

AGROCHEMICAL EFFECTS

Pervasive sublethal effects of agrochemicals on insects at environmentally relevant concentrations

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Insect biomass is declining globally, likely driven by climate change and pesticide use, yet systematic studies on the effects of various chemicals remain limited. In this work, we used a chemical library of 1024 molecules—covering insecticides, herbicides, fungicides, and plant growth inhibitors—to assess the impact of sublethal pesticide doses on insects. In *Drosophila melanogaster*, 57% of chemicals affected larval behavior, and a higher proportion compromised long-term survivability. Exposure to sublethal doses also induced widespread changes in the phosphoproteome and changes in development and reproduction. The negative effects of agrochemicals were amplified when the temperature was increased. We observed similar behavioral changes across multiple insect species, including mosquitoes and butterflies. These findings suggest that widespread sublethal pesticide exposure can alter insect behavior and physiology, threatening long-term population survival.

During the past decade, numerous reports have documented global declines in insect biodiversity (1–3). Changes in species diversity and high rates of compositional turnover have been reported worldwide (4, 5). Field studies have identified several factors associated with this decline, including habitat loss due to extensive farming and urbanization, global warming, and the widespread use of pesticides (1–3).

Sublethal concentrations of pesticides have been proposed to be a major driver of the loss of insect biodiversity (2). Case studies have outlined how sublethal doses of these agricultural chemicals can alter multiple aspects of insect biology, including metabolism, development, immunology, fecundity, and behavior (6). Thus, even when using pesticides with low nontarget activity (i.e., $LD_{50,pest\ spp.} << LD_{50,nonpest\ spp.}$, where LD_{50} indicates median lethal dose), side effects on nonpest insect species may still occur, as sublethal impacts are generally not accounted for during safety and efficacy testing (7). However, a lack of systematic experimental studies limits our mechanistic understanding of the scale and magnitude of this problem. To explore the sublethal effects of agrochemicals on insects, we established a quantitative analysis platform using *Drosophila melanogaster*—an insect model system for toxicology studies (8)—to assess the effects of these molecules on larval behavior, physiology, and fitness.

Behavior screen using an agrochemical library

We focused on the larval stage for precise compound delivery (8) and increased assay throughput (9). We exposed ~25 third-instar larvae per replicate (three replicates per pesticide and concentration) to three concentrations (2 μ M, 20 μ M, and 200 μ M) of 1024 different agrochemicals (table S1) for 16 hours in multiwell plates containing liquid food (10) (Fig. 1, A to C). This setup ensured consistent delivery and mitigated toxin avoidance (11). Concentrations of 200 and 20 μ M are typical for field applications (12, 13), whereas 2 μ M reflects environmental levels, such as those found for glyphosate in US ditches and wetlands (14).

After incubation, we measured larval survival and captured videos to assess behavioral effects (Fig. 1C). Surviving larvae were transferred to pesticide-free solid food (see materials and methods), and the number of adult flies was counted after 10 days to assess long-term lethality (Fig. 1C).

Automated pipelines were established to analyze the videos (Fig. 1D), tracking individual larvae and characterizing their behavior by trajectory features and LarvaTagger (15) (materials and methods). Behavioral impact was tested at the population scale, with outliers detected using standard methods (Fig. 1D and materials and methods). Data were imported into an open-access, relational database with an interactive website (16) for downstream applications (<https://agrotoxin.embl.de>; fig. S1A).

Sublethal effects of agrochemicals on insect behavior and physiology

To visualize the chemical diversity within our library, we applied the uniform manifold ap-

proximation and projection (UMAP) dimensionality reduction method (17) to the Morgan molecular fingerprints of the compounds (Fig. 1E and fig. S1B). This enabled fast similarity comparisons for structure-activity relationships. Although some groups, such as pyrethroid insecticides, clustered based on the UMAP embedding, most groups showed strong overlap (fig. S1, B to D), which explains potential effects of noninsecticide pesticides on insect systems (2).

Although higher concentrations generally increased acute lethality (fig. S2A), only a few chemicals produced strong (>50%) acute or long-term toxicity at the highest concentration (Fig. 1F). Each condition was tested in triplicate, showing high reproducibility for acute lethality (fig. S2B), although long-term lethality had higher variability (fig. S2, C and D) possibly because of survivorship bias. Acute and long-term lethality are not strongly correlated [Fig. 1F; coefficient of determination (R^2) = 0.06 at 200 μ M], with some pesticides showing strong acute lethality but little long-term effect.

Chemicals classified as insecticides (i.e., organophosphates, pyrethroids, carbamates, etc.; Fig. 1, G and H) caused substantial lethality even at the lowest concentrations. However, long-term lethality was widespread across the library, with many noninsecticide pesticides affecting larval survivability after overnight exposure (Fig. 1, I and J), even at the lowest tested concentration. A considerable fraction of the library (57%) affected larval behavior ($P < 0.05$; materials and methods, Fig. 1K, and fig. S1E), altering stereotypic behaviors (35%), trajectories (6%), or both (16%), even at sublethal concentrations (acute lethality < 10%) (Fig. 1K).

Insecticides were overrepresented among the molecules that lead to acute lethality (fig. S3), but all pesticide types affected behavior, with 382 noninsecticide pesticides significantly altering larval behavior (fig. S3). Abnormal wandering (materials and methods) showed the highest correlation with impaired long-term survivability (fig. S4), highlighting that some of these altered behavioral states are associated with impaired physiological processes that are critical to survival (18, 19). Furthermore, data from ToxCast (20) indicated that the effects on survivability and behavior do not correlate with the reported level of non-specific cell toxicity (table S1 and fig. S5), which suggests molecule-specific mechanisms.

Neuroactive, nonagrochemical molecules (i.e., caffeine) were tested as controls and caused significant behavioral changes that were more pronounced than changes due to different food sources or genetic backgrounds (fig. S6). To control for inbreeding depression in our laboratory strain (21), we compared its sensitivity to these molecules with two iso-female

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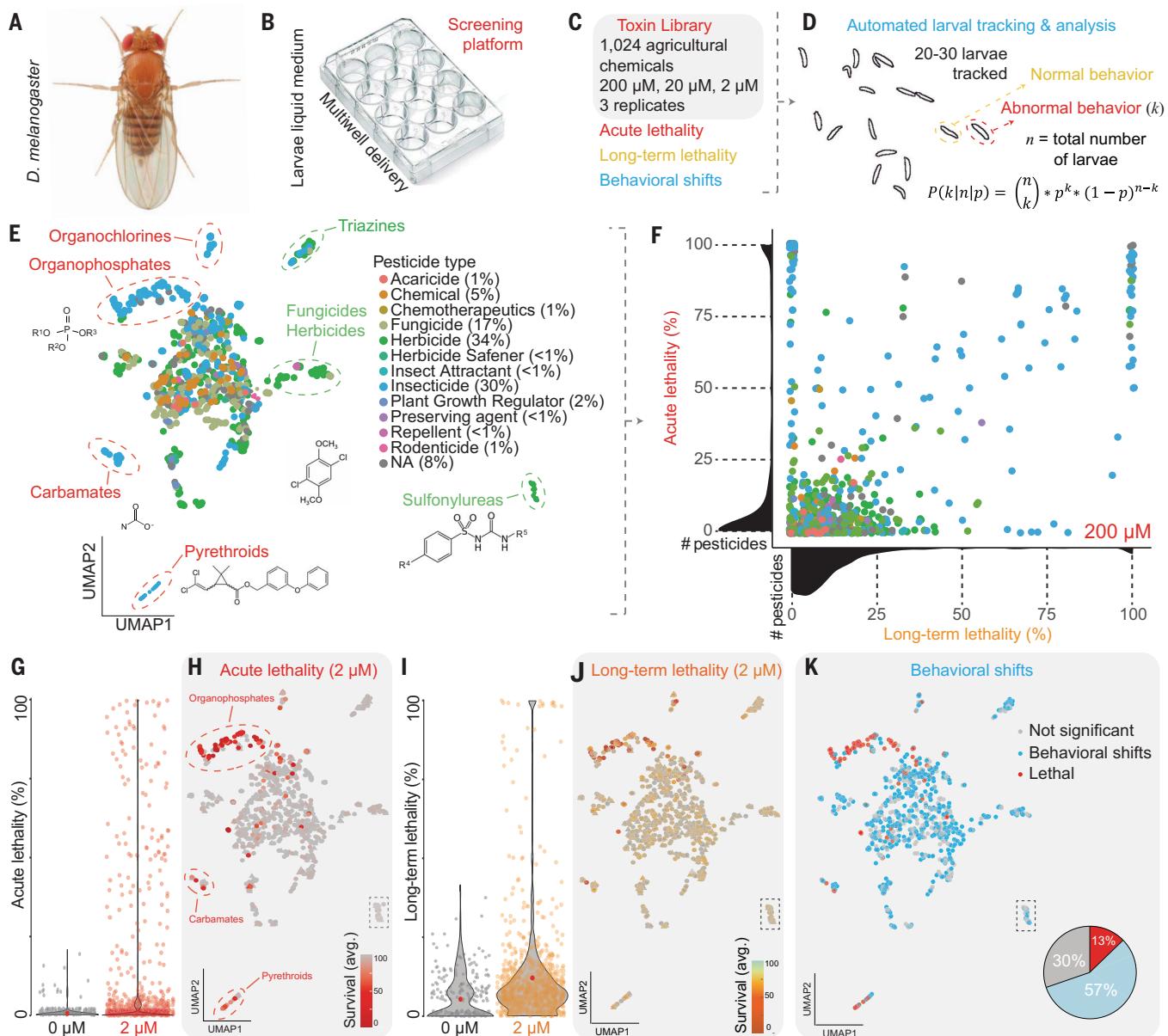


Fig. 1. Agrochemicals alter larval development and behavior at sublethal concentrations. (A to D) Schematic of the screen used for analyzing sublethal effects of pesticides. Third-instar *D. melanogaster* larvae (A) were exposed overnight in liquid medium (B) to a broad agrochemical panel, at multiple concentrations (C), and (immediate) acute lethality, long-term lethality, and the behavioral state of the treated populations were quantified after the exposure. The identification of aberrant behavior was based on the statistical comparison in each treatment with control populations (D) (materials and methods). (E) UMAP projections of the Morgan molecular fingerprints of the different molecules encompassed in the library, color coded according to their pesticide type. (F) Average ($N = 3$) assays per molecule and concentration, $n \sim 20$ larvae per assay acute and long-term lethality values observed for all of the molecules in

the library when used at 200 μM . The color code represents the pesticide type, as in (E). The axes also show the distribution of values for each of the variables. (G) Average ($N = 3$, $n \sim 20$) of acute lethality observed for all of the tested pesticides when used at 2 μM . Red dots show the mean across all molecules. (H) Acute lethality [as in (G)] mapped on top of the UMAP described in (E). (I) Average ($N = 3$, $n \sim 20$) of long-term lethality observed for all of the tested pesticides when used at 2 μM . Red dots show the mean across all molecules. (J) Long-term lethality [as in (I)] mapped on top of the UMAP described in (E). (K) Classification of pesticides as lethal at 2 μM (red), significantly affecting behavior at sublethal concentrations ($P < 0.05$ for at least the median number of replicates at that concentration; positive; light blue), or with no significant effect on behavior (negative; gray). This classification is shown as a color code on top of the UMAP described in (E).

lines from *D. melanogaster* flies collected near Naples, Italy (STR and BTI). We selected a subset of 49 pesticides (table S2) with high environmental prevalence in Europe [KEMI database (22); table S1] or in the US [Environ-

mental Protection Agency (EPA) (23) and the US Geological Survey (24); table S1] to ensure a representative sample across chemical identities and pesticide types (fig. S7A). The response profiles across these compounds were highly

similar (fig. S7, B to D). Only four molecules (in blue in fig. S7D) showed statistically significant differences in acute lethality at 2 μM , highlighting that outside of a few organophosphate compounds, the chemical response profiles are

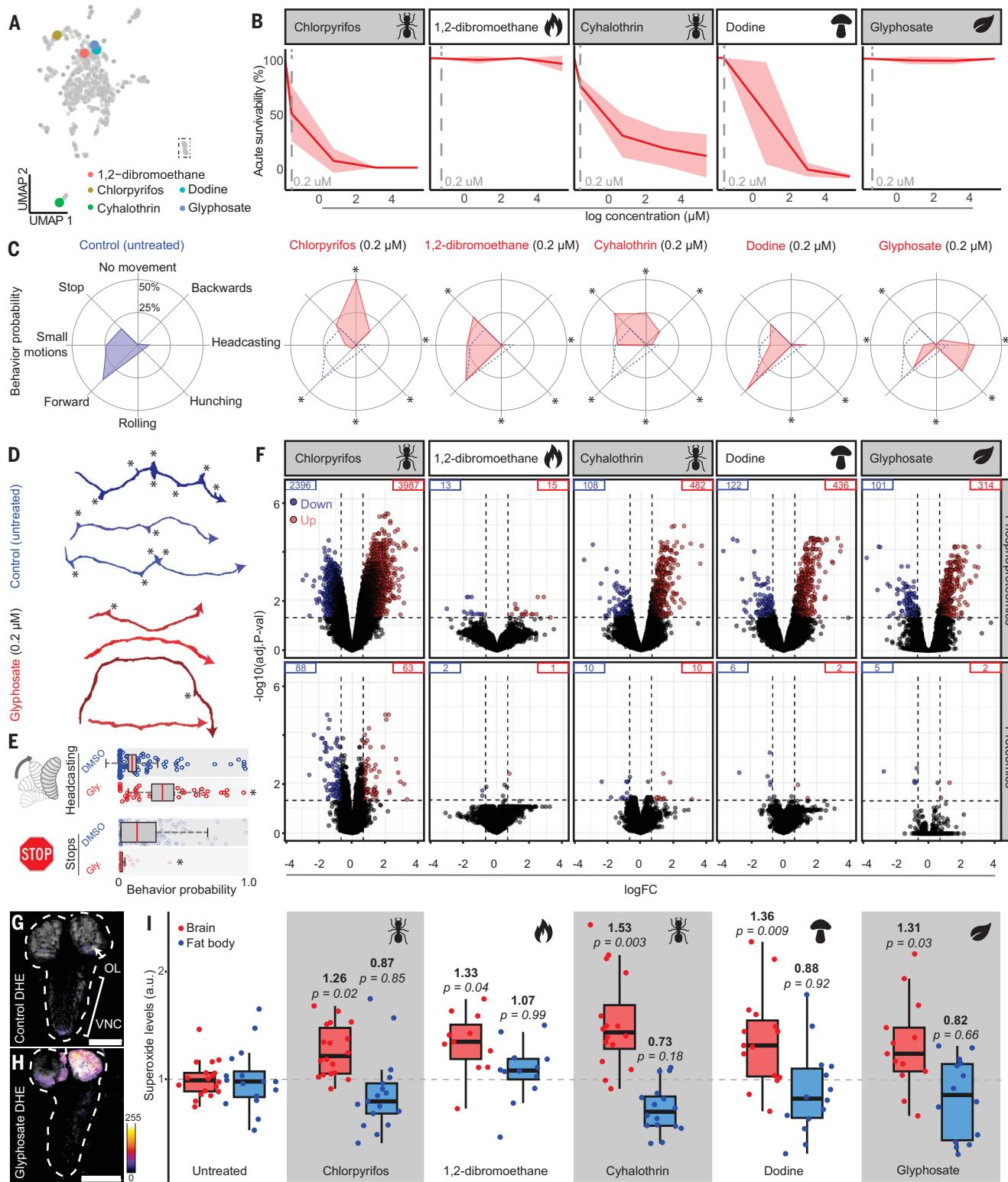


Fig. 2. Different classes of pesticides induce pervasive changes across larval systems. (A) Pesticides that were selected for phosphoproteomic analysis. Their position in the UMAP described in Fig. 1E is highlighted using colored dots. (B) Survivability dose-response curves for these five compounds. The concentration used for the phosphoproteomic characterization of the treated larvae—0.2 μM —is highlighted by the gray dashed line. $N = 2, n \sim 25$.

(C) Radar charts describing the behavioral repertoire of control (blue) or treated (red) larval populations. Each axis represents the frequency of stereotypical behaviors, noting significant changes. $*P < 0.05$; Tukey's test, one-way analysis of variance (ANOVA). $N = 2, n \sim 25$. (D) Examples of the trajectories of control (blue) or glyphosate-treated (red) larval populations. Each line depicts the path of a different larva. Asterisks (*) point out the stops. (E) Frequencies of

headcasting and stops in control (blue) or treated (red) populations. Each dot represents a different larva. $*P < 0.05$; Tukey's test after one-way ANOVA. $N = 2, n \sim 25$. (F) Volcano plot analysis showing significant changes in the phosphoproteome (upper row) and proteome (bottom row) of larvae treated overnight with different pesticides (column headers) at $0.2 \mu\text{M}$. The y axis represents the significance of the changes as minus log-transformed adjusted P value. Horizontal dashed lines mark the $P < 0.05$ threshold. The x axis indicates the magnitude of the changes as log-transformed fold change (FC). Vertical dashed lines mark the FC > 1.5 threshold. The number of annotations for

which significant changes were detected is shown within each panel (down in blue, up in red). (G and H) Brains of third-instar larvae exposed to dimethyl sulfoxide (DMSO) (G) or $0.2 \mu\text{M}$ glyphosate (H) for 2 hours and stained with dihydroethidium (DHE). The color code shows the intensity of the DHE signal. Scale bar, $100 \mu\text{m}$. OL, optic lobe; VNC, ventral nerve cord. (I) DHE signal in optic lobes (red) and fat bodies (blue) of larvae exposed to the indicated pesticides at $0.2 \mu\text{M}$ for 2 hours. $N = 2, n > 8$. Numbers in bold show the mean values for each treatment. P values come from Tukey's test after one-way ANOVA. a.u., arbitrary units.

similar across the strains. Additionally, five reconstituted populations from the *Drosophila* Outbred Synthetic Panel (25) (Dros-OSP) showed similar sensitivity to three of these molecules (fig. S7, E to G). Because these populations encompass a wide range of genetic diversity, these results indicate that our laboratory strains have not become particularly susceptible to pesticides.

To cross-validate the sublethal effects of pesticides and to identify molecular signatures of exposure, we analyzed the phosphoproteome of larvae treated overnight with five prevalent pesticides (three chemically diverse insecticides, a fungicide, and an herbicide; Fig. 2A). Posttranslational modification of preexisting proteins is rapid and can occur before transcriptional responses to toxic stimuli (26). We exposed larvae at $0.2 \mu\text{M}$ —an even lower concentration, well within environmental levels. Untreated adult flies did not show a lower preference for laying eggs in agar plates containing these molecules at $0.2 \mu\text{M}$ (fig. S8), which implies that *D. melanogaster* larvae might face these conditions in a natural context. Despite varying acute lethality (Fig. 2B), all pesticides significantly affected larval behavior at this lower concentration (Fig. 2C). For example, glyphosate—a widely used herbicide across the world (27) that is not lethal at $0.2 \mu\text{M}$ (Fig. 2B)—increased the frequency of turns (headcasting) and decreased stops, altering larval trajectories (Fig. 2, D and E, and fig. S9, A and B). Only chlorpyrifos, the most toxic compound (Fig. 2B), caused broad changes in protein levels. However, all but 1,2-dibromoethane altered the protein phosphorylation status in the treated larvae (Fig. 2F)—including those that are not lethal at the concentration used (Fig. 2B). Most changes were detected in the phosphorylation pattern of proteins associated with muscle and actin physiology, which might reflect important aspects of the stress response to reactive oxygen species (ROS) and xenobiotics (28, 29) (fig. S9C).

Consistent with previous studies showing that short-term exposure to pesticides can increase the levels of ROS in the larval nervous system (30), all five molecules led to O_2^- accumulation in the brain but not in the fat body (Fig. 2, G to I, and fig. S10) and reduced larval eating rates (fig. S11), potentially affect-

ing energy intake in natural contexts. These findings suggest that sublethal doses of these chemicals can have pervasive effects on larval populations.

Pesticides can affect life-history traits in more realistic experimental conditions

The previous experimental designs for high-throughput screens only partially simulate natural conditions for *Drosophila* larvae. To test sublethal pesticides' effects in more realistic environments, we used a mix of nine pesticides (table S3) detected in air samples across Germany (31) (Fig. 3, A and B). After overnight exposure at $0.02 \mu\text{M}$ —well within

the standard environmental prevalence ranges of these chemicals (14, 32)—no acute lethality (Fig. 3B) or behavioral changes were observed (fig. S12). However, larvae fed solid food containing this mix from hatching to the third instar showed widespread behavioral changes (Fig. 3C and fig. S12), development delays of about 1 day to pupariation ($P < 0.001$, two-tailed t test; Fig. 3D), and reduced egg-laying rates in emerging adults ($P < 0.001$, two-tailed t test; Fig. 3E). Although the developmental delay is within the expected variation among natural populations, the effect on egg laying goes beyond the physiological range measured in the above-described wild-type strains (fig. S13).

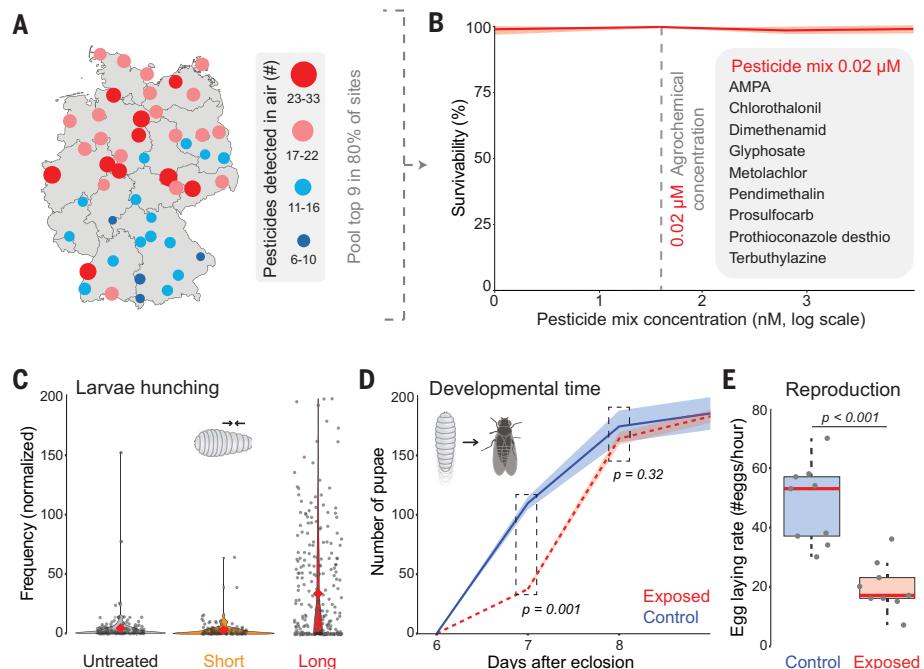


Fig. 3. Long-term exposure to pesticide mix causes changes in life-history traits. (A) Map of Germany showing the number of pesticides detected in the air [based on Kruse-Plaß et al. (31)]. The number of detected pesticides in each site is depicted in the color code and the radius of each circle. (B) Average ($N = 2, n \sim 20$) survivability (percentage of surviving larvae) after overnight exposure to the noted pesticide mix at different concentrations. (C) Hunching frequencies (normalized) of larval populations exposed to the $0.02 \mu\text{M}$ pesticide mix for 16 hours (short) or for 5 days (long). Red dots show the mean across all molecules. (D) Accumulated number of pupae observed each day after hatching on populations ($N = 3$) of 200 larvae exposed to the pesticide mix at $0.02 \mu\text{M}$ from eggs onward. *** $P < 0.01$; two-tailed t test. (E) Number of eggs laid per hour in populations ($N = 9$) of 100 flies (50 females) treated with the pesticide mix at $0.02 \mu\text{M}$ from eggs onward. Red dots show the mean across all molecules. *** $P < 0.001$; Wilcoxon test. The center line is the median, and the boxed region represents the interquartile range.

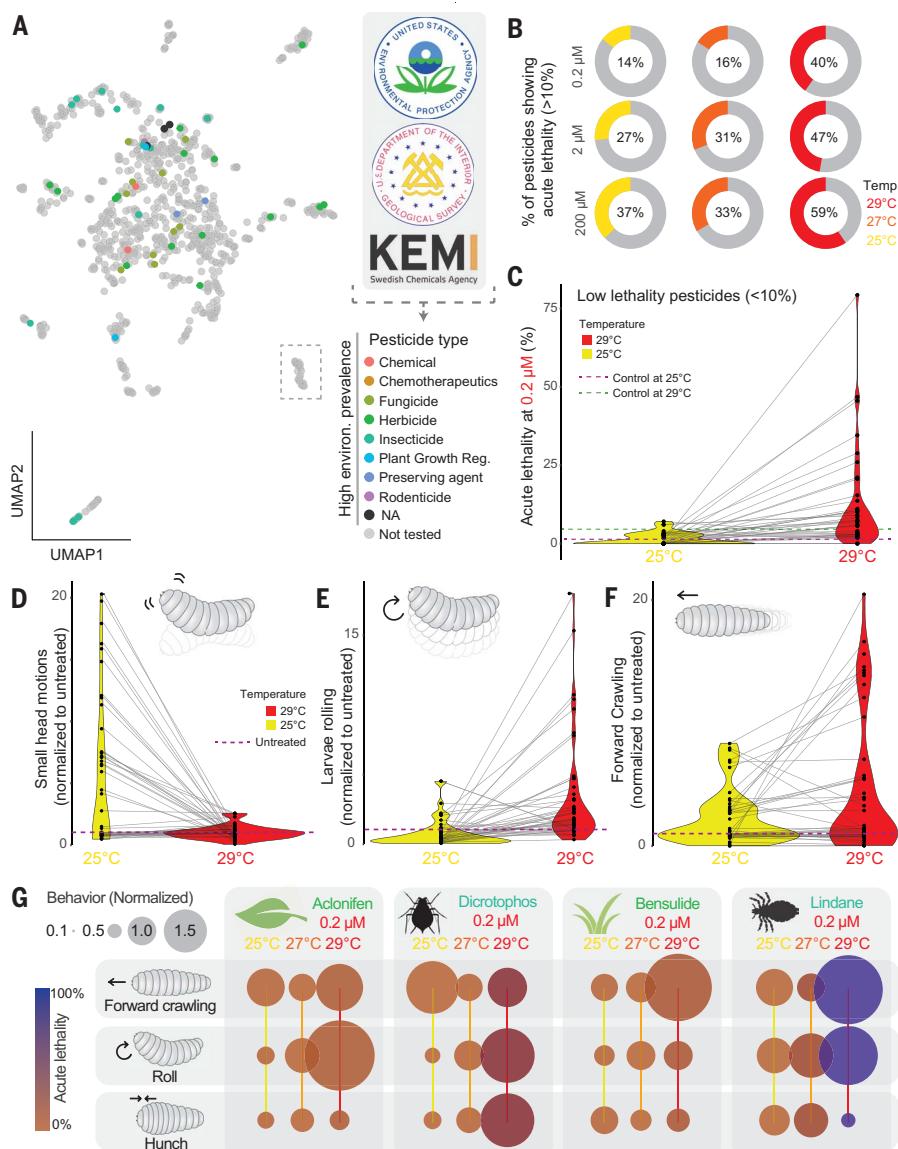


Fig. 4. Higher temperature increases acute lethality and modulates agrochemical-induced behaviors.

(A) Subset of agrochemicals selected on the basis of their high environmental prevalence (according to data from KEMI, the EPA, and the US Geological Survey) for their use in subsequent experiments. These molecules are color coded based on their agrochemical type on top of the UMAP described in Fig. 1E. (B) Percentage of agrochemicals that lead to acute lethality (>10% in two replicates) at three different concentrations and three different temperatures. (C) Average of acute lethality ($N = 2, n \sim 20$) for pesticides considered nonlethal (<10%) at 25°C. Each dot represents the average of a different molecule. Lines connect the values observed for the same molecule at different temperatures. (D to F) Average frequencies ($N = 2, n \sim 20$) of different stereotypic movements [small head motions in (D), rolling in (E), and forward crawling in (F)] in populations treated with the pesticides highlighted in (A). The frequencies are normalized to measurements from control populations at the indicated temperature. (G) Examples of temperature-dependent behavior alterations induced by agrochemicals. The radius of each circle is proportional to the average normalized frequency ($N = 2, n \sim 20$) of the indicated behavior. The color code shows the average acute lethality ($N = 2, n \sim 20$) measured for each condition.

None of the agrochemicals included in the mix are labeled as insecticides (table S3). Taken together, these findings demonstrate that—even under sublethal conditions and at concentrations found in natural habitats—diverse pesticides can compromise the fitness of insect populations.

Higher temperatures and nonlinear interactions amplify the effects of agrochemicals

The high-throughput platform allowed us to study the physiological effects of sublethal concentrations of pesticides and their inter-

action with other factors that may be driving the decline of insect populations, such as global warming (1, 2). We exposed larval populations to each of the 49 high-prevalence pesticides (Fig. 4A), increasing the overnight incubation temperature. Higher temperatures did not significantly affect larval survivability after a 16-hour exposure (fig. S14A). However, about twice as many compounds induced acute lethality at 29°C compared with at 25° or 27°C (Fig. 4B and fig. S14, B to L). Hence, many pesticides that showed low lethality (<10%) at 25°C started exhibiting significantly higher lethality when the environmental temperature was increased by just four degrees (Fig. 4C; $P = 0.300$ at 25°C versus $P = 0.008$ at 29°C, two-tailed t test).

For example, the insecticide lindane, non-lethal at 0.2 μ M at 25°C (0%), became strongly lethal (79%) at 29°C. Although many molecules altered behaviors at 25°C, the changes at 29°C were often radically different (Fig. 4, D to F, and fig. S14, M to P). Some molecules, such as the herbicides aclonifen and bensulfide, markedly altered behavior without affecting acute lethality (Fig. 4G), which highlights that behavioral effects might be missed if tests rely exclusively on lethality (6).

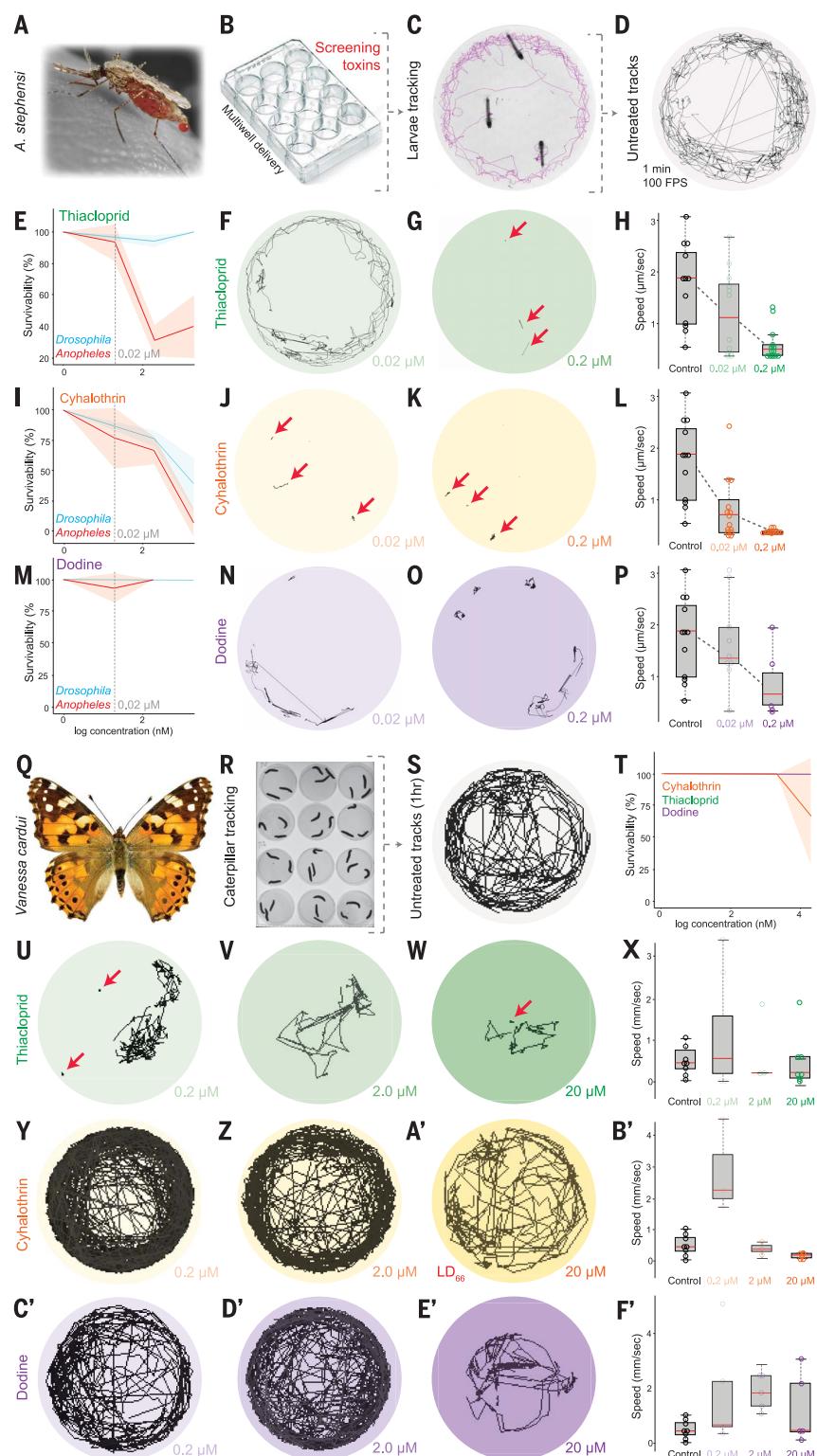
Pesticides are often used in combinations in industrial agriculture (33), which leads to animals being exposed to multiple chemicals simultaneously (34, 35). Previous studies have reported nonadditive effects of agrochemicals on arthropod systems such as *Daphnia* (36) and honey bees (37), but the frequency of these combinatorial effects across different chemical classes remains unclear.

To test possible combinatorial effects, we exposed larval populations to all pair-wise combinations of 22 highly prevalent pesticides (Fig. 4A) at 0.2 μ M and measured acute and long-term lethality. We found evidence for nonlinear interactions between many combinations (fig. S15, A and B), which suggests that synergistic pesticides interactions may be widespread. Synergy on acute lethality was confirmed for two combinations through a checkerboard analysis (fig. S15, C and D).

Behavior is also affected by sublethal agrochemical doses in mosquitoes and butterflies

We explored whether our approach could detect sublethal effects of pesticides on the behavior of other insects. We focused on medically and economically relevant species, such as a malaria vector, the mosquito *Anopheles stephensi*, and a widely distributed pollinator, the butterfly *Vanessa cardui* (Fig. 5). Mosquito larvae were exposed overnight to varying concentrations of a neonicotinoid (thiacloprid), a pyrethroid (cyhalothrin), and a fungicide (dodine) on multiwell plates followed by recording and tracking their movement (Fig. 5, A to D). All

Fig. 5. Sublethal effects of pesticides on the behavior of mosquitoes and butterflies. (A to D) Schematic of the experimental design used for analyzing the impact of pesticides on the behavior of mosquito larvae. Fourth-instar larvae of *A. stephensi* (A) were exposed overnight to different pesticides at either 0.2 or 0.02 μM in multiwell plates (B). The larvae were then recorded in each well and tracked using Mtrack2 [(C) and (D)]. (E, I, and M) Survivability curves of *Drosophila* and *Anopheles* larvae exposed overnight to thiacloprid (E), cyhalothrin (I), or dodine (M) in a liquid environment. (F to H) Effects of thiacloprid on the trajectories [(F) and (G)] and speed (H) of *Anopheles* larvae. (J to L) Effects of cyhalothrin on the trajectories [(J) and (K)] and speed (L) of *Anopheles* larvae. (N to P) Effects of dodine on the trajectories [(N) and (O)] and speed (P) of *Anopheles* larvae. $N = 3$, $n = 3$. (Q to S) Schematic representation of the experimental design used for analyzing the effect of pesticides on the behavior of caterpillars. Fifth-instar *V. cardui* caterpillars (Q) were exposed overnight to different pesticides at 20, 0.2, or 0.02 μM in solid food. The larvae were then transferred to agar plates, recorded (R), and then tracked using Mtrack2 (S). (T) Survivability curves of *Vanessa* caterpillars exposed overnight to thiacloprid (red), cyhalothrin (green), or dodine (purple) in a liquid environment. (U to X) Effects of thiacloprid on the trajectories [(U) to (W)] and speed (X) of *Vanessa* caterpillars. (Y to A') Effects of cyhalothrin on the trajectories [(Y) to (A')] and speed (B') of *Vanessa* caterpillars. (C' to F') Effects of dodine on the trajectories [(C') to (F')] of *Vanessa* caterpillars. $N = 3$, $n = 3$. Arrows across all panels highlight larvae that are alive but barely moving. In all box plots, the center line is the median, and the boxed region represents the interquartile range.



pesticides caused larvae to move significantly slower at concentrations with negligible or low lethality (Fig. 5, E to P). For *V. cardui*, we exposed fourth-instar caterpillars to solid food containing these same three molecules. Their movement was recorded in agar plates and tracked (Fig. 5, Q to S). Although only

cyhalothrin showed some lethality (Fig. 5T), all three molecules affected the movement patterns of the treated caterpillars (Fig. 5, U to F'). These results highlight that sublethal concentrations of pesticides can also affect the behavior of species with high ecological, economic, and clinical relevance.

Discussion

Agrochemicals are currently important tools for global food security and vector-borne disease control but with potential for deleterious side effects. For effective conservation efforts to mitigate the global decline in insect populations (1, 2), the relative effects of each

potential driver must be carefully disentangled. Experimental work with controlled variables is crucial (7), especially for studying synergic relationships between chemicals (33, 38, 39). This approach can provide policy-makers and chemical companies with necessary information to improve precision in agrochemical targeting and use so as to mitigate negative impacts (40).

Previous studies have linked pesticide exposure to behavioral alterations in nonpest arthropods (6, 41, 42). Building on previous phenomics assays (43), we developed a high-throughput phenotyping platform to characterize the behavioral effects of more than 1000 agrochemicals at varying concentrations. We found that 57% of these agrochemicals significantly affects *Drosophila* larvae behavior when exposed for a short time period at sublethal concentrations (Fig. 1K). These effects are broader than those from different feeding regimes or genetic backgrounds (Fig. 2, C to E, and fig. S6). Although further research is needed to investigate the longer-term impact on the population fitness, key traits—such as egg-laying rates—were significantly reduced by some of these molecules at concentrations that are orders of magnitude below sublethal concentrations (Fig. 3). Higher temperatures also increased pesticide-induced lethality and behavioral changes (Fig. 4), which emphasizes the need for chemical testing under realistic environmental conditions, especially given rising global temperatures.

Our results underscore the complex physiological mechanisms behind these phenomena, with no correlation between acute and long-term lethality (Fig. 1F), which suggests different pharmacodynamic processes (e.g., clearance, bioaccumulation, etc.). Future research can explore these processes—and the role that the gut microbiome plays in them—on a molecule-by-molecule basis. Together, these observations emphasize the potential for ecological developmental biology toward discussions on sustainability because it can provide quantitative information across life stages on how biological systems react to human-driven environmental perturbations (40).

Our findings highlight that many agrochemicals with high environmental prevalence can induce behavioral changes across insect species, even at sublethal levels. For example, dodine—a guanidine fungicide with no reported mechanism of action, which is currently sold in the US at 20 mM (44)—induced broad changes in the phosphoproteome of *Drosophila* larvae. Consistent with these changes, dodine altered larval behaviors in flies, mosquitoes, and butterflies (Fig. 2C and Fig. 5) when used at concentrations several orders of magnitude below the spraying concentration [EPA report (23)]. Notably, although low concentrations of dodine (0.2 μ M) increased the locomotor activity in *Drosophila* (Fig. 2C) and *Vanessa* (Fig. 5, C'

and F'), it inhibited movement in *Anopheles* (Fig. 5, O and P). This observation highlights how the same molecule at the same concentration can trigger almost opposite effects in different species. Dodine appears to have a stimulatory effect at lower concentrations and an inhibitory effect at higher ones (Fig. 5, C' to F'). The effective concentration of a xenobiotic depends on multiple factors, including the size of the animal and the method and time of exposure (45). Thus, smaller aquatic animals—such as *Anopheles* larvae—have an effectively higher exposure to environmental molecules, which can lead to different physiological effects in cases of molecules with nonmonotonic dose-response curves, such as dodine (Fig. 5F'). Therefore, our findings suggest that the next generation of pesticides should be subjected to more comprehensive testing focused on sublethal effects across different representative species. Notably, these types of assays provide more precise data on how to target pest control for medically important vector species without negatively affecting overall insect biodiversity.

The reduced egg-laying rate detected in fly populations exposed to pesticides reveals that even under sublethal conditions and at concentrations found in natural habitats, these molecules can compromise the fitness of insect populations. As such, we provide empirical evidence supporting the potential role of agrochemicals as a driver of the collapse of insect populations, which may be further exacerbated by climate change (2, 38, 46). We suggest that assays on chemical safety inclusive of fitness parameters outside of lethality could contribute to improving chemical safety assessments to better protect the environment, secure food supplies, and safeguard animal and human health.

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Competing interests: The authors declare that they have no competing interests. **Data and materials availability:** The fly strains generated in this study (BTI and STR) will be available upon reasonable compensation by the requestor for its processing and shipping. Imaging data (original videos and processed files) have been deposited at the BioImage Archive (<https://www.ebi.ac.uk/bioimage-archive>) and are publicly available as of the date of publication

(accession no. S-BIAD970). The code for the behavioral analysis pipeline and statistical analysis are publicly available through Git repositories: <https://gitlab.com/larvataggerpipelines/Pesticides> and <https://git.embl.de/grp-crocker/agrotoxin>. Proteomics data have been deposited in the ProteomeXchange Consortium with the dataset identifier PXD046850. **License information:** Copyright © 2024 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. <https://www.science.org/about/science-licenses-journal-article-reuse>

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S15

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