Received: 5 July 2011 Revised: 16 December 2011 Accepted article published: 24 January 2012 Published online in Wiley Online Library: 4 April 2012

(wileyonlinelibrary.com) DOI 10.1002/ps.3290

Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria

James E Cresswell,[a*] Nicolas Desneux[b] and Dennis vanEngelsdorp[c]

Abstract

BACKGROUND: Honey bees are important pollinators of both crops and wild plants. Pesticide regimes that threaten their sustainability should therefore be assessed. As an example, evidence that the agricultural use of neonicotinoid pesticides is a cause of the recently observed declines in honey bees is examined. The aim is to define exacting demographic conditions for a detrimental factor to precipitate a population decline, and Hill's epidemiological 'causality criteria' are employed as a structured process for making an expert judgement about the proposition that trace dietary neonicotinoids in nectar and pollen cause population declines in honey bees.

RESULTS: In spite of the absence of decisive experimental results, the analysis shows that, while the proposition is a substantially justified conjecture in the context of current knowledge, it is also substantially contraindicated by a wide variety of circumstantial epidemiological evidence.

CONCLUSION: It is concluded that dietary neonicotinoids cannot be implicated in honey bee declines, but this position is provisional because important gaps remain in current knowledge. Avenues for further investigations to resolve this longstanding uncertainty are therefore identified.

Keywords: agrochemicals; imidacloprid; insects; neurotoxin; pollination; pollutants

1 INTRODUCTION

Since the publication of Rachel Carson's book *Silent Spring*[1] there has been widespread public awareness that pesticides can have unintended detrimental effects on non-target biota.[2] Carson's book focused on DDT and precipitated a period of scientific and regulatory scrutiny that eventually led to restrictions on the chemical's use in the United States and other countries. Carson's warning resonates today, but science-based risk appraisals normally conclude that pesticides should play a role in crop protection because the benefits of increased productivity outweigh the risks to non-target organisms.[3] The regulation of pesticides is typically based on assessing these risks. In Europe, for example, the approval of crop protection products is governed by EU Regulation 1107/2009,[4] which does not require that agrochemical pesticides are ecologically harmless, but instead specifies that member states may not authorise a crop protection product unless it has no unacceptable effect on the environment, including non-target species. A full exploration of what counts as an unacceptable effect is not within the scope of the present review, but it is taken as granted that a pesticide's use is unacceptable if it seriously threatens a non-target species that contributes to human well-being by delivering an important ecosystem service. Here, an examination is made of the case of the neonicotinoid pesticides, which are implicated by some as a cause of the widely observed declines among honey bee populations[5,6] and as a serious threat to valuable pollination services. Imidacloprid is focused upon in particular because it is extensively used in agriculture, and, among the neonicotinoids, its effect on honey bees has been the most studied.

Honey bees (*Apis mellifera* L.) are important pollinators of both crops and wild plants, and they provide a highly valued ecosystem service.[7] On this basis, pesticide regimes that threaten the sustainability of honey bee populations are candidates for being deemed unacceptable. Below, the acceptability of the agricultural use of neonicotinoid pesticides is considered by examining the evidence that they contribute to the recently observed declines in honey bee populations of the United States and Europe.[8,9] Firstly, a brief review is given of the problem's context, and a structured procedure is introduced that can be employed to implicate a factor as a cause of a phenomenon in the absence of conclusive experimental evidence.

* *Correspondence to: James E Cresswell, College of Life and Environmental Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4 PS, UK. E-mail: j.e.cresswell@ex.ac.uk*

a *College of Life and Environmental Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4 PS, UK*

b *French National Institute for Agricultural Research (INRA), UR 880, 400 Route des Chappes, 06903 Sophia-Antipolis, France*

c *Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania, PA 16802, USA*

1.1 Context of the controversy

Systemic neonicotinoids, such as imidacloprid, are currently among the most widely used insecticides in crop protection.[10] They are neurotoxic to insects and disrupt their nervous system, causing paralysis and death.[11,12] Neonicotinoids are applied as foliar sprays and as seed dressings,[13] after which the chemical pervades the plant systemically to protect it against insect pests that consume sap and tissues. Bees are non-target insects that may be harmed either by direct contact with neonicotinoid products or by ingesting them in nectar and pollen from the flowers of treated crops. Treated crops whose nectar and pollen contain neonicotinoid residues include oilseed rape (canola), *Brassica napus* L.,[14,15] and sunflower, *Helianthus annua* L.,[14,16] where the residues occur at trace levels (here defined as less than 10 µg AI kg^{-1}). The existence of neonicotinoid residues in nectar and pollen suggests that trace dietary intake by the bees that forage on mass-flowering crops is inevitable, which necessarily raises concern over the potential impact of these pesticides on bee health.

In 1994, imidacloprid was the first of the neonicotinoids to be approved for use as an agricultural pesticide, but since then its use has increased greatly (Fig. 1), as has use of other members of the family, such as clothianidin and thiacloprid. The increased use of neonicotinoids has coincided with a period of continual decline in the numbers of managed honey bee colonies in some parts of the world. In the United States, for example, the number of colonies has fallen steadily from a post-war high of almost 6 million to its current level of approximately 2.5 million colonies, and the decline has been accompanied by high rates of annual colony loss, these being on average 30% in the years 2006–2008.[17] More recently, colony collapse disorder (CCD) has been recognised as a pathological syndrome among honey bees in the United States,[18] and it accounts for up to 10% of the colonies that are lost each winter.[19] The population declines of honey bees are accompanied by widespread concern over the sustainability of pollinator services to agriculture[20,21] and wild plants.[22,23] The increasing imperative to protect pollination services has raised the pressure to find the factors that threaten them, and pesticides are identified by some as important culprits.[5] Neonicotinoid pesticides in particular have attracted attention against this backdrop of heightened tension, as follows.

Imidacloprid was first identified publicly as a threat to bees in 1999, when its use in France was restricted by the French government after claims by beekeepers that agricultural use of its commercial formulation, Gaucho®, was responsible for widespread losses of hives.[24] Since then, many investigations into the effects of imidacloprid on honey bees have been published. Initially, these efforts were led by French government institutes[25] and by the laboratories of a pharmaceutical company that produces neonicotinoid products, Bayer.[16] However, there was no consensus about the potential impact of trace dietary imidacloprid on honey bees[26] because the studies were methodologically varied and produced conflicting results.[27] For example, one study indicated that mortality rates in adult bees were greatly increased at oral doses that were below the environmentally realistic trace range,[28] whereas another study failed to detect increased mortality even at much higher doses.[29] Moreover, while many laboratory tests found that trace dietary imidacloprid harmed adult honey bees, field tests found no detrimental effects on honey bee colonies either by imidacloprid[30] or by another neonicotinoid, clothianidin.[31] Outside France, government regulators rarely

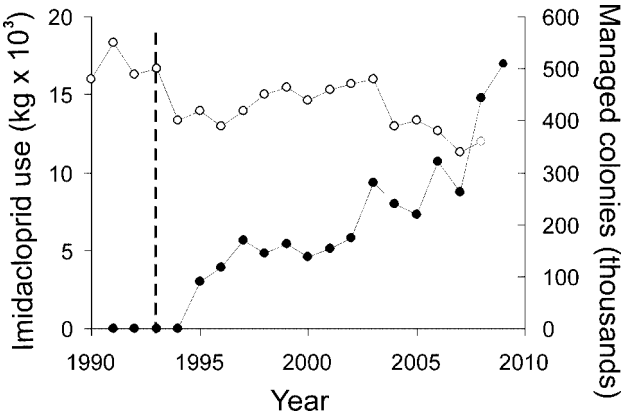

Figure 1. Annual amounts of imidacloprid (tonnes) applied to honey-bee-visited crops in California in 1991–2008 (filled symbols, leftmost *y*-axis) and number of managed honey bee colonies (thousands) in California in 1990–2008 (open symbols, rightmost *y*-axis). The dashed vertical line indicates 1994, when neonicotinoid use first began in California. The imidacloprid-treated crops are: alfalfa; almonds; apples; apricots; beans; bell peppers; blackberries; blueberries; cantaloupe; cherries; citrus; dried beans; grapefruit; lemons; limes; melons; nectarines; oranges; peaches; peas; plums; prunes; pumpkins; rape seed; succulent beans; tangelos; tangerines; watermelons. Data on imidacloprid use were compiled from the Pesticide Action Network (PAN) Pesticide Database (see http://www.pesticideinfo.org), which draws on the records of the California Department of Pesticide Regulation. The data on colony numbers are from the United States Department of Agriculture's National Agricultural Statistics Service (USDA-NASS).

restricted the agricultural use of neonicotinoids (but see Bortolotti *et al.*[32] and Forster[33]), presumably giving the null results of field trials precedence over the positive findings of laboratory studies.[34]

Public concern over the detrimental effects of neonicotinoids was inflamed in 2008, however, when clothianidin caused the mass mortality of honey bees in Baden-Württemberg, Germany. In contrast to the preceding controversy about trace dietary residues, this alarm was caused by improper agricultural practice, which released clouds of insecticidal dust during seed drilling of treated maize.[35] The potential for a recurrence of this catastrophe was greatly reduced by technological changes to drilling equipment and by improvement of the adhesive used to apply the neonicotinoid dressing to the seed.[36] After strengthening the legislation regulating seed drilling, the German government lifted part of its restrictions on neonicotinoid seed dressing.

Public concern reignited in 2009, however, when a new route for exposure of honey bees was identified, namely guttation fluid in seedling maize and other crops.[37] For a few weeks after germinating, seedling maize plants exude fluid droplets along their leaf margins by the process of guttation, and, in crops treated with a neonicotinoid seed dressing, the concentration of the insecticide in guttation fluid can reach levels that are lethal to a honey bee that ingests it.[38] The critical question of whether bees commonly consume the fluid under field conditions is currently unresolved,[37] but concern over the potential impact of neonicotinoid residues in leaf exudates has led to restrictions on the planting of maize with neonicotinoid seed dressings in Germany.[39]

In summary, the agricultural use of neonicotinoids is associated with three separate modes of exposure for bees: direct exposure by dispersal in particulate clouds during seed drilling; oral

ingestion of residues in guttation fluid of seedling maize; trace dietary residues in nectar and pollen. Here, a model for a risk assessment of neonicotinoids is presented that focuses exclusively on the potential impacts of trace dietary residues in nectar and pollen.

1.2 Definition of terms used in the evaluation

Throughout, the term 'population decline' will be used to refer to a decrease in the number of honey bee colonies in a particular region, and the term 'colony decline' will be used to refer to the decrease in the number of individual bees in a single hive. Before proceeding, the conditions necessary for a factor to precipitate a population decline will be set out using the general theory of population dynamics. Classically, ecologists model the dynamics of a resource-limited population by a logistic equation[40] that defines the per capita contribution to population change (units of individuals per individual per unit time) as

$$\frac{1}{N}\cdot\frac{\delta N}{\delta t} = r\left(1-\frac{N}{K}\right) \tag{1}$$

where t denotes time, N is the population size, K is the carrying capacity of the population's environment and r is the species' intrinsic per capita capacity for population change. Normally, r takes a positive value in a sustainable population and the population declines when $r < 0$, even when $N < K$. Present interest is in the case where a detrimental factor causes r to change from positive to negative. Let r denote the intrinsic rate of increase in the absence of the factor, and let r^* denote the rate in its presence. A factor harms individuals when

$$r > r^* \tag{2}$$

For a factor to precipitate population decline, it is required that

$$r > 0 > r^* \tag{3}$$

Therefore, not all factors that cause individual harm can precipitate population decline, because the inequality (2) does not imply inequality (3). For a factor to precipitate population decline, it is necessary that it causes harm [inequality (2)], but the level of harm must also be sufficient to fulfil condition (3), which is termed the 'sufficient harm' condition. To realise the demographic basis of this condition, it is recognised that the intrinsic growth parameter, r, reflects a difference between the intrinsic per capita birth and death rates, denoted by b and d respectively, which collectively are known as the demographic 'vital rates', i.e.

$$r = (b-d) \tag{4}$$

Given equation (1), population decline will occur whenever

$$(b-d) < 0 \tag{5}$$

With inequality (3) borne in mind, the 'sufficient harm' condition is rewritten as

$$(b-d) > 0 > (b^*-d^*) \tag{6}$$

where b and d denote intrinsic per capita birth and death rates in the absence of the detrimental agent, and b^* and d^* denote them in its presence. Thus, to precipitate population decline, the advent of dietary neonicotinoids must reduce r so as to cause 'sufficient harm' [inequality (3)], in spite of compensatory responses by beekeepers. The impact of neonicotinoids could be obscured if beekeepers compensated for an increase in the colony death rate by increased production of new colonies. In this case, a comparison between the values of d and d^* [inequality (6)], or the rate of colony losses, provides the pertinent indicator of neonicotinoid impact rather than the values of r and r^* [inequality (2)].

Note that inequality (6) can also be applied to an individual hive, in which case it is a question of whether neonicotinoids generate 'depopulation symptoms' by detrimentally affecting fecundity and survivorship [i.e. b^* and d^* in inequality (6)] sufficiently to cause colony decline, which creates a population decline, if prevalent.[41] In this case, the impact of neonicotinoids on the fecundity of queens (i.e. b versus b^*) and on the rates of individual mortality (d versus d^*) would provide the pertinent indicators.

1.3 Evaluatory procedure

Normally, the results of manipulative experiments are the hard currency of decisions about causality in natural science. In situations involving public concern over environmental change, however, decisions about causes sometimes must be made under political pressure, in spite of scientific uncertainties, which may include the lack of experimental evidence. In such circumstances, a scientific evaluation is nevertheless possible, but it uses a different process to manage uncertainty and to validate its conclusions. An example of this alternative process is the report of the Intergovernmental Panel on Climate Change (IPCC),[42] which was unable experimentally to test the effect of fossil-fuel-based emissions on global climate change because of the unavailability of a control group (i.e. Earth-like planets without anthropogenic emissions). Nevertheless, the IPCC's conclusions about the cause of global climate change are widely accepted as scientifically authoritative. Similarly, it is proposed to evaluate whether neonicotinoid pesticides cause population declines in honey bees without having recourse to decisive experiments. Specifically, Hill's causality criteria[43] are used as a structured process for making an expert scientific judgement that is open to critical inspection and repeatable by others. Hill's criteria are particularly appropriate for evaluating the cause–consequence relationship between certain pesticides and honey bee declines because they were devised to address epidemiological questions, such as whether there is sufficient evidence to support the proposition that a particular detrimental agent causes a particular disease.

Sir Austin Bradford Hill, a leading twentieth-century epidemiologist, identified nine types of information that provide 'viewpoints' from which to judge the verity of a cause–consequence relationship. These viewpoints have since become a widely used set of criteria for arriving at a verdict of causation,[44] and techniques for producing quantitative scores of 'certainty' have been developed.[45] The nine criteria include not only experimental evidence, which typically will be equivocal or lacking when the criteria are used as a resort, but also eight other kinds of evidence that fall into two categories (Table 1) as follows. Firstly, the theoretical criteria: coherence, plausibility and analogy. Secondly, the associational criteria: temporality, consistency, specificity, biological gradient and strength.

The goal has been to assign certainty scores to each of the criteria to reflect the degree to which available evidence supports the hypothesis that neonicotinoids cause honey bee declines. A brief description of the criteria and a summary of the scores are given in Table 1.

Table 1. The nine criteria that are evaluated in the present study, each with a brief indicative description – for a fuller exposition, see Hill.[43] The rightmost column contains the evidence-based score given to each criterion, with positive scores in favour of the hypothesis that dietary neonicotinoids cause population declines in honey bees (maximum score of 5), negative scores against and a zero score indicating that the evidence is equivocal. For a justification of the scores, see the text

Criterion	Brief description	Score
1. Experimental evidence		−1
2. Coherence	Fails to contradict established knowledge	+3
3. Plausibility	Probable given established knowledge	+2
4. Analogy	Similar examples known	+3
5. Temporality	Cause precedes effect	−4
6. Consistency	Cause is widely associated with effect	−4
7. Specificity	Cause is uniquely associated with effect	−5
8. Biological gradient	Monotonic dose–response relationship	−4
9. Strength	Cause is associated with a substantive effect	−2

To produce a quantitative score of certainty for each criterion, previously formulated descriptors[45] describing the level of conviction (slight, reasonable, substantial, clear and certain) with which an evaluator holds a cause–effect hypothesis to be true are adapted. These descriptors are associated with numerical values to create an 11-point scale for each criterion that returns a positive value (maximum 5) if the evidence suggests that the factor (trace dietary neonicotinoid) certainly causes population decline, a negative value (maximum −5) if the factor certainly does not and a zero value if the evidence is equivocal or lacking. For example, if the evidence for the *i*th criterion gives a reasonable indication that neonicotinoids do not cause population declines in honey bees, the score for that criterion will be $C_i = -2$, and so on. No attempt will be made to present an exhaustive review of evidence. Instead, evidence that, in the present authors' opinion, best supports the score of greatest absolute magnitude for each criterion will be cited.

The initial evaluation will focus exclusively on the proposition that neonicotinoid pesticides are capable in their own right of causing population declines in honey bees. This approach is taken initially because parsimony dictates that the more complex hypothesis that neonicotinoids act in concert with other stressors needs to be considered only once the simpler case is dismissed. The topic of interactions among stressors will be addressed in the concluding discussion.

2 EVALUATION OF CRITERIA AND JUSTIFICATION OF SCORES

2.1 C_1: experimental evidence

The question as to whether the neonicotinoids cause bee population declines would be settled beyond reasonable doubt if realistically dosed honey bee colonies showed sufficient harm under field conditions at the level required by inequalities (3) and (6). At the time of writing, no such experimental results had been reported. One experimental investigation investigated the effect of exposure to neonicotinoid-treated crops on colony health under field conditions,[31] and it found no effect on either overwinter survival and its proxy variables (e.g. gain in colony mass) or the mortality rates of individual bees. Similarly, laboratory trials provide no evidence that dietary neonicotinoids affect vital demographic rates: environmentally realistic trace levels do not cause elevated rates of mortality,[27] and effects on fecundity are as yet unstudied. However, the laboratory trials have shown that doses of dietary neonicotinoid at trace levels are capable of harming individual honey bees. Exposure for at least 6 days to trace dietary imidacloprid is expected to reduce behavioural performance in adult honey bees by between 6 and 20%.[27] Two major uncertainties prevent this level of individual harm from being linked directly to population decline, however. Firstly, even if this reduction in individual performance translates into an equivalent reduction in colony performance, none of the published field and semi-field studies had sufficient statistical power to detect it.[27] Consequently, whether the results of laboratory trials are environmentally relevant is unclear. Secondly, even if a dietary neonicotinoid caused a laboratory-scale reduction in colony performance (e.g. in foraging success or fecundity) under field conditions, it is not clear that this meets the condition of sufficient harm [inequality (3)].

In summary, experimental evidence to date has not demonstrated that trace dietary imidacloprid causes population decline, but neither has the testing been stringent enough under environmentally relevant conditions to reject this causal hypothesis convincingly because of shortcomings in statistical power. Instead, the credibility of the hypothesis is sustained to some degree by the sublethal, harmful effects that are detected in laboratory tests. With the limitations of field trials (statistical power, use of proxy response variables) taken into account, their null results are taken as only a slight indication that neonicotinoids are not a cause of bee population decline, and this criterion is scored at $C_1 = -1$.

2.2 C_2: coherence

The coherence criterion asks whether invoking a factor as the cause of a particular phenomenon conflicts with established knowledge. There is no conflict inherent in the proposition that dietary intake of an insecticidal chemical, such as a neonicotinoid, could harm honey bees sufficiently to cause a population decline because xenobiotics have this effect on other species.[46] However, the parameters of inequalities (3) and (6) cannot be populated with well-justified, quantitative values that specify a threshold for the generation of sufficient harm. The lack of this specific threshold means that a quantitative incoherence with existing knowledge simply cannot arise. Overall, no conflict is found between existing knowledge and the proposition that neonicotinoids cause honey bee declines, but the quantitative shortcomings in current knowledge mean that this coherence provides only a substantial indication in favour of the proposition, and this criterion is scored at $C_2 = +3$.

2.3 C_3: plausibility

The plausibility criterion asks whether a reasonable scientist would, in principle, entertain the factor as the cause of the observed phenomenon. In evaluation of this criterion, each of a series of mechanistic links in the hypothesised causal chain between dietary neonicotinoids and honey bee decline is critically examined.

Firstly, it is certain that neonicotinoid residues are present in pollen and nectar in mass-flowering crops that have been systemically treated, and that these are collected by honey bees.[16,47] It is inevitable that some of these residues are subsequently ingested by bees in nectar and pollen,[15] but the precise level of dosage is uncertain, because the neonicotinoid-containing pollen and nectar may be mixed with the colony's existing stores or with pollen and nectar collected at the same time from sources other than the treated mass-flowering crop, which would dilute the dose.

Secondly, it is well established that adult worker honey bees are susceptible to harm by trace dietary neonicotinoids,[27,48] although there is no evidence to support the claim that honey bees are unusually sensitive among insects.[49] It is possible that larval stages are equally or more susceptible,[50] but the effect on queens and drones is currently unknown.

Thirdly, it is possible that dietary neonicotinoids may affect the demographic vital rates of honey bees. While dietary imidacloprid at trace levels does not cause increased mortality rates in adult workers,[27] it could have various sublethal impacts,[51] including interfering with important behaviours, such as navigation during foraging[52] or social hygiene. The resulting loss of foragers could deplete the workforce and slow colony growth, and poor hygiene may promote disease, but these effects are currently hypothetical. It is possible that a neurotoxin may disturb the bee's fundamental physiology,[53,54] perhaps by diverting resources towards detoxification and away from other necessary functions,[55] and thereby increase death rates generally or reduce the fecundity of the sexual caste, but this hypothesis is also not substantiated.

Fourthly, it is possible that the various potential harmful effects of a neonicotinoid (reduced foraging workforce, increased risk of disease, reduced fecundity of the sexual caste) could weaken the colony and thereby increase winter losses among hives (but see Faucon *et al.*[30] and Cutler and Scott-Dupree[31]), but whether these losses amount to sufficient harm [inequality (3)] is currently unknown.

The plausibility criterion is more exacting than the coherence criterion, because it must be bolstered by positive examples from existing knowledge. All of the preceding links in the causal chain are possible in principle, but the existence of several is purely conjectural, which diminishes their plausibility. It is therefore concluded that the proposition that trace dietary neonicotinoids cause honey bee declines is only reasonably plausible, and this criterion is scored as $C_3 = +2$.

2.4 C_4: analogy

The analogy criterion asks whether a judgement can be supported by an appeal to similar, well-resolved cases. It is known that other pesticides have caused population declines in non-target organisms. For example, the use of DDT was associated with an increased frequency of addled eggs in the nests of the American bald eagle that was sufficient in magnitude to be held responsible for population decline.[56] However, this example is not a close analogy for the impact of neonicotinoids on honey bees because the effect of DDT on birds of prey was promoted through biomagnification in the food chain, which can greatly increase the effective concentration of a toxin in the diet.[57] In contrast, biomagification of neonicotinoids in honey bee colonies has not been demonstrated. Investigations of the sublethal effects of pesticides on the population dynamics of insects are rare in demographic toxicology,[58] but systemic neonicotinoids at sublethal doses are known to reduce the intrinsic rate of increase in aphids[59] and the fecundity of bumblebees.[60,61] Although bumblebees are taxonomically a close match to honey bees, the experiments on them involved dosages well above the environmentally realistic trace range, which diminishes their value as an analogy. Comparison with the available analogies provides substantial evidence that trace dietary neonicotinoids could detrimentally affect vital demographic rates in honey bees, which scores this criterion at $C_4 = +3$.

2.5 C_5: temporality

The temporality criterion asks whether the putative cause precedes the consequence. The question is therefore whether the widespread use of neonicotinoid insecticides preceded the honey bee population decline. The neonicotinoids were first marketed in 1991, and imidacloprid was the first product to be launched.[10] In the United States, imidacloprid-based products were licensed for widespread use on crops pollinated by honey bees (e.g. apples, fruit, vegetables and oilseeds) in 1994, and their usage grew rapidly thereafter (Fig. 1) until they occupied a 16% share of the total pesticides market by 2005.[10]

In the United States, a substantial decline in the numbers of managed honey bee colonies preceded the introduction of neonicotinoid use; the number of colonies declined steadily from a peak of approximately 5.5 million in 1945 to approximately 3.5 million in 1990.[17] The neonicotinoids are evidently not responsible for the population decline over this period. Furthermore, the rapid expansion of the use of imidacloprid in California in 1994–2007, for example, was not associated with an increased rate of honey bee population decline in this region (Fig. 1); there is no statistically significant correlation between the annual incremental rise in use of imidacloprid in 1995–2007 and the incremental decrease in the number of honey bee colonies in either the same year (Kendall's correlation test, $\tau = -0.25$, df $= 12$, $P = 0.22$ n.s.) or the succeeding year (Kendall's correlation test, $\tau = 0.38$, df $= 11$, $P = 0.07$ n.s.). However, this finding is not equivalent to showing that neonicotinoids were not causing colony losses, because inequality (6) shows that a stable population can be maintained if an increase in death rates, d^*, is offset by a corresponding increase in birth rates, b^*. Thus, an increase in the production of new colonies by beekeepers may have compensated for the increased mortality, and a detrimental effect of neonicotinoids that would be apparent in increased depopulation symptoms is invisible when inspecting overall colony numbers. To resolve this, long-term data on rates of colony loss and/or replacement in 1995–2007 would be needed, but these are unavailable. Overall, the advent of trace dietary neonicotinoids clearly neither preceded nor apparently intensified the honey bee decline, and on this basis the temporality criterion is scored at $C_5 = -4$.

2.6 C_6: consistency

The consistency criterion asks whether the association between the putative cause and its consequence is repeated in space and time. The initial question is whether honey bee population

declines coincide with spatial variation in the use of neonicotinoid insecticides. Worldwide, honey bee declines are not ubiquitous, and, according to figures produced by the United Nations Food and Agriculture Organisation, the global stock of managed colonies has increased by 45% in the last 50 years, in spite of the declines in North America and Europe.[21] Even in Europe, stocks of colonies have increased in some countries, such as Spain, where the numbers have risen by over 50% to approximately 2 million since the neonicotinoids were introduced in the early 1990s.[21] In principle, this spatial variation can be used to test whether declines are associated with neonicotinoid usage, but it has not been possible to obtain the necessary data on national application rates. However, the authors are not optimistic that the association would have emerged. For example, it is doubtful that the growth of colony numbers in Spain is due to its unusually low levels of neonicotinoid use, because it produces massive quantities of citrus fruit and tomatoes (FAO 2011: http://faostat.fao.org/site/339/default.aspx), for which imidacloprid is a standard insecticidal treatment.[62]

The consistency criterion offers another opportunity for evaluation, however. If neonicotinoids are to precipitate a population decline, the prevalence of neonicotinoid residues among the colonies of a declining population should be expected. However, a recent survey of 350 pollen samples from North America found imidacloprid in less than 3% of samples.[63] It is therefore concluded that dietary neonicotinoids are clearly inconsistently associated with honey bee decline, and this criterion is scored at $C_6 = -4$.

2.7 C_7: specificity

The specificity criterion asks whether the consequence is both unmistakably defined and uniquely associated with the putative cause. The present case fully meets the former requirement by having unambiguous characteristics, which are either population declines (Fig. 1) or depopulation symptoms, such as changes to demographic vital rates [inequality (6)]. However, an important source of uncertainty arises from the difficulty of attributing a unique cause to these phenomena. There are many potential causes of bee declines other than dietary neonicotinoids, of which four main types are identified. Firstly, even if dietary pesticides cause bee declines, the neonicotinoids are only a few of the chemicals that could be involved. In a survey of pesticide residues in North American honey bee hives,[63] 98 pesticides and degradates were identified, with an average of approximately seven different residues per colony. Similarly, co-occurrence of different residues in Spanish citrus fruit indicates that treatment with various pesticides was the norm.[62] Secondly, other highly detrimental agents that affect honey bee colonies have increased in prevalence, coincident with the increase in the use of neonicotinoids, such as mites,[64] microspridian parasites[65] and viruses.[66] Thirdly, landscape-scale reductions in the area of suitable forage due to agricultural intensification may limit the availability of nectar and pollen to colonies and thereby detrimentally affect demographic vital rates.[67] Fourthly, the dominant influence on the population dynamics of a domesticated population, such as the honey bee, is likely to be the economically motivated activities of beekeepers, who may either reduce, sustain or increase their stocks.[21] The potential contemporaneous impact of these drivers makes it difficult to conclude that dietary neonicotinoids could be uniquely, or even primarily, responsible for honey bee declines. It is therefore concluded that the putative cause, dietary neonicotinoids, is certainly not uniquely associated with population decline in honey bees, and the specificity criterion is scored at $C_7 = -5$.

2.8 C_8: biological gradient

This criterion asks whether an increase in the power of the putative cause is reflected by an increased effect. Currently, the necessary information to test whether global variation in the severity of population declines is associated with variation in the levels of neonicotinoid use is not available, but there is evidence on a smaller scale. In Europe, maize pollen can be a major component of the honey bee diet, comprising approximately 20% of the pollen harvested by honey bees over the entire flowering period.[68] A survey in Belgium found that the frequency of various depopulation symptoms, including colony mortality, in apiaries decreased as the neighbouring area of neonicotinoid-treated maize increased.[69] It is therefore concluded that the available evidence relating to biological gradient clearly contraindicates dietary neonicotinoids as a cause of honey bee decline, and this criterion is scored at $C_8 = -4$.

2.9 C_9: strength

This criterion asks whether the magnitudes of the effects that coincide with the action of the putative cause are so uncommonly large as to defy explanation by either chance or other artefact. To clarify his original definition, Hill[43] referred to cancer rates in people engaged in a particular practice (e.g. smoking), and he proposed that the implication of a causal relation is increasingly justified as the cancer rate, or degree of harm, associated with the practice deviates further from the norm.

While neonicotinoids are capable of making a devastating impact on bees when delivered at a sufficient dosage, as was observed in the accident at Baden-Württemberg,[35] their impacts on honey bees as trace dietary residues are much less evident. In laboratory trials, trace doses do not cause elevated death rates, but instead cause sublethal effects on behaviour, namely decreased performance in learning tasks in the range 6–20%.[27] The largest field trial conducted to date failed to detect detrimental effects of trace dietary neonicotinoids on colonies.[31] While this trial lacked the statistical power to detect small detrimental effects on performance ($\leq 20\%$), it should have detected more substantive impacts ($>33\%$).[27] It is therefore concluded that the failure to detect a strong detrimental impact of trace dietary neonicotinoids under field conditions is a reasonable indication against their implication in honey bee declines, and the criterion is scored at $C_9 = -2$.

3 DISCUSSION

In the absence of decisive experimental evidence ($C_1 = -1$), the cause–consequence relationship between trace dietary neonicotinoids and honey bee declines was evaluated using the other eight of Hill's criteria. The proposition that dietary neonicotinoids cause honey bee declines scored positively on all three of the theoretical criteria, C_2 to C_4 (mean = 2.7, SD = 0.6, $n = 3$), which makes it a reasonably justified conjecture in the context of current knowledge and indicates why these pesticides have been widely viewed as credible culprits. In contrast, the proposition scored negatively on the associational criteria, C_5 to C_9, on which the circumstantial epidemiological evidence was judged as substantially contraindicative (mean = −3.8, SD = 1.1, $n = 5$). The residual uncertainty concerning the associational

criteria arises largely from doubt over the strength of the putative effect. Overall, however, virtually all of the circumstantial evidence clearly contraindicates the proposition.

Weighted evenly, the scores of Hill's criteria imply that the evidence is almost equivocal (mean $= -1.3$), but the variation among criteria is high (SD $= 3.2$, $n = 9$), which may account for the vigour of the controversy over neonicotinoids, because different constituencies use the evidence differently.[26] There is no *a priori* reason to give equal weight to the nine criteria, and furthermore the widespread primacy given to experimental evidence in science suggests that an unequal weighting is normal. Hill[43] refused to provide hard-and-fast rules for weighting the criteria, but a quantitative analysis of known cause–consequence relationships[70] found that the criteria of experimental evidence, strength and consistency contributed most to the correct attribution of cause. If these findings generalise to the present case, the scores on these criteria (-1, -2 and -4 respectively) begin to contraindicate a cause–consequence relationship. It is conventional to favour material evidence over conjecture, which implies that the associative criteria have precedence, and these substantially contraindicate the proposition that trace dietary neonicotinoids cause population declines in honey bees, although not with clear certainty.

It may be argued that neonicotinoids act not as independent agents but as synergists of other stressors, such as poor forage, disease and the presence of other agrochemicals. There are two responses to this. Firstly, even if it is assumed that neonicotinoids are synergists, their detrimental effect still lacks epidemiological perceptibility, and this will not change the negative scores on most of the associative criteria, which are determined by the lack of response of the rate of bee decline to the advent of neonicotinoid use ($C_5 = -4$), the low prevalence of the pesticides in honey bee hives ($C_6 = -4$), the multiplicity of detrimental agents ($C_7 = -5$) and the absence of a biological gradient ($C_8 = -4$). Secondly, it is not yet proven that neonicotinoids at environmentally realistic trace dosages interact synergistically with other stressors. Laboratory experiments have shown that dietary imidacloprid synergises the effect of disease (*Nosema* infection) in harming individual bees,[71] but only at dosages of 70 µg AI kg^{-1}, which are substantially above environmentally realistic levels.

3.1 Directions for future work

Based on the present evaluation, it is concluded that trace dietary neonicotinoids are not implicated in population declines of honey bees. The evaluation is provisional, however, because important gaps remain in current knowledge. What information will be most valuable in further reducing uncertainty? Four avenues are suggested for further investigation. Firstly, experimental investigations of the effects of dietary neonicotinoids on the vital demographic rates of colonies and individuals are needed. Secondly, a quantitative demographic model for honey bee population dynamics is essential in order to evaluate the condition of sufficient harm [inequalities (3) and (6)], but, although valuable progress towards a predictive model has been made,[72] empirical measurements of the impacts of dietary neonicotinoids on the appropriate parameters are still required. Thirdly, epidemiological analyses of the association between the rates of neonicotinoid application and colony loss will be incisive. Fourthly, it is necessary to determine whether trace dietary neonicotinoids are synergists of co-acting stressors.

In closing, the authors commend the use of Hill's criteria. Since their inception over 40 years ago and subsequent widespread use, none of the criteria has been abandoned and none added,[70] which means that they provide a stable and well-established infrastructure in which to process scientific evidence. Their use formalises the evaluation of cause–consequence associations, and the scoring method proposed is subjective but transparent and repeatable. In the authors' experience, the nine criteria cover exhaustively the range of evidence that is used in the debate over neonicotinoids and honey bees. It is very much hoped that others will repeat the present evaluation in the light of new evidence, and that these iterations will eventually resolve this longstanding uncertainty.

REFERENCES

1 Carson R, *Silent Spring*. Houghton Mifflin, Boston, MA (1962).
2 Devine GJ and Furlong MJ, Insecticide use: contexts and ecological consequences. *Agric Human Values* **24**:281–306 (2007).
3 Berenbaum M, Brusseau M, DiPietro J, Goodman R, Gould F, Gunsolus J, *et al*, *The Future Role of Pesticides in US Agriculture*. National Academy Press, Washington, DC (2000).
4 *Concerning the Placing of Plant Protection Products on the Market*. [Online]. EEC Regulation 1107/2009 (2009). Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF [14 December 2011].
5 Isenring R, *Pesticides and the Loss of Biodiversity*. Pesticide Action Network Europe, London, UK (2010).
6 Caton M, Neonicotinoid pesticides. *Hansard* **522**:67WH–76WH (2011).
7 *Insect Pollination*. Parliamentary Office of Science and Technology, Millbank, London, UK (2010).
8 vanEngelsdorp D, Hayes J, Underwood RM, Caron D and Pettis J, A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. *J Apic Res* **50**:1–10 (2011).
9 vanEngelsdorp D and Meixner MD, A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J Invertebr Pathol* **103**:S80–S95 (2010).
10 Elbert A, Haas M, Springer B, Thielert W and Nauen R, Applied aspects of neonicotinoid uses in crop protection. *Pest Manag Sci* **64**:1099–1105 (2008).
11 Tomizawa M and Casida JE, Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu Rev Pharmacol Toxicol* **45**:247–268 (2005).
12 Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M and Sattelle DB, Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol Sci* **22**:573–580 (2001).
13 Sur R and Stork A, Uptake, translocation and metabolism of imidacloprid in plants. *Bull Insectol* **56**:35–40 (2003).
14 Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER and Colin ME, Quantification of imidacloprid uptake in maize crops. *J Agric Food Chem* **53**:5336–5341 (2005).
15 Rortais A, Arnold G, Halm MP and Touffet-Briens F, Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* **36**:71–83 (2005).
16 Schmuck R, Schoning R, Stork A and Schramel O, Risk posed to honey bees (*Apis mellifera* L. Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* **57**:225–238 (2001).
17 Pettis JS and Delaplane KS, Coordinated responses to honey bee decline in the USA. *Apidologie* **41**:256–263 (2010).
18 Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, *et al*, A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**:283–287 (2007).
19 Williams GR, Tarpy DR, vanengelsdorp D, Chauzat MP, Cox-Foster DL, Delaplane KS, *et al*, Colony collapse disorder in context. *Bioessays* **32**:845–846 (2010).
20 Garibaldi LA, Aizen MA, Klein AM, Cunningham SA and Harder LD, Global growth and stability of agricultural yield decrease with pollinator dependence. *Proc Natl Acad Sci USA* **108**:5909–5914 (2011).
21 Aizen MA and Harder LD, The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr Biol* **19**:915–918 (2009).

22 Biesmeijer JC, Roberts SPM, Reemer M, Ohlemuller R, Edwards M, Peeters T, *et al*, Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **313**:351–354 (2006).
23 Decourtye A, Mader E and Desneux N, Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie* **41**:264–277 (2010).
24 Doucet-Personeni C, Halm M, Touffet F, Rortais A and Arnold G, Imidaclopride utilisé en enrobage de semences (Gaucho) et troubles des abeilles – Rapport final du Comité Scientifique et Technique de l'etude multifactorielle des troubles des abeilles. Ministère de l'Agriculture, de la Pêche et des Affaires Rurales, Paris, France (2003).
25 Suchail S, Guez D and Belzunces LP, Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environ Toxicol Chem* **19**:1901–1905 (2000).
26 Maxim L and van der Sluijs JP, Expert explanations of honeybee losses in areas of extensive agriculture in France: Gaucho compared with other supposed causal factors. *Environ Res Lett* **5**:12 (2010).
27 Cresswell JE, A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* **20**:149–157 (2011).
28 Suchail S, Guez D and Belzunces LP, Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem* **20**:2482–2486 (2001).
29 Nauen R, Ebbinghaus-Kintscher U and Schmuck R, Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag Sci* **57**:577–586 (2001).
30 Faucon JP, Aurieres C, Drajnudel P, Mathieu L, Ribiere M, Martel AC, *et al*, Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manag Sci* **61**:111–125 (2005).
31 Cutler GC and Scott-Dupree CD, Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *J Econ Entomol* **100**:765–772 (2007).
32 Bortolotti L, Sabatini A, Mutinelli F, Astuti M, Lavazza A, Piro R, *et al*, Spring honey bee losses in Italy. *Julius-Kuhn-Archiv* **423**:148–152 (2009).
33 Forster R, Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008. *Julius-Kuhn-Archiv* **423**:126–131 (2009).
34 Thompson HM and Maus C, The relevance of sublethal effects in honey bee testing for pesticide risk assessment. *Pest Manag Sci* **63**:1058–1061 (2007).
35 Pistorius J, Bischoff G, Heimbach U and Stahler M, Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. *Julius-Kuhn-Archiv* **423**:118–126 (2009).
36 Nikolakis A, Chapple A, Friessleben R, Neumann P, Schad T, Schmuck R, *et al*, An effective risk management approach to prevent bee damage due to the emission of abraded seed treatment particles during sowing of seeds treated with bee toxic insecticides. *Julius-Kuhn-Archiv* **423**:132–148 (2009).
37 Thompson HM, Risk assessment for honey bees and pesticides – recent developments and 'new issues'. *Pest Manag Sci* **66**:1157–1162 (2010).
38 Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, *et al*, Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *J Econ Entomol* **102**:1808–1815 (2009).
39 Benjamin A, *Pesticides: Germany Bans Chemicals Linked to Honeybee Devastation*. [Online]. Available: http://www.guardian.co.uk/environment/2008/may/23/wildlife.endangeredspecies [1 July 2011].
40 Gotelli N, *A Primer of Ecology*. Sinauer Associates, Inc., Sunderland, MA (2008).
41 Higes M, Martin-Hernandez R, Martinez-Salvador A, Garrido-Bailon E, Gonzalez-Porto AV, Meana A, *et al*, A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. *Environ Microbiol Rep* **2**:243–250 (2010).
42 *Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, ed. by Core Writing Team, Pachauri RK and Reisinger A. IPCC, Geneva, Switzerland (2007).
43 Hill A, The environment and disease: association or causation? *Proc R Soc Med* **58**:295–300 (1965).
44 Hofler M, The Bradford Hill considerations on causality: a counterfactual perspective. *Emerg Themes Epidemiol* **2**:11 (2005).
45 Weiss C, Can there be science-based precaution? *Environ Res Lett* **1**:7 (2006).
46 Hendriks AJ, Maas-Diepeveen JLM, Heugens EHW and Van Straalen NM, Meta-analysis of intrinsic rates of increase and carrying capacity of populations affected by toxic and other stressors. *Environ Toxicol Chem* **24**:2267–2277 (2005).
47 Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME and Bengsch ER, A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Anal Chem* **75**:2027–2033 (2003).
48 Thany SH, Decourtye A and Devillers J, Ecotoxicity of neonicotinoid insecticides to bees, in *Insect Nicotinic Acetylcholine Receptors*, ed. by Thany S. Landes Bioscience and Springer Science + Business Media, LLC, New York, NY, pp. 85–95 (2010).
49 Hardstone MC and Scott JG, Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Manag Sci* **66**:1171–1180 (2010).
50 Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, Padovani E, *et al*, Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. *J Apic Res* **49**:52–59 (2010).
51 Desneux N, Decourtye A and Delpuech JM, The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* **52**:81–106 (2007).
52 Yang EC, Chuang YC, Chen YL and Chang LH, Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* **101**:1743–1748 (2008).
53 Scott JG and Wen ZM, Cytochromes P450 of insects: the tip of the iceberg. *Pest Manag Sci* **57**:958–967 (2001).
54 Scott J, Insect cytochrome P450s: thinking beyond detoxification, in *Recent Advances in Insect Physiology, Toxicology and Molecular Biology*, ed. by Liu N. Research Signpost, Kerala, India, pp. 117–124 (2008).
55 Riddell CE and Mallon EB, Insect psychoneuroimmunology: immune response reduces learning in protein starved bumblebees (*Bombus terrestris*). *Brain Behav Immun* **20**:135–138 (2006).
56 Grier JW, Ban of DDT and subsequent recovery of reproduction in bald eagles. *Science* **218**:1232–1235 (1982).
57 Henny CJ, Kaiser JL and Grove RA, PCDDs, PCDFs, PCBs, OC pesticides and mercury in fish and osprey eggs from Willamette River, Oregon (1993, 2001 and 2006) with calculated biomagnification factors. *Ecotoxicology* **18**:151–173 (2009).
58 Stark JD and Banks JE, Population-level effects of pesticides and other toxicants on arthropods. *Annu Rev Entomol* **48**:505–519 (2003).
59 Lashkari MR, Sahragard A and Ghadamyari M, Sublethal effects of imidacloprid and pymetrozine on population growth parameters of cabbage aphid, *Brevicoryne brassicae*, on rapeseed, *Brassica napus* L. *Insect Sci* **14**:207–212 (2007).
60 Tasei JN, Lerin J and Ripault G, Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera : Apidae), during a laboratory feeding test. *Pest Manag Sci* **56**:784–788 (2000).
61 Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G and Smagghe G, Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* **19**:207–215 (2010).
62 Blasco C, Font G and Pico Y, Evaluation of 10 pesticide residues in oranges and tangerines from Valencia (Spain). *Food Control* **17**:841–846 (2006).
63 Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, van Engelsdorp D, *et al*, High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *Plos One* **5**:e9754 (2010).
64 Sammataro D, Gerson U and Needham G, Parasitic mites of honey bees: life history, implications, and impact. *Annu Rev Entomol* **45**:519–548 (2000).
65 Paxton RJ, Klee J, Korpela S and Fries I, *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie* **38**:558–565 (2007).
66 Genersch E and Aubert M, Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). *Vet Res* **41**:20 (2010).
67 Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O and Kunin WE, Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evolut* **25**:345–353 (2010).
68 Sabugosa-Madeira B, Abreu I, Ribeiro H and Cunha M, Bt transgenic maize pollen and the silent poisoning of the hive. *J Apic Res* **46**:57–58 (2007).

69 Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J, Tuirionet B, *et al*, Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J Econ Entomol* **102**:616–623 (2009).
70 Swaen G and van Amelsvoort L, A weight of evidence approach to causal inference. *J Clin Epidemiol* **62**:270–277 (2009).
71 Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, *et al*, Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ Microbiol* **12**:774–782 (2010).
72 Khoury DS, Myerscough MR and Barron AB, A quantitative model of honey bee colony population dynamics. *Plos One* **6**:6 (2011).

