Carryover insecticide exposure reduces bee reproduction across years

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Insect life plays an unrivalled role in ecosystem function, and many insects are critical to agricultural production. However, insects face continuing threats from anthropogenic stresses related to habitat loss, pesticides and pollution, and climate change (1). No insect group captures as much public and scientific attention as pollinators, especially bees, which provide important agricultural and ecological services. While a variety of stresses have been blamed for their declines (2), pesticides and other agrochemicals have received the most scrutiny; unsurprisingly, hundreds of studies have measured how bees respond to agricultural chemical exposure. While many insecticides, fungicides, herbicides, and inert adjuvants have been investigated, neonicotinoid insecticides have garnered the most attention (3). Despite restriction in some parts of the world (4), neonicotinoids are among the most widely used insecticides and are commonly applied in many agroecosystems despite evidence that they can harm bees and other pollinators (5). In PNAS, Stuligross and Williams (6) use the solitary bee, *Osmia lignaria*, an important "alternative managed pollinator" with significantly different biology than the more commonly studied European honey bee, to discover an aspect of sublethal chemical exposure. Focusing on a formulation of the neonicotinoid imidacloprid that is commonly applied in California, they parse apart how past and current insecticide exposure affects vital rates and population growth, finding that sublethal exposure can affect insects over time scales spanning months or years (Fig. 1). These results have important implications on how we consider insecticide risks for nontarget organisms and can inform efforts to conserve insect biodiversity and ensure pollinator sustainability.

O. lignaria, the blue orchard bee, is a solitary, stem-nesting bee that goes through a single life cycle in a year. It builds nests inside existing wood cavities, where female bees provision cells with a ball of pollen. When an egg hatches, a single larva feeds on the provisioned pollen ball, pupates inside the cell, and then overwinters as an adult. The following year, these adults arise to repeat the cycle. In their study, Stuligross and Williams (6) created a controlled habitat for *O. lignaria* inside of small mesh flight cages, where they planted wildflowers that provide high-quality nutrition for bees. They applied a common commercial imidacloprid soil treatment or a control (no agrochemical) treatment to their enclosures. Five weeks after this application, *O. lignaria* were introduced to the enclosure and allowed to found nests, forage on flowers, and provision their offspring (year 1). The larvae reared under these conditions developed into adults and overwintered. The next spring (year 2), female bees with these known pesticide exposure histories were introduced into the same two habitat enclosure treatments—one with and one without insecticide application. Thus, the authors were able to partition the result of developmental and/or maternal exposure to agrochemicals (year 1), adult exposure to agrochemicals (year 2), and the combination of the two (Fig. 1). Throughout both years, they measured several different facets of *O. lignaria* nesting success, including total offspring produced, the probability that the bees nested successfully, the sex ratio produced, and the nesting rate (the number of brood cells completed by each female per day).

Insecticide exposure in year 2 reduced total overall reproduction, reduced nesting probability, biased the sex ratio toward males, and reduced nesting rate no matter what the bees experienced in year 1 (6). Bees from the "worst case scenario," with insecticide exposure both years, performed significantly worse than bees that only experienced exposure as adults. Perhaps most importantly, bees exposed only in year 1 also showed significant declines in reproduction, even though their adult environment was free of pesticide contamination! Using these data, the authors then calculated population change for these bees. They predict that, under field conditions where nutritional stress and predators would be present, carryover effects would lead to declines

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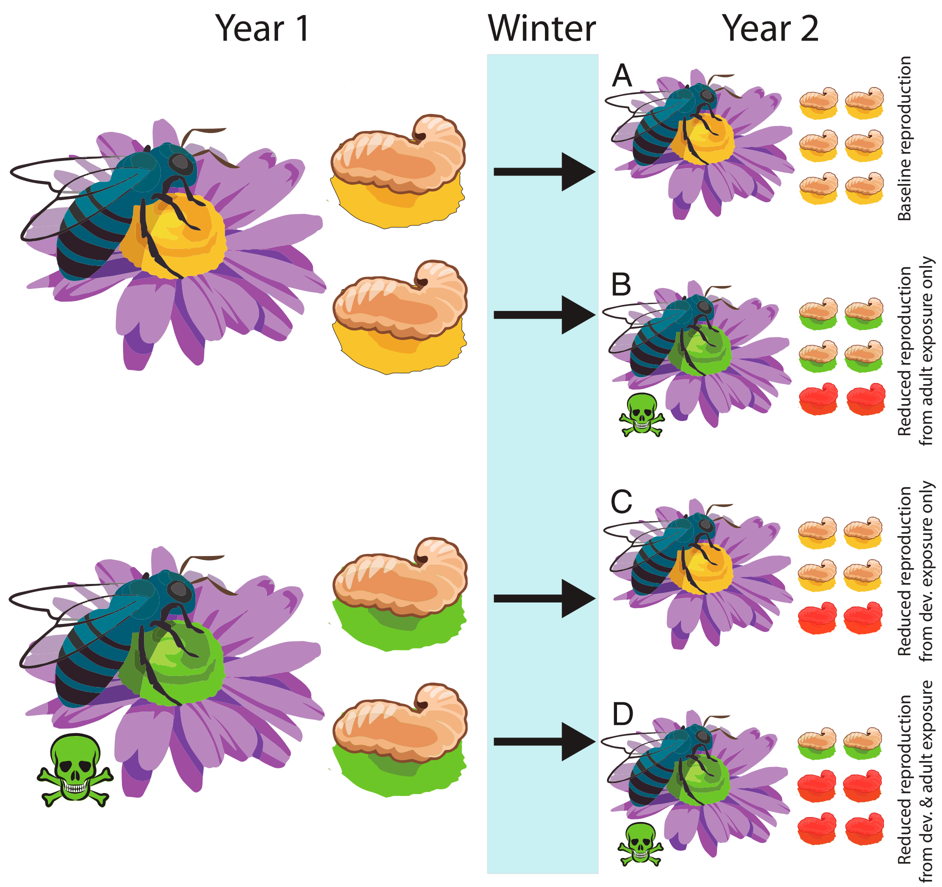

Fig. 1. **Insecticide exposure causes carryover effects resulting in reduced reproduction across years. In year 1, female *O. lignaria* feeds were exposed to control (yellow-centered flowers) or imidacloprid-contaminated (green-centered flowers) floral environments, and reared their larvae (developmental environment) on these food sources (yellow, control pollen; green, contaminated pollen). These individuals then overwinter (blue rectangle over arrows). In year 2, when offspring from the control group become adults and found nests, they produce a baseline number of offspring (scenario A; arbitrarily denoted by six larvae). If they are exposed to the contaminated environment, their reproductive output suffers (scenario B, showing red overlaid larvae). When females reared in a pesticide-contaminated environment found their nests in year 2, their previous experience affects reproduction. Even under pesticide-free conditions, they exhibit reduced reproductive output (scenario C), and, if they experience a second year of impidacloprid exposure, their reproduction declines even further (scenario D). Under field conditions, this phenomenon could result in long-term declines in bee population. Illustrations by A.G.D.**

in bee populations over time, even if the bees do not encounter pesticides as adults. Thus, without ever seeing a bee die from acute pesticide exposure, we still may see long-term declines in their populations.

So what does this all mean for protecting pollinators, and how do we use studies, like the work of Stuligross and Williams (6), to inform best management practices, policy decisions, and other actions? One route is to encourage better use of integrated pest management (IPM), that is, using chemical treatments for pest insects only when truly warranted by observing predetermined pest thresholds. For example, a recent study showed that using IPM in both a wind-pollinated field crop (maize) and a pollinator-dependent specialty crop (watermelon) resulted in no maize yield losses and improvements in watermelon yield. Further, within just a single year of using IPM, wild bee visitation of watermelon increased (7). Thus, an IPM approach could result in reduced insecticide use and improved yields and result in a rapid response by pollinators. Could such an approach be used in other cropping systems? Certainly, each has its own challenges and attributes, but this work (7) suggests that the types of effects observed by Stuligross and Williams could be reduced with expanded IPM practices in areas where better bee stewardship is needed.

Another way to improve protection of beneficial insects is to continue documenting the varied effects that different chemicals and agricultural practices have on managed and wild insect life. Then, these data can inform policies and regulations that determine how agrochemicals are used. For example, because "the [pesticide] label is the law" (8), changes in application rate, timing, etc., on pesticide labeling could reduce impacts on pollinators relatively quickly (7). Further, as new chemistries or formulations come on the market, Stuligross and Williams' (6) work underlines the importance of continuing to observe subtle effects and their causes. Their focal insecticide, imidacloprid, has been registered for use in the United States since 1994, but new products may soon replace it. For example, flupyradifurone is a relatively recently registered insecticide (9) that causes lower acute toxicity in honey bees than does imidacloprid or several other neonicotinoids (10) but has more-subtle effects on bees (9, 11, 12). Thus, the reality is that we simply do not yet know all the possible ramifications of chemistries just now coming to market, and the work by Stuligross and Williams shows that we still have a lot to learn, even with products that have been intensively studied for years.

Their work (6) also reminds us to carefully consider how we view agrochemical risk to bees. Honey bees are viewed by many regulatory agencies as a model for all bees. Widespread availability and knowledge of their biology make honey bees a practical model for assessing risk, and they likely do provide a good model for some effects (13). However, it is clear that their differences in biology result in our missing consequences that would be evident in other bee species. In our current example, Stuligross and Williams use a solitary bee to parse the different contributions of insecticide exposure at different times of an individual bee's life and then use those data to understand how such exposure would affect bee populations. Honey bee biology varies dramatically, with thousands of short-lived, functionally sterile workers that cooperate around a single reproductive queen that survives over multiple years. Observing reproductive effects and changes in population sizes would be impossible within the normal uses of honey bees for risk assessment, which primarily focuses on workers only. Therefore, we will need continued work to build the base of knowledge about comparative toxicology to inform risk assessments to better predict how new agrochemicals can affect wild bee populations (13).

Another potential way to protect pollinators is through providing greater access to high-quality habitat that provides refuge from agrochemical exposure. The use of insecticides in agroecosystems will likely always be needed to provide pest protection and ensure food security, but policies could also encourage creation of more habitat in agroecosystems. Programs like the Conservation Reserve Program can provide these kinds of refuges in the form of different habitat types, like Pollinator Habitat (CP42) and Prairie Strips (CP43), and refuges from pesticide exposure can provide important benefits to nontarget insects (14). If these refuges also provide valuable nesting habitat and food resources for bees, as is the case with CP42 and CP43 (15), they could be doubly powerful. Without care, however, pesticide exposure can still occur in these refuges, which could turn a potential conservation tool into an ecological trap (16, 17). Insecticide residues could result in chronic (18) or sublethal stress (19), even though insecticide contamination may be comparatively low in these habitats (20).

As we try to plan a future of pollinator protection and sustainability, Stuligross and Williams (6) remind us that pesticide exposure outside of what we normally consider "risky" could still result in subtle but long-lasting effects on pollinators and their populations that cannot be easily observed. Therefore, future work is needed both to identify agrochemical residues in different agricultural and conservation systems and to continue to experimentally test how different products affect a range of insect species.

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