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1. Varki, A. *Glycobiology* **27**, 3–49 (2017).
2. Li, S. *et al. Angew. Chem. Int. Edn* https://doi.org/10.1002/anie.201807054 (2018).
3. Miyawaki, A. *Nature Rev. Mol. Cell Biol.* **12**, 656–668 (2011).
4. Hori, Y. & Kikuchi, K. *Curr. Opin. Chem. Biol.* **17**, 644–650 (2013).
5. Haga, Y. *et al. Nature Commun.* **3**, 907 (2012).
6. Lin, W., Du, Y., Zhu, Y. & Chen, X. *J. Am. Chem. Soc.* **136**, 679–687 (2014).
7. Belardi, B. *et al. Angew. Chem. Int. Edn* **52**, 14045–14049 (2013).
8. Doll, F. *et al. Angew. Chem. Int. Edn* **55**, 2262–2266 (2016).
9. Laughlin, S. T. & Bertozzi, C. R. *Nature Protocols* **2**, 2930–2944 (2007).
10. Wu, N., Bao, L., Ding, L & Ju, H. *Angew. Chem. Int. Edn* **55**, 5220–5224 (2016).
11. Hui, J. *et al. Angew. Chem. Int. Edn* **56**, 8139–8143 (2017).

ECOLOGY

A systemic problem with pesticides

Exposure to a sulfoximine-based pesticide has substantial adverse effects on bumblebee colonies. This finding suggests that concerns over the risks of exposing bees to insecticides should not be limited to neonicotinoids. SEE LETTER P.109

NIGEL E. RAINE

Agricultural intensification has increased our reliance on pesticides, including insecticides. Although insecticides are useful for controlling crop damage caused by insect pests, they can also affect beneficial insects, potentially impairing their ability to control pests and pollinate crops[1] — qualities on which farmers rely. Indeed, increases in insecticide use are one of several major factors implicated in the worldwide declines of insect pollinators[2]. A commonly used class of insecticide called neonicotinoids has hit the headlines because of its impacts on bees. Siviter *et al.*[3] report on page 109 that a potential neonicotinoid replacement, the sulfoximine-based insecticide sulfoxaflor, also harms these crucial pollinators.

Insect pollinators that forage on neonicotinoid-treated plants can be exposed to small amounts of insecticide each time they or their larvae feed on pollen and nectar[4,5]. Although such chronic neonicotinoid exposure typically does not kill bees, it can have sublethal effects — impairing a range of behaviours such as learning and foraging[4–8], affecting nesting success, colony development and reproduction[7–12], and reducing pollination levels[13]. Because of this, substantial restrictions on neonicotinoid use have been introduced in some regions of the world, particularly Europe. Such restrictions might seem to be good news for bee health — but only if the insecticides that replace neonicotinoids are less harmful to insect pollinators.

Similar to neonicotinoids, sulfoximine-based insecticides are absorbed and systemically distributed throughout the plant. Sulfoxamines are one candidate to replace neonicotinoids[14], and have already been widely approved for use. Siviter and colleagues set out to assess the sublethal effects of sulfoxaflor on the agriculturally important pollinator *Bombus terrestris*. This bumblebee is common in the wild, and is also reared commercially for crop pollination. Although it is convenient to use commercially reared colonies for experiments, the authors chose to use wild colonies — a decision that should be lauded because it enhances the ecological realism of their study.

Siviter *et al.* collected 332 wild queen bumblebees, assessed them for parasites and used 249 uninfected individuals to start colonies in the laboratory. The authors succeeded in rearing colonies from 52 queens, providing a robust sample size for their experiment. They then randomly allocated pairs of size-matched bee colonies to either control or insecticide-exposure groups. The colonies fed at will for two weeks on either sugar water alone or sugar water containing five parts per billion of sulfoxaflor (a concentration found in the nectar of crops sprayed with sulfoxaflor), before being moved outdoors, so that the researchers could monitor bee behaviour and colony development under field conditions.

The team found that sulfoxaflor exposure had substantial and consistent effects on the rate of colony growth, which became apparent after just two to three weeks in the field. Sulfoxaflor-exposed colonies produced fewer female workers than did control colonies. They also produced 54% fewer reproductive offspring. This substantial difference was predominantly driven by a decrease in the total number of males produced, but also reflects the fact that all of the 36 new queens produced came from just 3 of the control colonies. Such strong variation in queen production among control colonies is not unexpected, but the lack of queen production by any of the insecticide-exposed colonies is concerning, because queens are needed to start new colonies in the following year.

These impairments in colony growth and reproduction are similar to those observed in comparable neonicotinoid-exposure studies[8–10, 12,15,16]. This similarity might be expected, given that both insecticide classes affect insects by binding to the same neurotransmitter receptors[14]. But whereas the

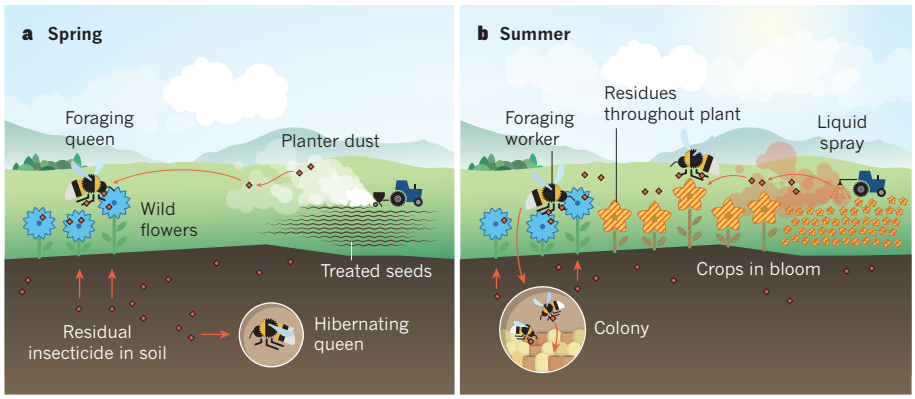

Figure 1 | Routes of bumblebee exposure to insecticides. Siviter *et al.*[3] have investigated how exposure to the insecticide sulfoxaflor affects bumblebee colonies, using a combined laboratory–field protocol. There are multiple potential routes of exposure to systemic insecticides. **a**, In spring, insecticide-treated seeds are sown. Contaminated dust from seed planters drifts across fields, and lands on wild flowers (insecticide residues are indicated by red diamonds, routes of spread by red arrows). Residual insecticide in the soil from the previous year might affect queen bumblebees hibernating in the soil, or be taken up by wild flowers, leading to exposure of foraging queens that consume contaminated nectar and pollen. **b**, In summer, crops grown from treated seeds bloom, producing contaminated nectar and pollen (red stripes). Spray treatments can increase insecticide levels on crops and on nearby wild flowers. Foraging worker bees ingest insecticide-laced nectar and pollen from both treated crops and contaminated wild flowers[17,18], and are exposed through contact with sprayed plant tissue when foraging on crops. Workers take insecticide-laced pollen and nectar back to the colony, where it is ingested by larvae (not shown).

effects on bumblebee colonies exposed to neonicotinoids seem to be driven by impaired pollen foraging[7,8] (leading to limited nutrition for larvae), the authors found no evidence that sulfoxaflor exposure caused significant differences in foraging performance. Perhaps early-stage colony growth and subsequent reproductive output were affected by sulfoxaflor toxicity to developing larvae, or by some other indirect mechanism — either way, the timing of declines in colony growth rate suggests that chronic sublethal stress at an early stage resulted in substantially reduced colony reproduction[15].

Correctly determining the effects of insecticides relies on accurate assessments of exposure, which varies depending on whether chemicals are applied by spray, soil drench or seed treatment (Fig. 1). For example, spray applications can lead to relatively high levels of exposure for a few days, whereas seed treatments can result in low-level, chronic exposure through residues in nectar and pollen[4,5]. The authors based exposure to sulfoxaflor in their experiment on a scenario in which bees ingest nectar from crop flowers following a spray application — currently, the most common mode of application for this insecticide class.

However, this scenario discounts any exposure from contact with plant tissues or dietary exposure from crop pollen, and assumes that bees forage only on sulfoxaflor-treated crops — all factors that could affect exposure levels. Moreover, exposure profiles would probably differ if sulfoxaflor were applied as a soil drench or seed treatment (an increasingly likely outcome following recent and probable future neonicotinoid regulation). Exposure could also be affected if sulfoxaflor, applied as a seed treatment or soil drench, moves outside crop fields and is absorbed by wild plants and contaminates their nectar and pollen, as reported for neonicotinoid seed treatments[17,18]. More data on sulfoxaflor concentrations in the nectar and pollen of bee-attractive crops are needed for an accurate assessment of the implications of sulfoxaflor use.

Nonetheless, Siviter *et al.* provide a valuable first step towards understanding the effects of sulfoxaflor exposure on bees. Future discussions must be broader than two-way comparisons of neonicotinoids and sulfoximines, because other classes of systemic insecticide (such as butenolides and anthranilic diamides) are also in agricultural use. It is vital to ascertain which of these insecticide classes represents the lowest potential risk to pollinators. A major part of the answer depends on how comparative risk assessments are undertaken, including which of the 20,000 living bee species are considered, because there is substantial variation in physiology, behaviour and ecology between these species. Such differences — particularly the extent to which species are social — might affect the bees' sensitivity to insecticides[10,12,19]. For instance, low-level insecticide exposure might have more impact on solitary bees than on highly social colonies that have an abundance of workers.

Finally, commercially reared pollinators (particularly honeybees) feature prominently in global agriculture, but cannot provide all of the crop-pollination services needed[20]. Wild pollinators, including bumblebees and solitary bees, have a crucial, undervalued role that is likely to become increasingly important as our crop-pollination demands rise[1,20]. Our understanding of the risks to pollinators, and the choices we make about pest control, must evolve to reflect and balance these realities. There are no risk-free choices, but with more information such as that provided by Siviter and colleagues, we can make the most appropriate decisions about how to produce the food we need without inflicting irreparable damage on the global environment and the essential ecosystem services (such as pollination) on which we depend. ■

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1. Garibaldi, L. A. *et al.* Science **339**, 1608–1611 (2013).
2. Vanbergen, A. J. & the Insect Pollinators Initiative. *Front. Ecol. Environ.* **11**, 251–259 (2013).
3. Siviter, H., Brown, M. J. F. & Leadbeater, E. *Nature* **561**, 109–112 (2018).
4. Godfray, H. C. J. *et al. Proc. R. Soc. B* **281**, 20140558 (2014).
5. Godfray, H. C. J. *et al. Proc. R. Soc. B* **282**, 20151821 (2015).
6. Stanley, D. A. & Raine, N. E. *Funct. Ecol.* **30**, 1132–1139 (2016).
7. Gill, R. J., Ramos-Rodriguez, O. & Raine, N. E. *Nature* **491**, 105–108 (2012).
8. Stanley, D. A., Russell, A. L., Morrison, S. J., Rogers, C. & Raine, N. E. *J. Appl. Ecol.* **53**, 1440–1449 (2016).
9. Whitehorn, P. R., O'Connor, S., Wackers, F. L. & Goulson, D. *Science* **336**, 351–352 (2012).
10. Rundlöf, M. *et al. Nature* **521**, 77–80 (2015).
11. Baron, G. L., Jansen, V. A. A., Brown, M. J. F. & Raine, N. E. *Nature Ecol. Evol.* **1**, 1308–1316 (2017).
12. Woodcock, B. A. *et al. Science* **356**, 1393–1395 (2017).
13. Stanley, D. A. *et al. Nature* **528**, 548–550 (2015).
14. Brown, M. J. F. *et al. PeerJ* **4**, e2249 (2016).
15. Bryden, J., Gill, R. J., Mitton, R. A. A., Raine, N. E. & Jansen, V. A. A. *Ecol. Lett.* **16**, 1463–1469 (2013).
16. Ellis, C., Park, K. J., Whitehorn, P., David, A. & Goulson, D. *Environ. Sci. Technol.* **51**, 1727–1732 (2017).
17. Tsvetkov, N. *et al. Science* **356**, 1395–1397 (2017).
18. Nicholls, E. *et al. Environ. Sci. Technol.* https://doi.org/10.1021/acs.est.7b06573 (2018).
19. Arena, M. & Sgolastra, F. *Ecotoxicology* **23**, 324–334 (2014).
20. Aizen, M. A. & Harder L. D. *Curr. Biol.* **19**, 915–918 (2009).

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STRUCTURAL BIOLOGY

Spotlight on proteins that aid malaria

The multiprotein complex PTEX enables malaria-causing parasites to survive inside red blood cells. Studies reveal how PTEX assembles, and identify a function for one of the complex's proteins, EXP2. SEE ARTICLE P.70

TANIA F. DE KONING-WARD

Malaria is caused by the parasite *Plasmodium falciparum*. For part of its life cycle, this organism resides inside human red blood cells in a membrane-bound compartment called a vacuole. To survive, multiply and evade an immune response in this environment, *P. falciparum* must transport nutrients and proteins across the vacuolar membrane[1]. On page 70, Ho *et al.*[2] report the structure of the parasite PTEX complex, which resides on the vacuolar membrane and facilitates the export of proteins from the vacuole to the cytoplasm of red blood cells[3]. And in a paper in *Nature Microbiology*, Garten *et al.*[4] reveal that the protein EXP2, which forms part of the PTEX protein-conducting channel located in the vacuolar membrane, can also form a channel that facilitates nutrient transfer across the membrane. These insights into the structure and function of key proteins that aid the survival of *P. falciparum* might help efforts to develop new antimalarial drugs.

PTEX consists of five proteins[3]: HSP101, PTEX150, EXP2, PTEX88 and TRX2. Multiple HSP101, PTEX150 and EXP2 molecules assemble to form the core part of PTEX[3,5]. It has been predicted that HSP101 unfolds proteins destined for export, and provides the energy needed for cargo to pass through the vacuolar-membrane-spanning part of the channel, which is proposed[3,6] to consist of EXP2. PTEX150 is thought[5] to have a structural role, connecting HSP101 and EXP2.

Reduced expression[7] of HSP101 or PTEX150, or inhibition[8] of the assembly of HSP101 into the PTEX complex, results in parasite death. PTEX is specific to species of the genus *Plasmodium* and is not made by humans. It is an attractive drug target because it provides the only known route by which parasite proteins enter the cytoplasm of a red blood cell. However, PTEX's relative