Une faible dose d'un insecticide provoque la disparition des butineuses

Axel Decourtye, Maxime Beguin, Jean-François Odoux, Fabrice Requier, François Brun, Pierrick Aupinel, Pascal Jourdan, Mickaël Henry

Evaluation du risque

<u>Aujourd'hui :</u> Effets à court terme sur adulte

<u>Demain :</u> Effets sur larves, chroniques, sublétaux, modulés en fonction de co-exposition

Danger

+

Exposition

→ Risque

<u>Aujourd'hui :</u> « *Vrai partout et tout le temps* »

<u>Demain :</u> Territoire par territoire

<u>Aujourd'hui :</u> Dose d'usage ou scénarii d'exposition

<u>Demain</u> : Selon pratiques, butinage

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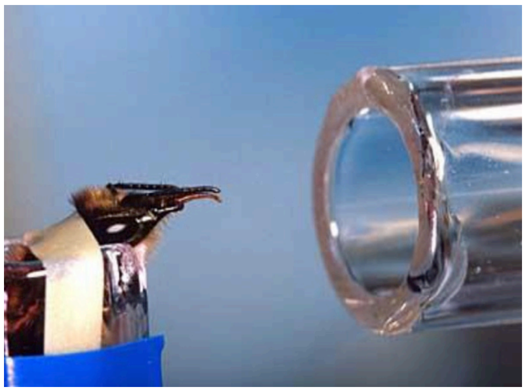
Les effets sublétaux des pesticides : de plus en plus d'exemples

Pour revue : Desneux et al. 2007 Ann. Rev. Entomol. ; Cresswell 2011 Ecotoxicol.

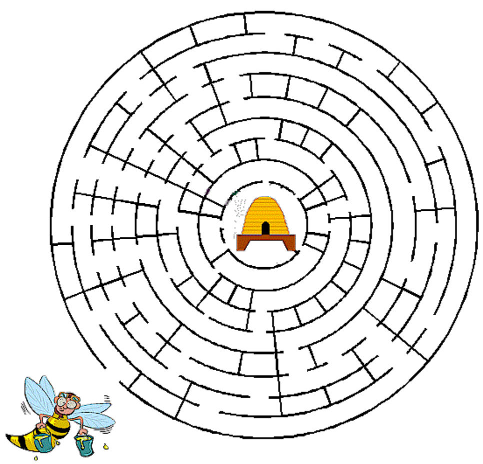


Apprentissage

Abramson et al., 1999
Decourtye et al., 2003, 2004, 2005
Kacimi El Hassani et al., 2005

Comportement de butinage

Cox & Wilson, 1984
Colin et al., 2004
Decourtye et al., 2004

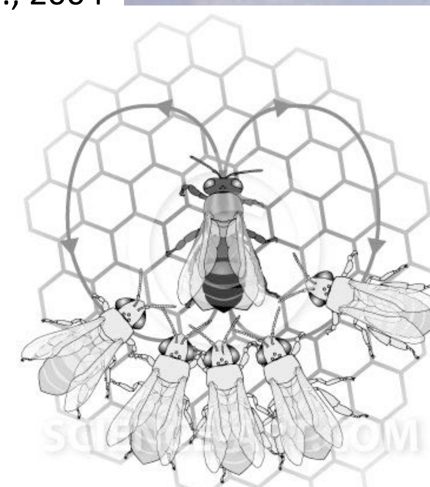


Mémoire / Orientation

Decourtye et al., 2009

Danse des butineuses

Schricker & Stephen, 1970
Kirchner, 1999



Peu d'études en conditions réelles

- Tests en milieu contrôlé (laboratoire, serre ou tunnel)
- Pas de technologie permettant le suivi automatisé des individus

Derniers progrès technologiques: RFID

(Decourtye et al. 2011; Schneider et al. 2011)

UMT PrADE

M. Béguin

Le projet « TECHBEE »

REPORTS

A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

Mickaël Henry,[1,2]* Maxime Béguin,[2,3] Fabrice Requier,[4,5] Orianne Rollin,[2,6] Jean-François Odoux,[5] Pierrick Aupinel,[5] Jean Aptel,[1,2] Sylvie Tchamitchian,[1,2] Axel Decourtye[2,6]

Nonlethal exposure of honey bees to thiamethoxam (neonicotinoid systemic pesticide) causes high mortality due to homing failure at levels that could put a colony at risk of collapse. Simulated exposure events on free-ranging foragers labeled with a radio-frequency identification tag suggest that homing is impaired by thiamethoxam intoxication. These experiments offer new insights into the consequences of common neonicotinoid pesticides used worldwide.

Colony collapse disorder (CCD) is a recent, pervasive syndrome affecting honey bee (*Apis mellifera*) colonies in the Northern hemisphere, which is characterized by a sudden disappearance of honey bees from the hive (*1*). Multiple causes of CCD have been proposed, such as pesticides, pathogens, parasites, and natural habitat degradation (*2–4*). However, the relative contribution of those stressors in CCD events remains unknown. Some scientists and beekeepers suspect pesticides to hold a central place in colony-weakening processes (*1*) or at least in interaction with other stressors (*5*, *6*). In modern cereal farming systems, honey bees are readily exposed to pesticides because they rely heavily on common blooming crops, such as oilseed rape (*Brassica napus*), maize (*Zea mays*), or sunflower (*Helianthus annuus*), that are now routinely treated against insect pests (*3*). Systemic pesticides in particular diffuse throughout all the tissues as plants grow up, eventually contaminating nectar and pollen (*7*). Foraging honey bees are therefore directly exposed, but so is the rest of the colony as returning foragers store or exchange contaminated material with hive conspecifics (*7*, *8*). Those exposure pathways are of important concern, and pesticide manufacturers pay special attention to reduce nonintentional intoxications in field conditions. Pesticide authorization procedures now require running mortality surveys to ensure that doses encountered in the field remain below lethal levels for honey bees.

However, a growing body of evidence shows that sublethal doses—doses that do not entail direct mortality—still have the potential to induce a variety of behavioral difficulties in foraging honey bees, such as memory and learning dysfunctions and alteration of navigational skills (*9*). Neonicotinoid pesticides used to protect crops against aphids and other sap-sucking insects are especially liable to provoke such behavioral troubles. They are highly potent and selective agonists of nicotinic acetylcholine receptors, which are important excitatory neurotransmitter receptors in insects (*10*, *11*). Effects of sublethal neonicotinoid exposures in honey bees may include abnormal foraging activity (*12–14*), reduced olfactory memory and learning performance (*15–17*), and possibly impaired orientation skills (*18*). Yet, the consequences of such behavioral difficulties on the fate of free-ranging foragers and on colony dynamics are extremely difficult to assess in the field and remain poorly investigated.

In this study, we tested the hypothesis that a sublethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees. We focused our attention on thiamethoxam, a recently marketed neonicotinoid substance (*19*) currently being authorized in an increasing number of countries worldwide for the protection of oilseed rape, maize, and other blooming crops foraged by honey bees. We proceeded in two steps. First, we assessed mortality induced by homing failure (m_{hf}) in exposed foragers. This was achieved by monitoring free-ranging honey bees with radio-frequency identification (RFID) tagging technology (*14*, *20*). Second, we assessed the extent to which m_{hf}, in combination with natural forager mortality, may upset colony dynamics. For that purpose, m_{hf} was introduced into a model of honey bee population dynamics (*21*).

We used a custom-made RFID device (*20*) to monitor the fate of 653 individual free-ranging foragers in the course of four separate treatment-versus-control homing experiments (*22*). The study was conducted in an intensive cereal farming system of western France, as a part of the ECOBEE monitoring facility (Zone Atelier Plaine et Val de Sèvre, Centre d'Études Biologiques de Chizé) and in a suburban area in Avignon, southern France. To simulate daily intoxication events, foragers received a field-realistic, sublethal dose of thiamethoxam (a real dose of 1.34 ng in a 20-μl sucrose solution) and were released away from their colony with a microchip glued on their thorax (Fig. 1A). RFID readers placed at the hive entrance (Fig. 1B) were set to detect on a continual basis tagged honey bees going through the entrance. Mortality due to postexposure homing failure, m_{hf}, was then derived from the proportion of nonreturning foragers. To further discriminate m_{hf} from other causes of homing failure in treated foragers—such as natural mortality, predation, or handling stress—we simultaneously released equal numbers of control foragers fed with an untreated sucrose solution. Hence, m_{hf} was calculated as the proportion of nonretuning treated foragers relative to expectations given by the proportion of returning control foragers. Depending on the experiment, tagged honey bees were released up to 1 km away from their respective colony, a distance usually covered by foragers during normal foraging flights (*23*). Experiments were conducted on individuals from three different colonies (*22*).

Our strategy was not to get an estimate of m_{hf} per se. Instead, we assessed its upper and lower bounds, depending on whether foragers were familiar or not with the foraging site in which they might get intoxicated. Indeed, one might expect that foragers familiar with the pathway back to the colony are less prone to homing failure than are unfamiliar foragers. Under field conditions, many foragers are probably familiar with the pathway back to the colony because they repeatedly forage on the same

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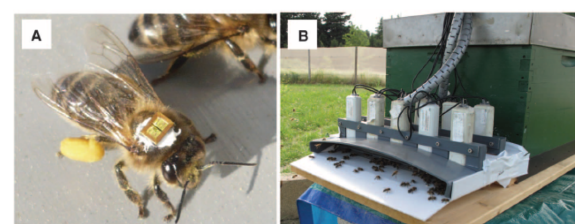


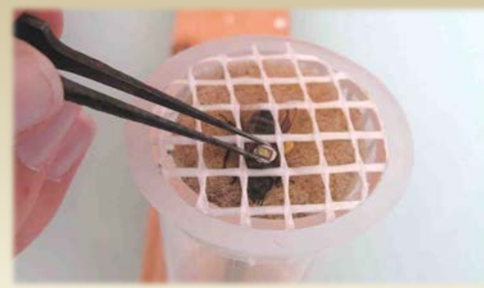
Fig. 1. Honey bee RFID monitoring equipment. **(A)** A pollen-forager honey bee fitted with a 3-mg RFID tag. **(B)** A hive entrance equipped with RFID readers for detecting returning marked foragers.

Expériences de vol de retour

653 individus suivis pendant 1 semaine

Prélèvement de butineuses

Jeûne 1,5 h



Pose d'un RFID

Contamination – 1,3 ng/ind.

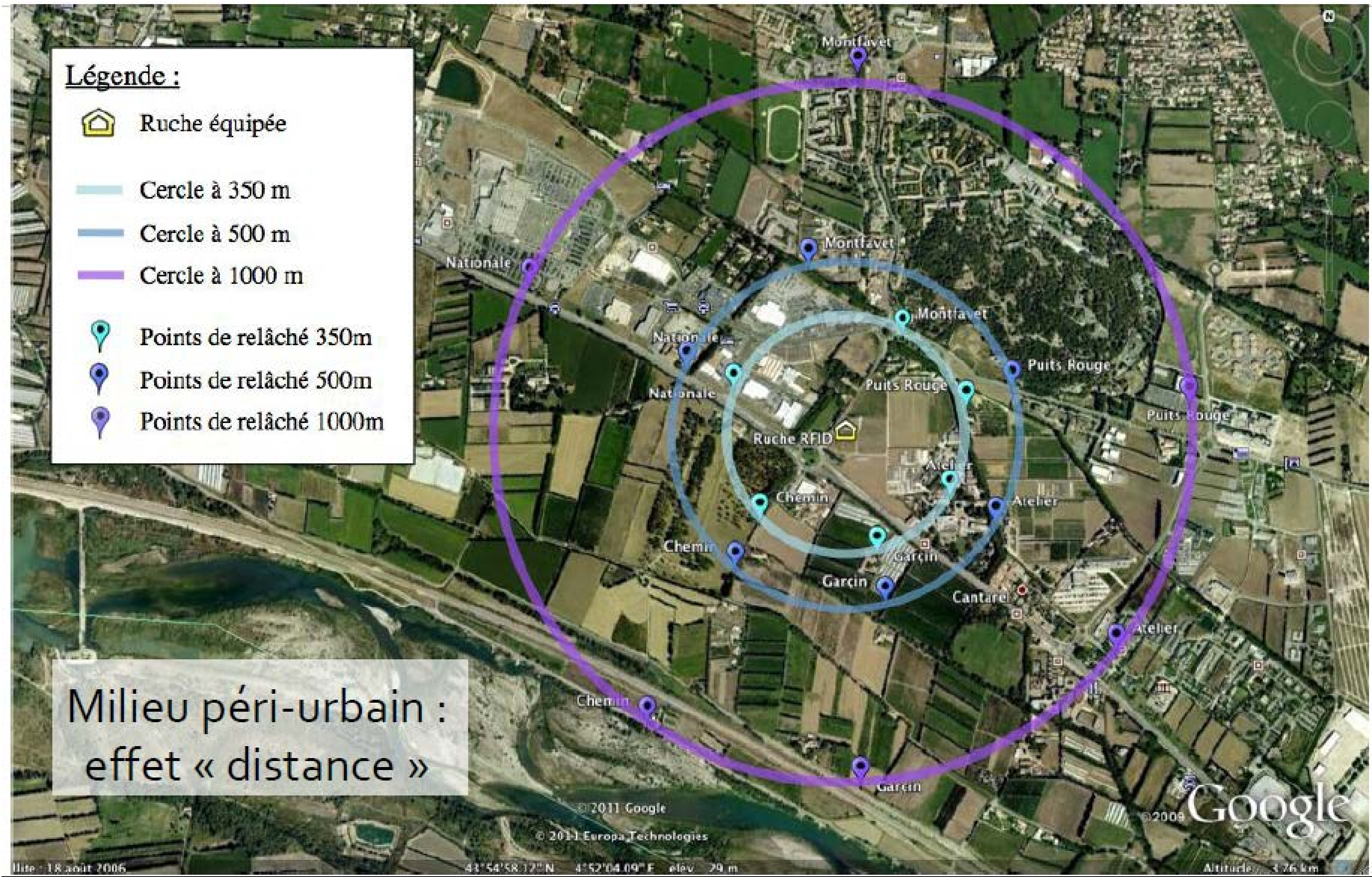
« 0,000 000 001 g »

40 min

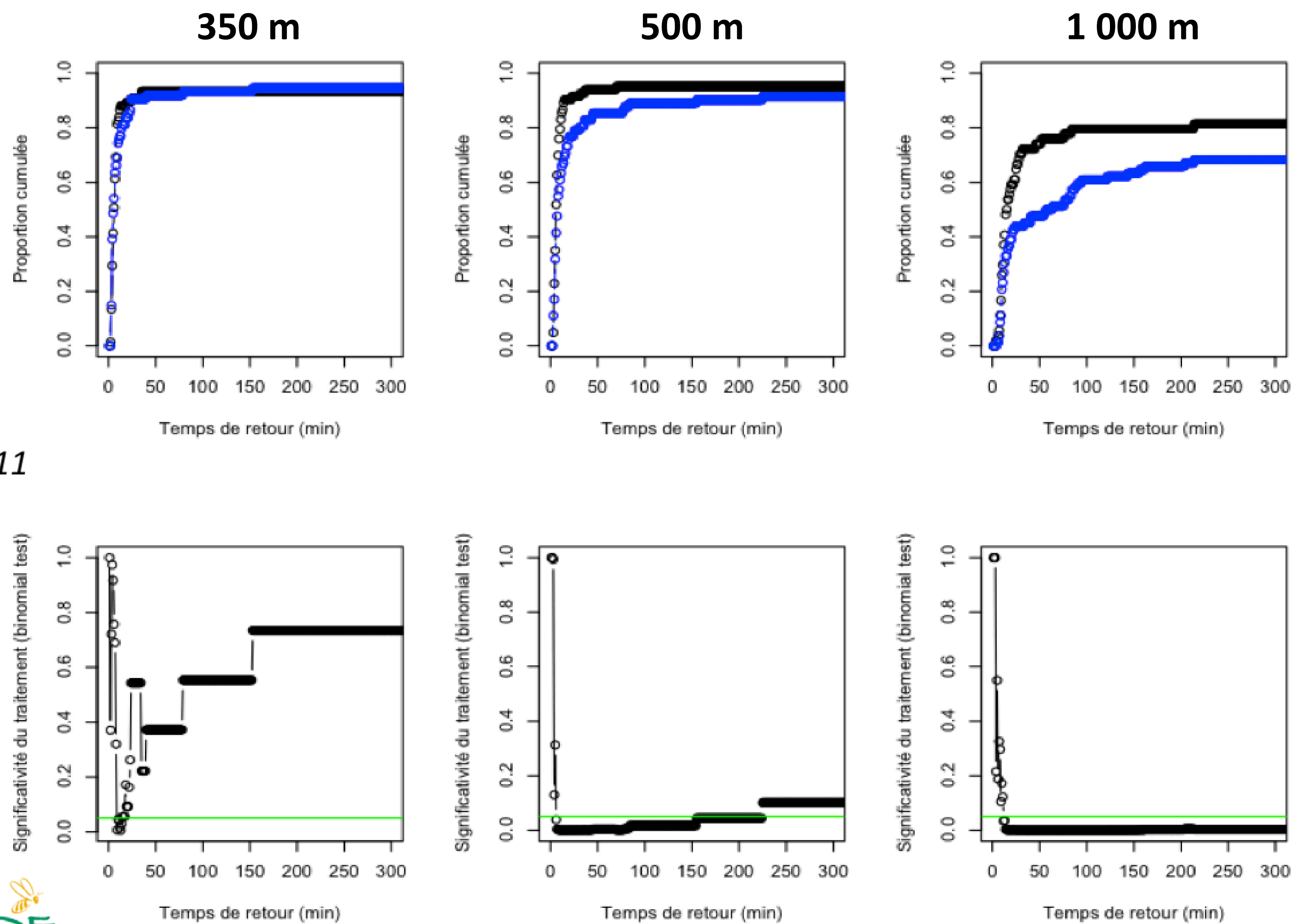
Relâchés à 1 km de la ruche

Photos : Maxime Béguin

Site expérimental – Agroparc, INRA d'Avignon



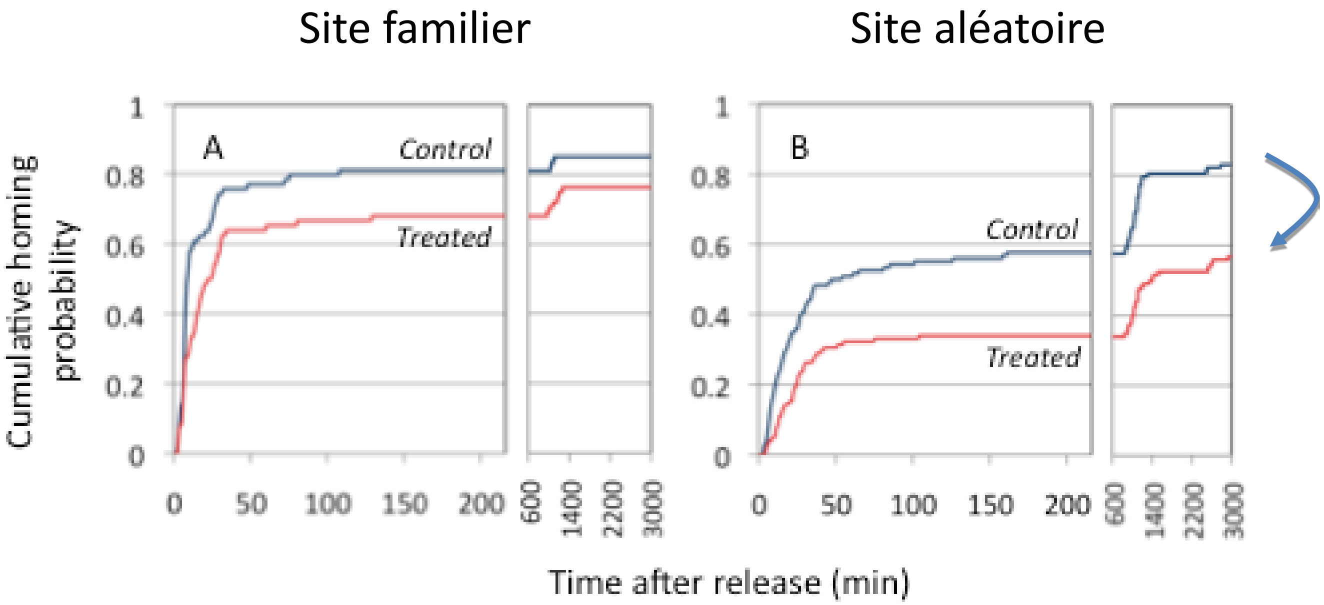
Temps de retour en fonction de la distance



Béguin 2011

Site expérimental – Zone Atelier Plaine et Val de Sèvres

Un effet très significatif sur la probabilité de retour à la ruche:
L'exposition à une faible dose provoque indirectement la disparition des butineuses



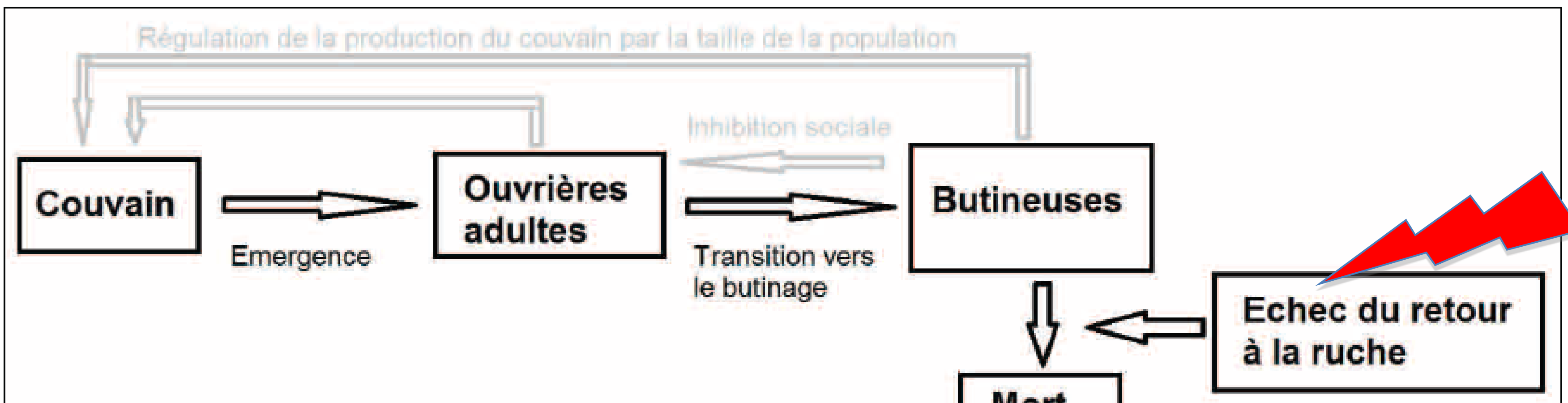
Retours à la ruche en fonction du temps

Probabilité de disparition des butineuses exposées:
10.2% à 31.6%

Pour comparaison, le taux moyen de mortalité des butineuses non traitées/jour :
15.4%

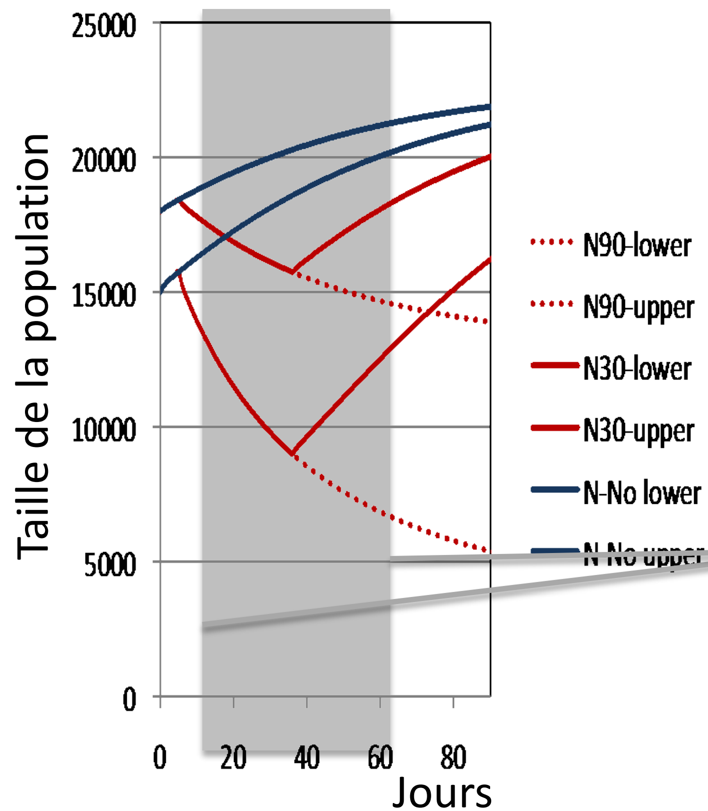
Quel impact sur la colonie dans son ensemble?

Un modèle mathématique pour explorer cette question



(Modèle adapté de Khoury et al. 2011, PLoS ONE)

Modèles démographiques évaluant les conséquences des pertes de butineuses sur le développement d'une colonie



Croissance de la population attendue (scénarii mini et maxi selon le taux de retour mesuré chez les contrôles)

Scénarii après exposition (10,2 % ou 31,6 % de non retours à la ruche)

Période d'exposition (1 mois)

TECHNICAL COMMENT

Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

James E. Cresswell[1,2]* and Helen M. Thompson[3]

Henry *et al.* (Reports, 20 April, p. 348) used a model to predict that colony collapse in honey bees could be precipitated by pesticide-induced intoxication that disrupts navigation. Here, we show that collapse disappears when the model is recalculated with parameter values appropriate to

The model's output is very sensitive to the value of *w* (Fig. 1). Like the model's originators (*9*), Henry *et al.* assumed that *w* = 27,000 (*8*), but this is unrealistic because a colony of 18,000 adult bees (*8*) then grows only by 11% in a month in the absence of pesticide (Fig. 1). In spring or early summer, which is when bees in Europe are typically exposed to neonicotinoid-treated mass-flowering crops such as oilseed rape (*Brassica napus*) (*8*, *11*), a colony of this size can increase by >40% over 30 days (*12*, *13*), which is consistent with *w* ≈ 16,000 (Fig. 1). Indeed, using *w* = 16,000 in the model very accurately predicts observations of adult life span on similarly sized colonies in the absence of

TECHNICAL COMMENT

Response to Comment on "A Common Pesticide Decreases Foraging Success and Survival in Honey Bees"

Mickaël Henry,[1,2]* Maxime Béguin,[2,3] Fabrice Requier,[4,5] Orianne Rollin,[2,6] Jean-François Odoux,[5] Pierrick Aupinel,[5] Jean Aptel,[1,2] Sylvie Tchamitchian,[1,2] Axel Decourtye[2,6]

Cresswell and Thompson have suggested an elegant way to improve honey bee colony simulations when forecasting the fate of colonies exposed to pesticides. Following their recommendations, we rescaled the model on a sound empirical data set. The adjusted forecast is bleaker than their tentative scenario.

Henry *et al.* (*1*) reported that sublethal doses of thiamethoxam, a neonicotinoid pesticide used on some common flow-

noted that our parameterization (*w* = 27,000) assumed an 11% growth only in the absence of pesticides and therefore predicts an excessive

monitored colonies only, and no indication is given on the use of oilseed rape in the vicinity (*4*). Given the tremendous variability one usually observes among colonies, any attempt to derive model parameters from empirical data deserves stronger support. Here, we followed Cresswell and Thompson's valuable suggestion to solve the calculation of *w*, using a strong empirical data set.

We reanalyzed the ECOBEE (Ecological Honeybee Colony Monitoring) data set used in our original study to set a range of realistic starting values for colony size (*1*). ECOBEE is managed by our research groups with the objective to provide ecologists with detailed honey bee colony dynamic data under real beekeeping management conditions. Colony monitoring data, including adult population size, are collected biweekly within a network of about 50 colonies per year. Over the 2008 to 2011 beekeeping seasons— i.e., before thiamethoxam was marketed for oilseed rape protection in our study area— a total of 208 colonies have been monitored. They

Doses d'exposition prédites dans l'environnement[1]

Cas des butineuses de nectar d'un colza d'hiver traité par Cruiser OSR

Scénario	Quantité de sucre consommée/h de vol (mg) [4]	Durée de vol/jour (h) [5,6]	Consommation de sucre/jour (mg)	% en sucres du nectar de colza d'hiver [7]	Consommation de nectar/jour (mg)	Concentration en résidus de thiaméthoxam dans le nectar (µg/kg) [2,3]	Dose d'exposition (ng/abeille/j)
mini	8 (mini)	4 (mini)	32	30	106,7	1,85 (moy)	**0,20**
Inter.1	12 (max)	4	48	10	480,0	1,85	**0,89**
Inter.2	12	10,7 (max)	128,4	20	642,0	1,85	**1,19**
maxi	12	10,7	128,4	10	1284,0	4,6 (max)	**5,91**
Anses[11]	12	10,7	128,4	50,5	254,3	1,3	**0,33**

[1] Rortais A., Arnold G., Halm M. P., Touffet-Briens F. (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees, Apidologie 36:71–83.AFSSA - dossier n°2009 - 1235 - CRUISER 350

[2] AFSSA - dossier n°2009 - 1235 - CRUISER 350

[3] Anses - dossier n°2007 - 3336 - CRUISER OSR

[4] Balderrama N.M., Almeida L.O., Núñez J.A. (1992). Metabolic rate during foraging in the honeybee. J. Comp. Physiol. 162:440–447.

[5] Ribbands C.R. (1953). The behaviour and social life of honeybees. London Bee Research Association, London, UK.

[6] Winston M.L. (1987). The Biology of the Honey Bee. Harvard University Press, Cambridge, UK.

[7] Pierre J., Mesquida J., Marilleau R., Pham-Delègue M.H., Renard M. (1999). Nectar secretion in winter oilseed rape, *Brassica napus* - quantitative and qualitative variability among 60 genotypes. Plant Breeding 118:360-365.

[10] Avis de l'Anses Saisine n° 2012-SA-0092

Répercussions : saisines, avis, arrêté

- **29 Mars 2012**: Publication

- **Mai 2012**: Evaluation de l'étude par l'ANSES et par l'Autorité Européenne de Sécurité des Aliments (EFSA)

- **Juin 2012 :**
  - ➢ Publication par EFSA d'un avis sur les procédures d'homologation des pesticides
  - ➢ Retrait de l'autorisation de mise sur le marché en France prononcée par le ministre de l'agriculture

- **Juillet 2012**: Interdiction du semis de semences de colza traitées avec du thiaméthoxam en France

Avis de l'EFSA sur les procédures d'évaluation de risque liés aux pesticides

« Les **effets sublétaux** devraient être pris en compte et observés en laboratoire. L'importance d'étudier les effets sublétaux a également été démontrée dans l'étude récente de Henry et al. (2012). Il faut étudier les moyens d'intégrer des **tests spécifiques** dans les premières étapes d'analyse. […] **De plus amples recherches** sont nécessaires pour intégrer le résultat de ces études dans les procédures d'évaluation de risque ».

efsa European Food Safety Authority

EFSA Journal 2012;10(5):2668

SCIENTIFIC OPINION

Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The PPR Panel was asked to deliver a scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). Specific protection goals options were suggested based on the ecosystem services approach. The different routes of exposure were analysed in detail for different categories of bees. The existing test guidelines were evaluated and suggestions for improvement and further research needs were listed. A simple prioritisation tool to assess cumulative effects of single pesticides using mortality data is suggested. Effects from repeated and simultanous exposure and synergism are discussed. Proposals for separate risk assessment schemes, one for honey bees and one for bumble bees and solitary bees, were developed.

KEY WORDS

Guidance Document, PPR opinion, honey bees, bumble bees, solitary bees, pesticide, risk assessment

[1] On request from the European Commission, Question No EFSA-Q-2011 00417, adopted on 18 April 2012.

[2] Panel members: Jos Boesten, Claudia Bolognesi, Theo Brock, Ettore Capri, Anthony Hardy, Andrew Hart, Karen Hirsch-Ernst, Susanne Hougaard Bennekou, Robert Luttik, Michael Klein, Kyriaki Machera, Bernadette Ossendorp, Annette Petersen, Yolanda Pico, Andreas Schäffer, Paulo Sousa, Walter Steurbaut, Anita Stromberg, Maria Tasheva, Ton van der Linden, Christiane Vleminckx. Correspondence: pesticides.ppr@efsa.europa.eu3

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Bee Risk Assessment (Robert Luttik, Gérard Arnold, Jos Boesten, James Cresswell, Andrew Hart, Jens Pistorius, Fabio Sgolastra, Noa Simon Delso, Walter Steurbaut, Helen Thompson) for the preparatory work on this scientific opinion,the hearing expert (Anne Alix) and EFSA staff (Franz Streissl, Domenica Auteri, Jean-Lou Dorne, Agnès Rortais, Klaus Swarowsky, Csaba Szentes) for the support provided to this scientific opinion.

Suggested citation: EFSA Panel on Plant Protection Products and their Residues (PPR); Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, *Bombus* spp. and solitary bees). EFSA Journal 2012; 10(5) 2668. [275 pp.] doi:10.2903/j.efsa.2012.2668. Available online: www.efsa.europa.eu/efsajournal

Conclusion

Exposition unique à 1,3 ng de thiaméthoxam/abeille :

- **Effets négatifs sur la capacité de retour à la ruche** (confirmation de précédents résultats non publiés, L. Belzunces)
- **La distance et le manque d'expérience des butineuses aggravent les effets.**

En cours d'analyse

- **Effets d'une exposition répétée au thiaméthoxam durant le stade larvaire** (test d'élevage en laboratoire ; Aupinel et al., 2005)
- **Graduation des effets d'une exposition ponctuelle au thiaméthoxam** en fonction de **facteurs extrinsèques** (météorologie, paysage)

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ITSAP – Institut de l'abeille

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