to imidacloprid. *J Agric Urban Entomol* **23**:195–200 (2006).
13 Gerry AC and Zhang D, Behavioral resistance of house flies, Musca domestica (Diptera: Muscidae) to imidacloprid. *US Army Med Dep J*: 54–59 (2009).
14 Hubbard CB and Gerry AC, Genetic evaluation and characterization of behavioral resistance to imidacloprid in the house fly. *Pestic Biochem Physiol* **171**:104741 (2021).
15 Hubbard CB and Gerry AC, Selection, reversion, and characterization of house Fly (Diptera: Muscidae) behavioral resistance to the insecticide Imidacloprid. *J Med Entomol* **57**:1843–1851 (2020).
16 Hubbard CB and Murillo AC, Concentration dependent feeding on Imidacloprid by behaviorally resistant house flies, Musca domestica L. (Diptera: Muscidae). *J Med Entomol* **16**:2066–2071 (2022).
17 Zahn LK, Gerry AC and Fly H, (Musca domestica) Rearing and Colony Maintenance [UC Riverside] Protocol.Io (2018). https://www.protocols.io/view/house-fly-musca-domestica-laboratory-rearing-proto-36wgq93dklk5/v1.
18 R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria (2021).
19 Burgess ER, King BH and Geden CJ, Oral and topical insecticide response bioassays and associated statistical analyses used commonly in veterinary and medical entomology. *J Insect Sci* **20**:1–9 (2020).
20 Kavi L, Kaufman PE and Scott JG, Genetics and mechanisms of imidacloprid resistance in house flies. *Pestic Biochem Physiol* **109**:64–69 (2014).
21 Kaufman PE, Nunez SC, Mann RS, Geden CJ and Scharf ME, Nicotinoid and pyrethroid insecticide resistance in houseflies (Diptera: Muscidae) collected from Florida dairies. *Pest Manag Sci* **66**:290–294 (2010).
22 Brown AWA, The Insecticide-Resistance Problem. *Bull World Health Organ* **18**:309–321 (1958).
23 Georghiou G and Garber MJ, Studies on the inheritance of carbamate-resistance in the housefly (Musca domestica L.). *Bull World Health Organ* **32**:181-196 (1965).
24 Georghiou GP, Insecticide resistance and prospects for its management. *Residue Rev* **76**:131–145 (1980).
25 Berticat C, Boquien G, Raymound M and Chevillon C, Insecticide resistance genes confer a predation cost on mosquitoes, Culex pipiens. *Genet Res* **79**:41–47 (2002).
26 Bourguet D, Guillemaud T, Chevillon C and Raymond M, Fitness costs of insecticide resistance in natural breeding sites of the mosquito Culex pipiens. *Evolution* **58**:128–135 (2004).
27 Rinkevich FD, Zhang L, Hamm RL, Brady SG, Lazzaro BP and Scott JG, Frequencies of the pyrethroid resistance alleles of Vssc1 and CYP6D1 in house flies from the eastern United States. *Insect Mol Biol* **15**:157–167 (2006).
28 Gordon JR, Potter MF and Haynes KF, Insecticide resistance in the bed bug comes with a cost. *Sci Rep* **5**:10807 (2015).
29 Jensen K, Wada-Katsumata A, Schal C and Silverman J, Persistence of a sugar-rejecting cockroach genotype under various dietary regimes. *Sci Rep* **7**:46361 (2017).
30 Alam M, Shah RM, Shad SA and Binyameen M, Fitness cost, realized heritability and stability of resistance to spiromesifen in house fly, Musca domestica L. (Diptera: Muscidae). *Pestic Biochem Physiol* **168**:104648 (2020).
31 Shah RM and Shad SA, House fly resistance to chlorantraniliprole: cross resistance patterns, stability and associated fitness costs. *Pest Manag Sci* **76**:1866–1873 (2020).
32 Shah RM and Shad SA, Inheritance, stability, cross-resistance, and life history parameters of a clothianidin-selected strain of house fly, Musca domestica Linnaeus. *Environ Pollut* **278**:116880 (2021).
33 Roush RT and Daly JC, The role of population genetics in resistance research and management, in *Pesticide Resistance in Arthropods*. Springer, Berlin pp.97–152 (1990).
34 Abbas N, Khan H and Shad SA, Cross-Resistance, Stability, and Fitness Cost of Resistance to Imidacloprid in Musca Domestica L., (Diptera: Muscidae). *Parasitol Res* **114**:247–255 (2015).
35 Mullens BA, Gerry AC and Diniz AN, Field and laboratory trials of a novel Metaflumizone house Fly (Diptera: Muscidae) bait in California. *J Econ Entomol* **103**:550–556 (2010).
36 Murillo AC, Gerry AC, Gallagher NT, Peterson NG and Mullens BA, Laboratory and field assessment of cyantraniliprole relative to existing fly baits. *Pest Manag Sci* **71**:752–758 (2014).
37 Roush RT and McKenzie JA, Ecological genetics of insecticide and Acaricide resistance. *Annu Rev Entomol* **32**:361–380 (1987).
38 Zaka SM, Abbas N, Shad SA and Shah RM, Effect of emamectin benzoate on life history traits and relative fitness of Spodoptera litura (Lepidoptera: Noctuidae). *Phytoparasitica* **42**:493–501 (2014).

