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Behavioral resistance to insecticides: current understanding, challenges, and future directions

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Identifying and understanding behavioral resistance to insecticides is vital for maintaining global food security, public health, and ecological balance. Behavioral resistance has been documented to occur in a multitude of insect taxa dating back to the 1940s, but has not received significant research attention due primarily to the complexities of studying insect behavior and a lack of any clear definition of behavioral resistance. In recent years, a systematic effort to investigate the mechanism (s) of behavioral resistance in pest taxa (e.g. the German cockroach and the house fly) has been undertaken. Here, we practically define behavioral resistance, describe the efforts taken by research groups to elucidate resistance mechanisms, and provide insight on designing appropriate bioassays for investigating behavioral resistance mechanisms in the future.

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Introduction

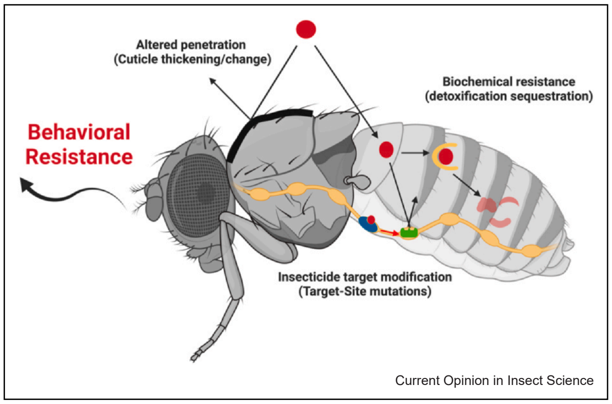
Plant and animal production, public health, and pest management programs have relied extensively on insecticides to control insects to ensure food security, reduce vector-borne diseases, and reduce bothersome environmental pests [1•]. Insecticides differ in mode of action, knockdown speed, toxicity, off-target effects, and environmental persistence [2]. They can be formulated and applied in many forms, including as sprays, dusts, seed coatings, pour-ons, ear tags, and baits [3,4]. Unfortunately, the failure of insecticides to control insect populations is a common occurrence, with the first case of insecticide resistance documented in San Jose scale (*Quadraspidiotus perniciosus*) more than 115 years ago [5].

The World Health Organization defines insecticide resistance as “the development of an ability in a strain of an organism to tolerate doses of toxicant which would prove lethal to the majority of individuals in a normal (susceptible) population of the species” [6]. The development of insecticide resistance occurs when there is high insecticidal pressure, lack of chemical class rotation, and a lack of refugia from insecticide exposure [7–9••]. Mechanisms conferring insecticide resistance can broadly be categorized as physiological, biochemical, or behavioral [7] (Figure 1).

Physiological and biochemical resistance mechanisms, which include target site insensitivity, altered penetration of insecticides, and increased metabolic detoxification, have been well- studied and characterized across a wide range of insect taxa [10–12]. Over the last 70 years, methodologies for testing and identifying physiological and biochemical resistance mechanisms have been developed, including dose–response bioassays, biochemical assays, genetic linkage analyses, and molecular assays to detect resistance alleles [13–18•]. Behavioral resistance has been noted to occur in many insect groups dating back to the 1940s [19], but despite its documentation, behavioral resistance has not received significant attention from the toxicological, ethological, or general entomological communities. The lack of attention is in part due to 1) the complexity of insect behavior and its study, 2) disagreement on the definition of behavioral resistance, 3) bioassays screening for insecticide resistance not detecting changes in insect behavior (i.e. topical bioassays), and 4) the field of behavioral genetics/genomics not being fully developed [20–22•].

Despite the challenges associated with qualifying and quantifying behavioral resistance, this mechanism is as important as physiological or biochemical resistance when understanding insecticide failure and its implications in pest and vector management. Behavior at its core is observable physiology and is rooted in the underlying physiological mechanisms [20]. If researchers and product manufacturers continue to ignore behavioral resistance as a serious resistance mechanism, they risk making uninformed management decisions/recommendations, increasing costs for users, and applying

Figure 1



The three significant mechanisms of insecticide resistance are broadly characterized as physiological, biochemical, or behavioral. Physiological and biochemical resistance mechanisms, including altered insecticide penetration due to cuticular thickening, target site insensitivity, and increased metabolic detoxification, have been thoroughly studied across many insect taxa. In contrast, investigation of behavioral resistance mechanisms has only recently received attention despite being first documented in the late 1940s (Created with BioRender.com).

ineffective materials, leading to unnecessary environmental and personnel exposure.

The purpose of this article is to practically define behavioral resistance, highlight different examples of behavioral resistance across several taxa, provide a framework for the development and execution of future studies examining behavioral resistance, and discuss the future of behavioral resistance research in the field of pest and vector management.

Definition of behavioral resistance

Behavioral resistance can be generally defined as "those actions, evolved in response to the selective pressures exerted by a toxicant, that enhance the ability of a population to avoid the lethal effects of that toxicant" [23]. Behavioral resistance can be categorized as either stimulus-independent or stimulus-dependent [7]. Stimulus-independent behavioral resistance comes from a behavior that leads to the natural avoidance of an environment or situation where an insect might be exposed to an insecticide, which is likely caused by evolution in innate behavioral patterns/pathways [24,25]. In contrast, stimulus-dependent behavioral resistance involves the heightened ability of an insect to detect and limit contact with a toxic substance before acquiring a lethal dose due to a repellent or irritant property of the toxic substance, its formulation, or presentation leading to an aversive response [7,9••,26–29]. Stimulus-dependent behavioral resistance likely is caused by evolutionary changes to specific receptor or neural pathways, which is then observed by a change in the insect's behavior to a specific stimulus [20]. Behavioral *resistance* implies that the avoidance or irritancy before lethal contact is enhanced by insecticidal selection over time, whereas 'protective avoidance' refers to natural irritability or avoidance that is innate within a population of insects [30].

In the broader evolutionary context, the development of behavioral resistance represents a dynamic interplay between proximate mechanisms and ultimate outcomes, reflecting an adaptive response to environmental pressures. The physiological and genetic adaptations that underlie stimulus-independent and stimulus-dependent behavioral resistance drive the evolution of insect populations in real-time. As these behavioral modifications become more prevalent within a population, they illustrate a clear evolutionary trajectory that is selecting for traits that confer an enhanced survival advantage when insects are exposed to a toxicant or insecticide formulation. Over successive generations, these selective pressures change the genetic makeup of the population, favoring alleles that promote behaviors that result in the avoidance of the toxicant or insecticide formulation.

Zalucki and Furlong [22•] make the argument that in many cases where behavioral resistance is reported, it may be an innate behavior (protective avoidance) that causes the avoidance behavior, but because "comprehensive pre-control surveys of [insect] populations in areas targeted for control are rarely done, we don't know initial conditions of various traits in field populations," therefore it is not genuinely behavioral resistance. Documenting this behavior change in response to the insecticide would require a population of insects to be screened before insecticide use began, which is not practical. Additionally, when evaluating a population of insects for physiological resistance to an insecticide of interest, the historical resistance profile of a population is generally not considered. Instead, the population is compared to a known susceptible population of insects or a dose that resulted in mortality previously [31–33]. Perhaps, it is more realistic to compare the behavioral response of a population in the presence of the insecticide to a *typical* response from that taxon. We propose that,

> "*Behavioral resistance is, therefore, an evolved response to the selective pressures exerted by a toxicant or formulation that enhances the ability of a population to avoid the lethal effects of that toxicant or formulation and exhibit different behavior typical for that insect taxon.*"

True behavioral resistance examples

Over the last 70+ years, since the widespread use of Dichlorodiphenyltrichloroethane (DDT) in agricultural

Box 1 Early cases of behavioral resistance to insecticides (excito-repellency response).

The earliest examples of behavioral resistance were documented to the insecticide DDT as an excito-repellency response. Behavioral resistance to DDT was documented in 1949 by King and Gahan in the house fly (*Musca domestica*) [19]. The authors observed that in dairy barns where DDT residues were not giving satisfactory fly knockdown, many house flies rested on untreated floors, equipment, and feed troughs instead of on treated walls and ceilings.

Trapido [36] documented reduced efficacy in DDT residual house-spraying for the control of *Anopheles albimanus* in two experimental villages in Panama, but found that field-collected mosquitoes were as susceptible to DDT as a known susceptible mosquito colony. Owing to this lack of physiological resistance to DDT, Trapido concluded that any change in the effectiveness of the insecticide must be due to a change in the mosquito behavior [36]. While Trapido made assumptions that the mosquito behavior had been modified, leading to the reduced efficacy of DDT, Gerold and Laarman [37] showed through lab selection assays that colonies of *Anopheles atroparvus* could be selected to either escape or not escape from tubes impregnated with DDT after only ten generations.

Excito-repellency responses have been observed repeatedly in various arthropod species to various insecticides since the initial instances, notably in mosquitoes [24,35–39], horn flies [20,40], kissing bugs [41], spider mites [42], bed bugs [43], and house flies [26,44].

and commercial settings, numerous studies from an array of insect taxa have documented behavioral resistance. The early examples of documented behavioral resistance were examples of what is known as an excito-repellency response (Box 1). This response is a behavioral change elicited by an insect after coming near or making casual contact with a surface treated with an insecticide, which results in the organism's 'avoidance' of an area treated with an insecticide, caused by noncontact (spatial) repellency, or contact excitation (irritancy) [34,35]. Excito-repellency has been documented across many insect taxa (Box 1); the bioassays used to examine this form of behavioral resistance are often limited to tests that only examine if insects escape response from a test cage [38]. While this study design allows for the documentation of behavioral resistance, it does not allow for elucidating the fine-scale mechanisms conferring the resistance.

Aversion to insecticides or components formulated into toxic food baits

The most well-documented behavioral resistance phenotype is when an insect can detect and limit contact with a toxic food material. This limited contact reduces or eliminates the consumption of the toxicant, dramatically increasing the organism's survival. Behavioral resistance to insecticides or components of toxic food baits has been documented in numerous insect species such as the fungus-growing termite (*Macrotermes gilvus*) [45], German cockroach (*Blattella germanica*) [27,46–48•], fruit fly (*Drosophila melanogaster*) [49], and house fly (*Musca domestica*) [9••,17••,28,50–56]. While being documented in numerous insect taxa, the most thoroughly studied examples come from the German cockroaches and the house fly. These two examples, as discussed in-depth below, demonstrate novel bioassays that validate true behavioral resistance, which allows for the identification of the mechanisms conferring resistance, and examines the inheritance of the resistance (Figure 2).

German cockroach behavioral resistance (glucose aversion)

Corn syrup-based baits containing the insecticide hydramethylnon were first documented to have reduced efficacy against German cockroaches in 1988, as cockroaches were observed rejecting the diet in the field, whereas laboratory colonies readily fed on the bait [27]. Laboratory feeding bioassays documented that field-collected German cockroaches reduced consumption of corn syrup (fructose + glucose), and glucose alone, but would readily feed on fructose [27], indicating that a feeding aversion to glucose existed. Through no-choice feeding bioassays (only one food option provided to a group of cockroaches), aversion to glucose was documented to be so extreme that despite food deprivation (up to 9 days), glucose-averse cockroaches would not feed on glucose, resulting in high cockroach mortality [57].

Aversion to glucose was shown to be inherited as an incompletely dominant trait controlled by a single major gene on autosome 9, through standard F_1 backcross experiments between glucose-averse and glucose-susceptible German cockroaches. Glucose-averse or -susceptible individuals were identified by screening backcross progeny with a colorimetric feeding assay, where glucose consumption was quantified via a spectrophotometer [27,58].

Glucose aversion was further characterized through colorimetric two-choice preference assays, feeding response tests to tastants, dose-feeding response assays, and electrophysiological recordings [48••]. The results indicated that glucose aversion is processed through chemosensory appendages as glucose acted as a deterrent to feeding. Glucose was shown to stimulate both sweet and bitter gustatory receptor neurons (GRN) in the peripheral gustatory system, indicating that resistant cockroaches interpreted glucose as both a phagostimulant

Figure 2

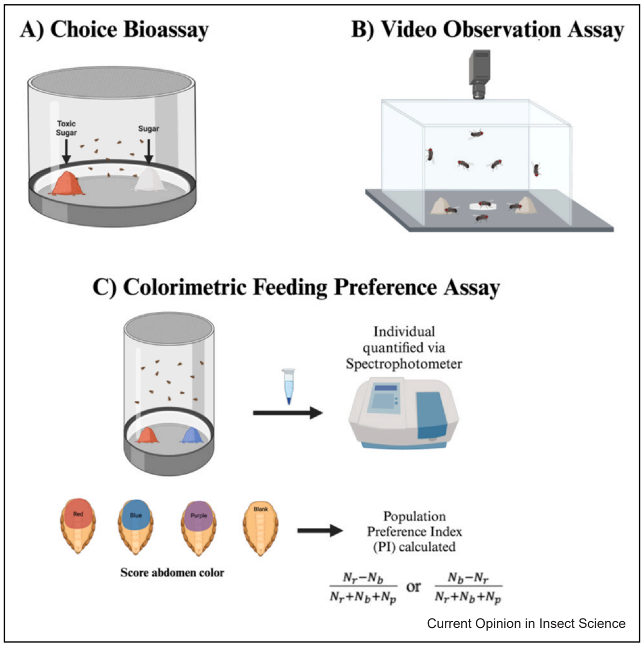


Illustration of standard behavioral resistance bioassays in insects. **(a)** Choice bioassay — presenting insects with treated and untreated food options to evaluate insect survival when given the option to ‘choose.’ **(b)** Video observation assay — monitoring insect behavior in the presence of an insecticide using video-recording. Common measurements during assays include insect visitation time, feeding length, and number of contacts with an insecticide. **(c)** Colorimetric feeding preference assay — assessing insect feeding choices based on the color of the abdomen or digestive tract, which indicates ingestion of food. This assay allows for either calculating a preference index at the population level or the quantification of an individual insect’s food intake using a spectrophotometer. (Created with BioRender.com).

and a deterrent [48••,49•]. Behavioral aversion to glucose has now been demonstrated in multiple field populations of cockroaches, including populations collected in Florida, Ohio, Puerto Rico, and Russia and has resulted in the failure of multiple insecticides containing glucose, necessitating the reformulation of these baits [48••,59].

House fly aversion to insecticides or components formulated into toxic food baits

In house flies, behavioral resistance has been documented and characterized to numerous different insecticides and formulations. Through choice feeding bioassays, behavioral resistance to malathion was detected as flies were shown to reduce feeding on malathion-treated sugar. Surviving flies (malathion-resistant) were shown to have consumed only small amounts of malathion-treated sucrose (P^{32} radiolabeled) compared with a susceptible house fly colony [60].

Freeman and Pinniger [50] examined behavioral resistance to the organophosphate bait (Alfacron®, A.I. azamethiphos) in house fly populations. Following single-fly no-choice feeding responses to blank bait (all inert ingredients of the Alfacron® bait), sugar, technical-grade azamethiphos, and the Alfacron® bait, the authors concluded that aversion was likely to the formulation components or contaminants in the insecticidal bait matrix instead of the active ingredient azamethiphos. The fly feeding response to the blank bait included the inhibition of the proboscis extension response (PER) and resulted in 0 total seconds feeding, whereas flies readily fed on the sugar and azamethiphos bait formulation.

Learmount et al. [51] examined field-collected house fly colonies from the United Kingdom for behavioral resistance to commercial bait formulations using choice bioassays where flies were provisioned commercial bait and granulated sugar. Behavioral resistance was shown to exist in 17 house fly populations to Alfacron®, and 9 house fly populations exhibited behavioral resistance to Golden Malrin®. Darbro and Mullens [52] documented a similar aversive response to methomyl-treated bait (Golden Malrin®) when flies from several California locations were tested in choice feeding assays.

House fly behavioral resistance to imidacloprid

House fly resistance to imidacloprid was reported soon after the commercial availability of imidacloprid fly baits [28]. Gerry and Zhang [28] assessed behavioral resistance to technical- grade imidacloprid from a field-collected cohort of house flies collected from a dairy in San Jacinto, California, using choice feeding assays. Flies exhibited high behavioral resistance to imidacloprid, with mortality rates never exceeding 35%, even at the highest concentrations. Mullens et al. [53] documented that in the field, flies seldom visited or fed on imidacloprid-containing baits and showed that during lab fly bait visitation/feeding assays that field flies spent significantly less time feeding on imidacloprid baits than susceptible laboratory flies.

Most recently, Hubbard and colleagues took a deliberate approach to attempt to uncover the phenotypic and genotypic mechanisms that confer behavioral resistance to imidacloprid [9••,17•,54•–56]. Using choice feeding bioassays, Hubbard and Gerry [9••] screened field-collected house flies for behavioral resistance to imidacloprid. While behavioral resistance was present in the population tested, fly survival in choice bioassays was variable. Selection for behavioral resistance was then performed *without increasing the physiological resistance profile* of the flies. Behavioral resistance was rapidly selected, with fly survival being > 90% in male and female flies following the 10th selection. The successful selection of fly strains for increasing behavioral resistance indicated that behavioral resistance to imidacloprid is a heritable trait. The behavioral resistance phenotype was then characterized via video observation assays and feeding preference assays. Behavioral resistance to imidacloprid was found to be contact-dependent, specific to imidacloprid, and concentration-dependent [9••,55•].

Following the characterization of resistance, the genetics and linkage of behavioral resistance was investigated via chromosomal linkage analysis and genetic backcross experiments [17,54•]. Resistance was shown to be inherited as a polygenic trait that was neither fully dominant nor recessive trait [17]. Behavioral resistance was further linked to factors on autosomes 1 and 4 [54•]. This type of analysis before this study, had only been completed to examine physiological resistance factors in the house fly.

Developing appropriate bioassays to evaluate behavioral resistance

Behavioral resistance should be considered a potential mechanism of resistance when insecticides in the field are documented to have reduced efficacy or fail completely. It is especially important to consider evaluating an insect population for behavioral resistance when inconsistencies between the control efficacy in the field and the physiological resistance profile of the insects are observed (low levels of control efficacy in the field, but highly susceptible to the insecticide through physiological resistance testing) [9,29,56].

Designing appropriate bioassays to evaluate behavioral resistance to insecticides is critical to understanding how insects interact with these chemicals and formulations and to manage resistance. When selecting or designing bioassays, several considerations should be kept in mind. For an in-depth overview of techniques for behavioral bioassays, please see the excellent chapter written by Baker and Cardé [61••].

When investigating behavioral resistance, researchers should first consider the objective of the bioassay and the type of response being identified. It is best to identify a single objective at a time and measure behavioral response (e.g. feeding, landing, and flying). The design of a bioassay should be tailored to the specific organism and the insecticide or formulation in question. For example, when working with a flying insect, one should allow enough space for the insect(s) to behave as they would in the field. Insects may be tested in groups or individually, and this decision should again be based on the taxa, insecticide formulation/product, and realistic field conditions. It is important to consider how individual insects or groups of insects would interact with the insecticide/product in the field, or if their interaction may impact insecticide visitation (e.g. the 'fly factor' [62]). If possible, bioassays should be conducted in tandem with a known susceptible insect strain (e.g. not previously exposed to the insecticide in question) and the insect population in question to determine if differences in behavioral responses exist. Finally, when considering insect behavior, it is imperative to standardize the physiological state of the insects tested (e.g. age, sex, and satiety state) so that responses being measured during bioassays are from the external stimulus (i.e. response to the insecticide).

Future directions

As the global community strives to maintain food security, protect public health, and ensure ecological balance, addressing behavioral resistance becomes imperative. Without understanding how widespread behavioral resistance is, techniques to combat resistance cannot be developed. An interdisciplinary collaboration, including research groups, chemical research and development, and end users, should identify research priorities and work together to establish protocols for considering insect behavior when developing new insecticidal chemistries/formulations and evaluating the efficacy of products in the lab and field. Additionally, funding agencies and policymakers should recognize and prioritize this emerging challenge by supporting research and field studies focusing on behavioral resistance and behavioral responses of insects.

Behavioral resistance to insecticides poses significant challenges in the ongoing battle against insect pests and vectors [9••,63]. While most research to date has focused on investigating physiological and biochemical resistance mechanisms, behavioral resistance to insecticides has been documented in multiple taxa and to multiple insecticides or formulations. The examples provided, our modified definition of behavioral resistance, and recommendations for designing appropriate bioassays should provide researchers with a framework to conduct future studies investigating behavioral resistance to insecticides. Moving forward, it is paramount to develop a multifaceted approach to identify, characterize, and develop ways to combat behavioral resistance to insecticides. Protocols and bioassays should be established and shared open sources for insects of concern (major pests and vectors), so that research groups nationally and internationally can identify and combat behavioral resistance. Bioassays should be conducted to establish a baseline repository of typical behaviors elicited by a susceptible population of insects in response to different insecticidal chemistries and formulations. Last, future studies and collaborations must explore the field of behavioral genetics and genomics as it relates to behavioral resistance. This will assist us in comprehending the link between shifts in insect behavior caused by insecticides and alterations in their genome or transcriptome.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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In this article, the authors conducted a series of experiments to examine the inheritance and dominance of behavioral resistance to imidacloprid in the house fly. The authors used the F_1 male backcross method in conjunction with choice and preference assays to conduct their experiments. Results documented that behavioral resistance was inherited as a polygenic trait that was differentially inherited between male and female flies. Additionally, the authors report that feeding preference assays can be instrumental in genetic inheritance studies as they showed differential feeding behavior of different genetic cross progeny.

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