In Response: A perspective from academia

Insect pollination is an ecosystem service with a high economic value [1]. Many hymenopteran pollinators are threatened worldwide, and a pollinator decline (e.g., Potts et al. [2] and Winfree [3], but compare Ghazoul [4]) would, therefore, lead to higher production costs of crops. In addition, plant biodiversity and ecosystem stability may be at risk. Honeybees of the genus *Apis* are among the most important pollinating hymenopterans. Firstly, they form large colonies with several thousand individuals, which enable very efficient pollination of crops. Secondly, they are managed by beekeepers, who provide their services for agriculture wherever needed. The steady decline in colony numbers of *Apis mellifera* during the last decades has many causes. High annual winter losses of hives (15–30% of all managed hives in Europe), for example, are caused by parasites, microbial infections, malnutrition, pesticides, and climatic parameters. These factors interact, and beekeepers treat their colonies according to the representative threats. Although scientists regard the parasitic mite *Varroa destructor* as the main factor in colony losses, other anthropogenic stressors contribute. The role of the widely used neonicotinoids in colony losses is particularly controversially discussed in the public and scientific spheres.

Neonicotinoids act as agonists of the insect nicotinic acetylcholine receptor (nAChR). On binding to the synaptic nAChR, the channel opens and cations can permeate through the channel pore [5]. This cation flux depolarizes the postsynaptic membrane. Cholinergic synaptic transmission is disturbed by...
neonicotinoids either by a lasting postsynaptic depolarization or by blocking the binding of the natural transmitter to the nAChR. Nicotinic AChRs represent the major excitatory transmitter receptors within the insect central nervous system. Thus, interfering with nAChR impairs the behavior of insects. Although neonicotinoids are selective for insect nAChR over mammalian receptors, they are not very specific for any given insect species. Therefore, neonicotinoids also target nAChRs in pollinating hymenopterans. The agonist actions of various neonicotinoids on honeybee receptors have been described (reviewed by Godfray et al. [6]). It is neither surprising nor disputed that neonicotinoids also affect pollinator behavior. The question is, rather, whether neonicotinoids affect bee behavior in ways and at dosages that disturb efficient pollination and/or vitality of colonies.

Although many studies have been published recently, a distinct answer on the risks of neonicotinoids in the field under normal use is still missing. This is because of 3 major problems: 1) Which dose is relevant? 2) How do the behavioral deficits of individuals compromise the social behavior of the colony? and 3) Which species are relevant?

Which dose is relevant? The residue concentrations of pollen and nectar in samples from whole colonies are rather low [7]. In experiments where such low dosages were fed to bees, hardly any effects were observed. Similarly, field studies where hives were placed close to treated crop fields failed to show any detrimental effects on the whole colony (e.g., Blacquiere et al. [7]). However, several authors argue that the substance uptake by foragers is higher than the residues found in honey or bee bread. The argument is that foragers visit several flowers and accumulate the substances within their hemolymph on repetitive foraging trips. Because they also metabolize the substances, they unload lower residues than they encountered. Indeed, honeybees or colonies treated with such higher insecticide doses show various behavioral deficits such as foraging success [8]. Intoxicated foragers may get lost during their foraging trips rather than getting their nectar unloaded within the hive. The concentrations in honey and bee bread within the hive would then be lower than the actually encountered dosages of individuals, and the risks of field exposures to neonicotinoids would be underestimated. Field studies of full colonies typically lack information on concentration dependencies. This is due to experimental limitations because each hive represents n = 1 observation; it is impossible to keep many groups, each comprising several hives, under identical conditions simultaneously; and replications of field experiments can be performed only in successive years. In consequence, there is an obvious gap in our knowledge between the doses that cause behavioral deficits and those found in bee products.

How do the behavioral deficits of individuals compromise the social behavior of the colony? Experiments with individually neonicotinoid-treated bees indicate behavioral impairments on navigation, foraging, odor learning, and locomotion [6]. These effects occur at doses just above the field doses. Although the experimental situation in these studies does not represent the environmental reality, they are inevitable to identify physiological processes that are affected by neonicotinoids. These behavioral deficits probably also occur in hives in the field. The problem is that they often cannot be studied within the hive. By contrast, the hive parameters that are usually measured (number of bees, honey and pollen cells, weight, brood area, overwintering success, or pathogen loads) are too coarse to resolve subtle changes in behavior. Therefore, it remains an open question to what amount the deviating activity of a group of impaired workers affects the colony fitness. Foraging behavior, for example, may be impaired within a subpopulation of foragers [8,9], whereas the majority of workers are not affected: the contribution of the impaired bees to the overall collection of food is an open question. In field studies, bee colonies are placed close to crop fields treated with neonicotinoids (and untreated fields as a control), and their development is observed for an extended period of time. Today, results from experimental assays and field studies often do not correspond (but see Henry et al. [10]). High-resolution behavioral analyses of individual bees within the social activity of the hive are among the most urgently needed topics of future research.

Which species are relevant? Most honeybee populations are maintained by beekeepers. The numbers of honeybee hives are, therefore, adjusted to the demands of the market, and the numbers of beekeepers depend largely on the beekeepers’ activities. By contrast, the decline of wild bees (bumblebees and solitary bees) is not counterbalanced by beekeepers. They suffer from habitat loss or fragmentation, climate change, and pesticides [3]. Some species are specialized for certain flowers where honeybee pollination is less effective and, thus, they contribute significantly to pollination. Although some studies exist on the effects of neonicotinoids on bumblebees (e.g., Gill et al. [11]), studies on solitary bees are sparse (e.g., Jin et al. [12]). Because their worldwide decline is apparent, the maintenance of the biological diversity in many ecosystems is severely threatened, and immediate measures are needed to protect wild bee populations.

In conclusion, agricultural demands need to be balanced with ecosystem diversity and pollinator conservation. Hymenopteran pollinators come into contact with pesticides and are affected by them. Because their pollination service is indispensable for most ecosystems and for crop production, the use of pesticides should be reduced to a minimum to prevent potential risks for pollinators. More basic and independent research needs to be performed to better define these risks.

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REFERENCES
1. Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. Proc Biol Soc 274:303–313.
2. Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: Trends, impacts and drivers. Trends Ecol Evol 25:345–353.
3. Winfree R. 2010. The conservation and restoration of wild bees. Ann NY Acad Sci 1195:169–197.
4. Ghazoul J. 2005. Buzziness as usual? Questioning the global pollination crisis. Trends Ecol Evol 20:367–373.
5. Jeschke P, Nauen R, Beck ME. 2013. Nicotinic acetylcholine receptor agonists: A milestone for modern crop protection. Angew Chem Int Ed Engl 52:9464–9485.
6. Godfray HC, Blacquiere T, Field LM, Hails RS, Petrokofsky G, Potts SG, Raine NE, Vanbergem AJ, McLean AR. 2015. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc Biol Sci 282:20151821.
The debate surrounding the potential role, if any, of neonicotinoids in the decline in pollinator health has raised the issue of use and interpretation of laboratory versus field-based evidence. Recently published studies are discussed to demonstrate both the strengths and weaknesses of the different approaches under a regulatory risk-assessment framework.

**Laboratory Studies**

Laboratory studies are a very useful tool to investigate inherent toxicity, sublethal toxicity, and mechanisms of toxicity of insecticides to pollinators; and as such, they are used in the authorization process as a tier-1 screening step. Another benefit is the ability to reduce the confounding variables and include sufficient replication to facilitate statistical analysis of the data. However, adverse effects reported from such studies cannot be used in isolation for concluding a risk to pollinators under field conditions of use. For example, Kessler et al. [1] reported from a laboratory based 2-choice assay, including nectar-relevant concentrations, which both honeybees and buff-tailed bumblebees preferred sucrose spiked with imidacloprid and thiamethoxam. Even though in this experiment there were no effects on survival of bees at concentrations equivalent to 10 times what is reported from field pollen and nectar, it was still concluded that flowering crops treated with imidacloprid and thiamethoxam present a sizeable hazard to foraging bees. There was no discussion on the relevance of the behavior of these bees, which were confined in a laboratory in the dark, or the behavior of free-flying bees foraging on nectar and pollen in the agricultural landscape.

Similarly, Stanley et al. [2] investigated the proboscis reflex response of bumblebees to environmentally relevant thiamethoxam concentrations in spiked sucrose and concluded that chronically exposed bees learned more slowly and showed significantly impaired short-term memory. They suggested that these effects could be even worse under field conditions because bees would be exposed to both contaminated pollen and nectar. However, once again there is no discussion of how representative such results are from bees that have been fed thiamethoxam-spiked food continuously for 24 d. In addition, there is no discussion of how representative this response from bees individually restrained in harnesses for up to 24 h is for free-foraging bees under field conditions nor if this proboscis reflex response endpoint is a reliable measure to predict learning behavior in the field.

**Semi-Field Studies**

Semi-field studies include studies that are carried out in the field but where exposure is unrealistically forced, for example, tunnel studies and studies where treated sucrose or pollen is introduced directly into colonies or provided directly to bees. Such studies do introduce more environmental realism into the experiment than laboratory-based studies, can offer sufficient replication to facilitate statistical analysis of the data, and are valuable in refining risk assessments. However, once again care must be taken with regard to extrapolating adverse effects from such studies to actual exposure and risk to free-foraging bees under field conditions of use. For example, Henry et al. [3] used radiofrequency identification technology to conclude loss of homing behavior in honeybees exposed to thiamethoxam and predicted that exposed colonies would eventually collapse. However, in their study bees were not only individually fed with thiamethoxam-spiked sucrose prior to being released into the field, but they were also exposed to concentrations of thiamethoxam which were 36 times higher than residues typically measured in nectar and pollen under field conditions. In fact, a more recently published full field study by the same lead author [4] which exposed honeybees to thiamethoxam seed-treated oil seed rape indicated that the colony collapse predictions of Henry et al. [3] were in fact not realized under field conditions.

A more common semi-field trial design is the introduction into colonies of neonicotinoid-spiked sucrose/syrup feeding stations or pollen patties, followed by monitoring colony parameters under field or semi-field conditions. For example, Moffat et al. [5] reported that bumblebee colonies fed an environmentally relevant dose of imidacloprid and allowed to forage showed reduced colony growth. Similarly, Stanley et al. [6] used the same method of exposure of bumblebees to report reduced pollination service parameters (e.g., flower visitation rates) delivered to apples grown in tunnels. Williams et al. [7] reported compromised reproductive anatomy and reduced queen success from honeybee queens exposed to thiamethoxam and clothianidin. However, in all of these studies there is no discussion of the potential limitations and relevance of the route or method of exposure used (i.e., direct introduction into the colony of spiked food) to free-foraging bees under field conditions of use of the products in question. In fact, in every case the results of these studies are used to support wide-ranging claims on the potential threat neonicotinoids present to pollinators and pollination services in general. In addition, although all of these studies include statistical analysis, pseudoreplication starts to become an issue, which, unless specifically addressed, questions the robustness of the reported effects.

**Field/Landscape Studies**

True field or landscape studies reflect experiments where bees are actually exposed in the natural environment to crops treated under in-use conditions of the product being investigated. Such field studies are the most representative of risk under field conditions of use and thus the highest-tier studies in the risk assessment. However, clearly the control of non-treatment-related confounding factors is more difficult than for laboratory and semi-field studies, and the replication requirements for statistical analysis of the data are much more challenging. For example, Pilling et al. [8] reported no effects on honeybee colonies exposed for 4 consecutive years to oil seed
rape or maize commercially seed-treated with Cruiser (containing thiamethoxam). Even 2 yr after publication this study continues to be targeted for criticism (e.g., Hoppe et al. [9]). A comprehensive rebuttal of the criticisms was recently published [10]. Probably the most common criticism of Pilling et al. [8] has been that it lacked statistical analysis of the data. However, the lack of statistical analysis reflects the challenge of conducting field studies in the agricultural landscape, where treated fields, not colonies, are the true statistical replicate, with a need for clear separation between control and treated fields within an environment with no significant alternative forage. These factors limit the amount of true replication possible in the farming landscape and hence the statistical power of the study. Interestingly, a non-industry-funded honeybee field study, which was designed with statistical power to detect ≥20% effect, also reported no effects on honeybees from clothianidin seed-treated spring oil seed rape. In the same study, however, Rundlof et al. [11] did report effects on bumblebees and solitary bees. Other confounding aspects of this study were that other foliar insecticides were used on the crops and clothianidin residues in pollen and nectar were up to 1 order of magnitude greater than those reported in previous similar field studies [12]. This may be explained by the fact that the authors treated their own seeds, and interestingly, in the follow-up repeat study a year later by the same the authors, the reported residues were in fact significantly lower [13].

Conclusion
Clearly laboratory, semi-field, and field studies are all valuable tools that can help assess the risk of insecticides to pollinators in a tiered risk-assessment scheme. However, when it comes to making regulatory decisions, it is essential to derive conclusions on the level of risk under field realistic conditions of use of the product. In this context, although adverse effects reported in laboratory studies provide useful information on mechanisms of toxicity, they are likely to overestimate the risk under field conditions [14]. Similarly, adverse effects reported from semi-field studies, which typically will represent “high-end” unrealistic exposure conditions [15], also need to be treated with care. Although there are methodological challenges with designing and conducting true field studies, ultimately for insecticides they do provide the most representative study type for concluding on risk under real field conditions of use.

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REFERENCES
1. Kessler KC, Tiedeken EJ, Simcock KL, Derveau S, Mitchell J, Softley S, Stout JC, Wright GA. 2015. Bees prefer foods containing neonicotinoid pesticides. Nature 521:74–76.
2. Stanley DA, Smith KE, Raine NE. 2015. Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. Sci Rep 5:16508.
3. Henry M, Beguin M, Requier F, Rollin O, Odoux J-F, Aupinel P, Apel J, Tchamitchian S, Decourtey A. 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336:348–350.
4. Henry M, Cerruti N, Aupinel P, Decourtey A, Gayard M, Odoux J-F, Pissard A, Ruger C, Bretagnolle V. 2015. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. Proc Biol Sci 282:20152110.
5. Moffat C, Pacheco JP, Sharp S, Samson AJ, Boilan KA, Huang J, Buckland ST, Connolly CN. 2015. Chronic exposure to neonicotinoids increases neuronal vulnerability to mitochondrial dysfunction in the bumblebee (Bombus terrestris). FASEB J 29:2112–2119.
6. Stanley DA, Garratt MPD, Wickens JB, Wickens VJ, Potts SG, Raine NG. 2015. Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. Nature 528:548–550.
7. Williams GR, Troxier A, Retschgini G, Roth K, Yanez O, Shuler D, Neumann P, Gauthier L. 2015. Neonicotinoid pesticides severely affect honey bee queens. Sci Rep 5:14621.
8. Pilling E, Campbell P, Coulslon M, Ruddle N, Tornier I. 2013. A four-year program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS One 8:e77193.
9. Hoppe PP, Safer A, Amaral-Rogers V, Bonmatin J-M, Goulson D, Menzel R, Baer B. 2015. Effects of a neonicotinoid pesticide on honey bee colonies: A response to the field study by Pilling et al. (2013). Environ Sci Eur 27:28.
10. Campbell P, Coulslon M, Ruddle N, Tornier I, Pilling E. 2015. Authors’ response on Hoppe et al. (2015) “Effects of a neonicotinoid pesticide on honey bee colonies: A response to the field study by Pilling et al. (2013).” Environ Sci Eur 27:31.
11. Rundlof M, Andersson GKS, Bommarco R, Fries I, Hederstrom V, Herbertsson L, Jonsson O, Klett BK, Pedersen TR, Yourstone J, Smith HG. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521:77–79.
12. Cutler G, Scott-Dupree CD, Sultan M, McFarlane AD, Brewer L. 2014. A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success. PeerJ 2:e652.
13. Rundlof M, Bonmarco R, Fries I, Smith HG, Pederson TR. 2015. Inventing av risken för fogtving av bin med vaxtskyddsmedel av typen neonikotinoider under svenska forhållanden. Jordbruksverket Slutrapport 2015:24. [cited 2015 November 25]. Available from: https://www.jordbruksverket.se/download/18.1cc979d215215de0b0bb650/145215885294/Rapportbinochneonikotinoider.pdf.
14. Carrek NL, Ratnieks FLW. 2014. The dose makes the poison: Have “field realistic” rates of exposure of bees to neonicotinoid insecticides been overestimated in laboratory studies? Journal of Apicultural Research 53:607–614.
15. Godfrey HCJ, Blaquiére T, Field LM, Hails RS, Potts SG, Raine NE, Vanbergen AJ, McLean AR. 2015. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc Biol Sci 282:20151821.

In Response: A regulatory perspective from the United States

Each year, the Office of Pesticide Programs within the US Environmental Protection Agency (USEPA) makes thousands of registration decisions that encompass more than 600 existing conventional pesticides and approximately 30 new pesticides [1]. To register a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the USEPA must determine that the uses of a pesticide do not pose an unreasonable risk to human health or the environment (referred to as the “FIFRA standard”). In 2014, the USEPA published its Guidance for Assessing Pesticide Risks to Bees [2], which sets forth a tiered process for evaluating laboratory and field data to estimate risks of pesticides to bees. With a multitude of registration decisions which may be local or national in scope and require as little as several months (emergency uses) to several years (registered pesticides undergoing registration review) to complete, the risk-assessment process for bees must be not only accurate but also efficient.
A key step for ensuring efficiency in the risk-assessment process is the ability to quickly and reliably screen pesticides from further testing and evaluation. By design, this step relies on readily obtained laboratory toxicity data on individual bees (tier 1) and “high-end” estimates of exposure to determine the need for additional data and characterization. The goal of this step is to minimize false-negative outcomes while still enabling a reasonable number of pesticides to be screened for further evaluation. Laboratory data include acute and chronic toxicity endpoints associated with contact or oral exposures with the honeybee *A. mellifera*, which are then compared with high-end exposure estimates and conservative “levels of concern.” Toxicity data are typically derived from studies conducted according to internationally harmonized protocols (guidelines) which have undergone rigorous evaluation and been deemed fit for purpose. Although numeric “triggers” have been established to facilitate the decision to require additional data and refinement, other information such as the pesticide’s mode of action, data from the open literature meeting USEPA standards for inclusion in risk assessment [3], and occurrence of ecological incidents is also considered. The decision to transition to higher-tier studies does not depend entirely on the risk findings. It also depends on the risk manager’s need for additional characterization. For example, implementing risk-mitigation measures on the basis of tier-1 risk (e.g., restricting applications during or near bloom) may be preferable to requiring higher-tier information (and additional time) for refining risk estimates.

For eusocial bees, once the decision has been made to transition to a higher-tier studies (e.g., semi-field and full-field studies), the focus shifts from effects on individual bees to effects on the whole colony. Questions of the nature, magnitude, and duration of effects on the colony become central to the evaluation in addition to the ability of the colony to recover from pesticide-induced perturbation. Exposure assessment also shifts from use of model-derived estimates to measurement of pesticide residues in pollen and nectar in actual crops treated in the field. Although higher-tier studies offer the advantages of incorporating greater environmental realism and quantification of effects at a higher level of biological organization (colony level), these advantages do not mean that risks determined at lower tiers are automatically usurped by higher-tier data.

As shown in Table 1, each type of study exhibits strengths and limitations that must be carefully considered to develop an accurate interpretation of risks. For example, although semi-field (tier 2) tunnel studies can incorporate multiple routes of exposures (contact, oral) under “high-end” exposure conditions while bees are actively foraging, they may be limited by relatively short exposure durations (e.g., 10 d or less) because of confinement stress experienced by bees. Furthermore, high biological variability combined with a low number of replicates can result in poor statistical power with semi-field tunnel studies. Long-term exposures using tier-2 feeding study designs have attempted to overcome these limitations and have recently been required for more persistent, systemic insecticides. However, the resource demand for these studies is significant (e.g., 12 replicate sites/treatment and ~2 yr to complete), and uncertainties remain regarding the environmental realism of exposure from spiked pollen and/or nectar. Full field (tier 3) studies offer the greatest amount of environmental realism as applications are made to the specific crop(s) of interest and bees are freely foraging. However, actual exposure of bees to the treated crop(s) can be difficult to quantify, and resource limitations may preclude assessment of “high-end” exposure conditions which may occur elsewhere in the agricultural landscape given the large foraging distance of honeybees (e.g., up to 5 miles). Retrospective monitoring studies, for evaluation of either effects or exposure, offer an additional line of evidence for assessing risk but come with their own set of limitations in that causality is often difficult to rigorously establish.

Given the wide variety of study designs being considered for evaluating pesticide risks to bees, the question is not “whether” studies conducted at different assessment tiers provide different indications of risk. Rather, the questions are, when they do differ, “why” do such risk findings differ and is there a sufficient “concordance of information” across different levels of biological organization to support a given risk hypothesis? Such an assessment approach is very similar to the conceptual framework of an adverse outcome pathway [4] and relies on information from different disciplines and study types to support an underlying hypothesis of the progression of a key event across multiple levels of biological organization. With imidacloprid, for example, the nature of the effects on honeybee colonies fed contaminated sucrose solutions was consistent with other evidence linking imidacloprid exposure to reduced foraging success in bees [5,6], with dose-dependent reductions in pollen collection resulting in reductions in pupae and eventually reduced numbers of adult bees. Therefore, from a regulatory perspective, the decision to transition to higher assessment tiers is not based entirely on a set of predetermined numeric risk-based triggers. Instead, it is informed by risk-management concerns (e.g., potential mitigation measures, economic benefits, availability of pesticide alternatives) and requires careful evaluation of the unique set of strengths and limitations of each study to help determine, through evaluation of multiple lines of evidence, that the use of a pesticide will or will not meet the FIFRA standard.

Disclaimer—The views and opinions expressed in the present Perspectives are those of the authors and do not necessarily represent those of the US Environmental Protection Agency.

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REFERENCES

1. US Environmental Protection Agency. 2016. Implementing the Pesticide Registration Improvement Act—Fiscal Year 2015, Twelfth Annual Report. [cited 2016 September 01]. Available from: https://www.epa.gov/sites/production/files/2016-02/documents/table3.pdf

2. US Environmental Protection Agency, Health Canada Pest Management Regulatory Agency, California Department of Pesticide Regulation. 2014. Guidance for assessing pesticide risks to bees. [cited 2016 September 01]. Available from: http://www2.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

3. US Environmental Protection Agency. 2011. Evaluation Guidelines for Ecological Toxicity Data in the Open Literature. [cited 2016 September 01]. Available from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/evaluation-guidelines-ecological-toxicity-data-open

4. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA,
OECD = Organisation for Economic Co-operation and Development.

In Response: A regulatory perspective from Australia

Australian agriculture makes significant use of the neonicotinoid insecticides, particularly as seed treatments for broad-acre crops including canola. Australia is also 1 of the few countries currently still free of the V. destructor mite. Furthermore, winters in its temperate cropping zones are mild, with no (or very occasional and transient) snow. Hence, observations from Australian agricultural regions can provide a useful perspective on the ongoing debate about the effect of neonicotinoids on the global state of honeybee health, free of the significant impact...
that varroa mites (and their associated bee viral diseases) and harsh winters have on bees.

The European honeybee *A. mellifera* was introduced to Australia in the early 19th century for domestic and commercial honey production; that is, it is not native to Australia. It may come as a surprise to many that rather than being under threat, populations of feral *Apis* honeybees have proliferated to such an extent in some parts of Australia that their colonies present a very significant problem for native birds and small marsupial mammals. This proliferation can be at least partly attributed to the change in Australia’s broad-acre farming over the past several decades from an almost exclusive dependence on growing grass-based cereals (cereals are pollinated by wind and thus are not reliant on honeybees for seed set; because they do not need to expend energy to produce colorful petals, nectar, or an attractive odor, they are not bee-attractive crops: wheat, barley, and oats) to a greater reliance on flowering crops, particularly canola and lupines [1]. Canola is now Australia’s third-largest broad-acre crop after wheat and barley (~3.3 million ha sown in 2012–2013) [2]. In the temperate southern areas of Australia canola frequently provides some of the earliest springtime blossoms for both managed and feral honeybees, flowering from September to October; it produces abundant quantities of nectar and pollen with high protein content and thus is an important food resource for bees, in addition to native flora and flowering weeds. Canola crops provide a huge foraging resource for feral honeybees and stimulate them to swarm and form new colonies [3].

Many reports from agricultural areas of the southwest of Western Australia, in particular, provide evidence to counter the widely repeated claim that global populations of European honeybees are in serious decline and suggest that canola crops grown from seed treated with neonicotinoids do not appear to be causing any particular problems for honeybee colonies. Widespread canola cropping in the Western Australia wheat belt since the 1990s—now Australia’s largest canola-growing region—has coincided with a dramatic increase in feral bee numbers [4]. This area of Australia is also a native plant biodiversity hot spot [5,6]. Under these conditions, the proliferation of feral honeybee colonies has become a “major ecological problem” for a number of species of native birds [7] and small marsupial mammals [8]. This is primarily because feral bee colonies can compete with them for hollows in the trunks of old native trees, denying access to nesting sites in which they live and raise their young. The preference of feral bees for the large hollows used by cockatoos is threatening the ongoing survival of several species of black cockatoo in southwest Western Australia [9]. In addition to competing with birds for nesting spaces and access to water sources, *Apis* bee stings have been reported to kill adult nectar-eating birds (from being stung on the eye) and hatchlings in nesting hollows. Nectivorous Australian birds tend to avoid flowers being worked by *Apis* honeybees, although population-level effects on these native species are uncertain [4].

In addition to wildlife impacts, many Western Australia farms that have had continuous plantings of canola report multiple feral bee colonies infesting their farm buildings, machinery sheds, and associated equipment, whereas in the rangelands country north and east of Perth, farmers commonly report bees dying in “bucket loads” in livestock water troughs in summer; the presence of large numbers of feral bees prevents stock from accessing the water (R. Manning, Department of Agriculture and Food, Western Australia, personal communication).

The Australian Pesticides and Veterinary Medicines Authority has issued a number of permits in recent years to allow the of-f-label use of insecticides to destroy feral honeybee colonies; Western Australian land-care groups hold, or have held, permits to use fipronil-based product for this purpose [10]. The proliferation of feral honeybees is also an issue in the southeastern part of Australia; for example, the New South Wales state government has listed competition from feral *Apis* honeybees as a “key threatening process” in Schedule 3 of its Threatened Species Conservation Act [11].

Notwithstanding the above, there are a number of factors which can adversely impact populations of both commercially managed and feral *Apis* bees in Australia. These include heat waves (increasing summer temperatures have led to loss of many beehives as the wax combs have melted), droughts and extended dry weather (impacting plant flowering and directly affecting the health of bees and their ability to produce honey), loss of native flowering trees (as a result of bushfires, the creation of more conservation parks from which apiarists have been excluded, land clearing, “dieback” of flowering eucalypts as a result of fungal and insect attack, rising salinity in some areas, etc.), and loss of flowering weeds (from increased herbicide usage in low-till and no-till agriculture and from deliberate biological control of species which are toxic to stock). Furthermore, each season in Australia there are usually several reports of poisoning of managed beehives, arising from spray drift or overspray of agricultural or horticultural pesticides at or around crop flowering [12].

Thus, the information summarized above suggests that it is not correct to conclude, as many articles and reports have done, that *Apis* honeybee populations are in global decline. Furthermore, evidence from a major canola-growing area of Australia suggests that feeding on canola crops grown from neonicotinoid-treated seed is not having any obvious adverse impact on the proliferation of feral honeybee colonies. This is not to say that insecticides, compounds deliberately designed to kill or otherwise control pest insects, will not harm nontarget pollinating insects (including honeybees) attracted to flowering plants, if sufficient exposure occurs. However, observations from the southwest of Western Australia, in particular, indicate that regulatory decisions relating to the protection of pollinators should consider information gathered from observations and monitoring of representative agricultural landscapes in addition to results from laboratory-based tests, semi-field studies (including tunnel studies), and controlled field studies. Interestingly, a recent detailed study from France which tracked individual bees with radiofrequency identification technology suggests that *Apis* colonies can compensate for excess neonicotinoid-related mortality of individual bees, preserving population size and honey production [13].

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REFERENCES
1. Western Australian Museum. 2015. Feral European bees. [cited 2015 September 27]. Available from: http://museum.wa.gov.au/explore/online-exhibitions/cockatoo-care/feral-bees
2. Australian Bureau of Statistics. 2014. 7121.0—Agricultural Commodities, Australia, 2012–2013. Crops. [cited 2015 September 27].
3. Allan L. 2000. Increase in feral bees linked to canola. Department of Agriculture and Food Western Australia. [cited 2015 September 27]. Available from: http://agspsrv34.agric.wa.gov.au/ento/bee5.htm

4. Danks A. 2012. Feral bees in Western Australia. Proceedings, Forum on Feral Bees Albany, Western Australia, December 7, 2012. [cited 2015 September 27]. Available from: http://www.greenskills.org.au/pub/fb/fb.html

5. Australian Government, Department of the Environment and Energy. International biodiversity hotspots: Southwest Australia. [cited 2015 September 27]. Available from: https://www.environment.gov.au/biodiversity/conservation/hotspots

6. Critical Ecosystem Partnership Fund. 2015. The biodiversity hotspots: Southwest Australia. [cited 2015 September 27]. Available from: http://www.cepf.net/resources/hotspots/Asia-Pacific/Pages/Southwest-Australia.aspx

7. Johnstone RE, Kirkby T. 2007. Feral European honey bees: A major threat to cockatoos and other tree hollow users. West Aust Nat 25:252–254.

8. Trainor R. 1995. Sweet danger: How feral bees compete with hollow-using birds and mammals. Bird Observer 75:7–9.

9. Department of Environment and Conservation Western Australia. 2008. Forest black cockatoo (Baudin’s cockatoo Calyptorhynchus baudinii and forest redtailed black cockatoo Calyptorhynchus banksii naso) recovery plan. [cited 2015 September 27]. Available from: http://www.environment.gov.au/system/files/resources/48e4fc8c-9cb7-4c85-bc9f-6b847cf4c017/files/wa-forest-black-cockatoos-recovery-plan.pdf

10. Available from: https://portal.apvma.gov.au/permits (search on ‘PER14518’ and ‘PER80706’)

11. New South Wales Government, Office of Environment & Heritage. Competition from feral honeybees—Key threatening process listing. [cited 2015 September 27]. Available from: http://www.environment.nsw.gov.au/determinations/feralhoneybeesktplisting.htm

12. Australian Pesticides and Veterinary Medicines Authority. 2014. Overview report on neonicotinoids and honeybee health in Australia released. [cited 2015 September 27]. Available from: http://apvma.gov.au/node/12291

13. Henry M, Cerrutti N, Aupinel P, Decourtye A, Gayrard M, Odoux J-F, Pissard A, Rüger C, Bretagnolle V. 2015. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. Proc Biol Sci 282:20152110.