Possibilities for fast on-site detection of neonicotinoids

Jeroen Peters

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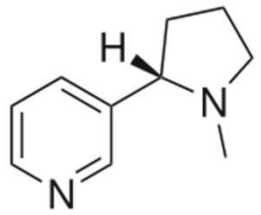
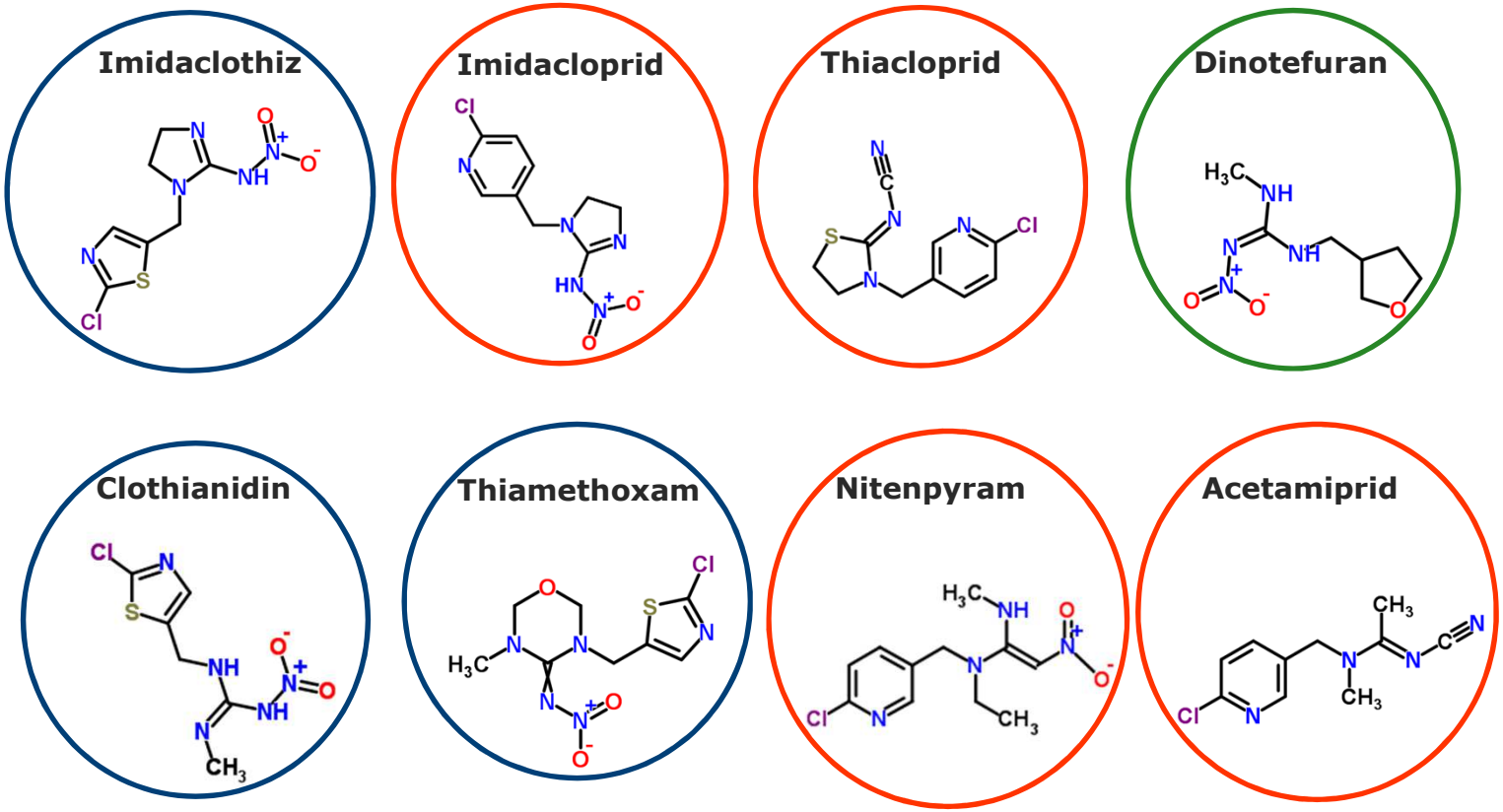
Neonicotinoids



nicotine



Type 1 (chlorothiazole ring)

Type 2 (chloropyridine ring)

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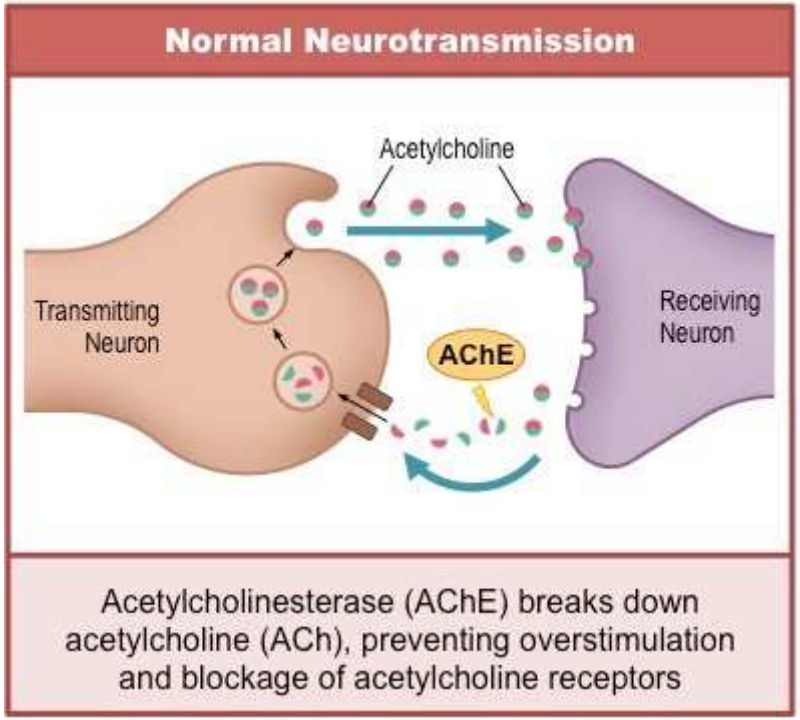
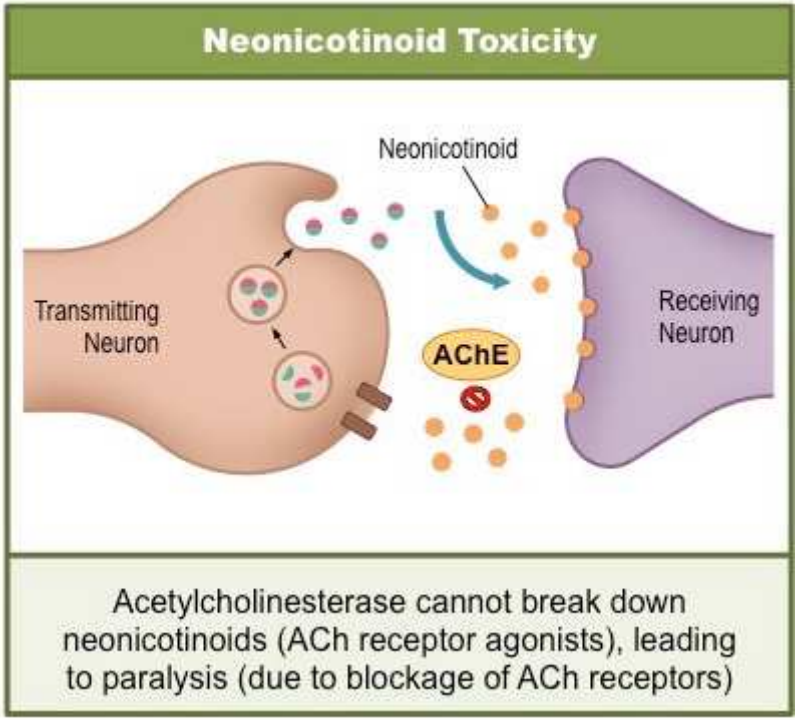
Neonicotinoids - neonics

- Water soluble pesticides
- Most widely used insecticide worldwide
- Bind to nicotine receptors





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Waterdrinker Aalsmeer

WATERDRINKER aalsmeer

- Global exporter of ornamental flowers
- 54 countries, 93 million plants per year
- 9000 species of plants
- Corporate Social Responsibility → Sustainability
- Residue monitoring
- Can we simply test our products for neonicotinoids?
- Can we guarantee a neonicotinoids free chain?

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Commercial immunoassays

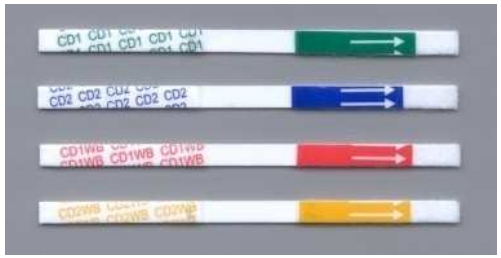
- QuantiPlate™ - Kit for **Imidacloprid** (EnviroLogix)
- **Imidacloprid** ELISA, Microtiter Plate (Abraxis)
- "SmartAssay Series" – **5 neonics** (Horiba)

Commercial immunoassays

Commercial ELISA tests found!

Small basic laboratory

No commercial striptest found!



Ingredients available

Creative Diagnostics (VS)

- **Imidacloprid-** mAb + BSA- + HRP-conjugate
- **Acetamiprid-** mAb + BSA- + HRP-conjugate

Unibiotest (China)

- **Acetamiprid** mAb IC_{50} 0.3 ng/ml
- **Imidacloprid** mAb IC_{50} 0.2 ng/ml

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Institute of Pesticide and Environmental Toxicology of the Zhejiang University (Hangzhou, China)

农药学学报 2016, 18(3): 337-343
Chinese Journal of Pesticide Science
http://www.nyxxb.com.cn

·研究论文·
DOI: 10.16801/j.issn.1008-7303.2016.0046

啶虫脒金标免疫速测试纸条研制及其在茶叶中的应用

赵 颖，杨 斌，柳 颖，方一画，司芳芳，郭逸蓉*，程敬丽，朱国念
(浙江大学 农药与环境毒理研究所，杭州 310058)

摘 要：阐述了一种基于直接竞争免疫层析法的啶虫脒金标速测试纸条的研制方法，及其在茶叶中快速检测与诊断啶虫脒残留的应用情况。通过制备啶虫脒人工抗原，获得了高灵敏度的单克隆抗体，抗体效价大于 1:10 000。据此研制的金标速测试纸条对啶虫脒肉眼判断的检出限为 10 ng/mL，检测时间为 10 min；可特异性地检测茶叶 (绿茶、红茶、铁观音) 中烟碱类农药啶虫脒的残留量，而对其他烟碱类农药 (吡虫啉、噻虫嗪、烯啶虫胺等) 无交叉反应，能满足中国茶叶中啶虫脒最大残留限量 (0.5 mg/kg) 下的检测要求，具有灵敏度高、使用便捷、结果准确、成本低等优点。该技术可以实现成品茶样品中啶虫脒的现场快速测试诊断。

关键词：啶虫脒；残留；抗体；金标试纸；茶叶；快速检测
中图分类号：S482.3　　文献标志码：A　　文章编号：1008-7303(2016)03-0337-07

Development and application of gold immunostrip for the detection of acetamiprid residue in tea samples

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(*Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou* 310058, *China*)

Abstract: The development of a gold immunostrip for the rapid detection of acetamiprid residue in tea samples was described. It was based on the principle of direct competitive immunochromatography. The artificial antigen of acetamiprid was prepared for immunization, and a highly-sensitive monoclonal antibody was obtained with the titer of 1:10 000. Subsequently, the gold immunostrip was developed and the visual detection limit of acetamiprid was 10 ng/mL. The detection time was just 10 min. No cross reaction with other neonicotinoid pesticides, such as imidacloprid, thiamethoxam, and nitenpyram, was detected. Moreover, this method has been used to determine the acetamiprid residue in tea (green tea, black tea and extra-strong tea) by spiked recovery tests. And results indicated that this method could meet the detection requirement of Chinese MRL of acetamiprid (0.5 mg/kg) in tea. Also, it possessed some advantages such as high efficiency, convenience, and low cost. Overall, the newly-developed immunostrip can be used in field tests for rapid screening of fresh tea before tea harvest and the quality control of tea products.

Keywords: acetamiprid; residue; monoclonal antibody; gold immunostrip; tea; rapid screening

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Zhejiang University: Pesticide & Ecotoxicology

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Exploration of the strip test (Hangzhou)

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Bees


"IF THE BEE DISAPPEARED OFF THE SURFACE OF THE GLOBE, THEN MAN WOULD ONLY HAVE FOUR YEARS OF LIFE LEFT....
~ ALBERT EINSTEIN
...NO MORE BEES
NO MORE POLLINATION
NO MORE PLANTS
NO MORE ANIMALS
NO MORE MAN"
SAVE THE BEES!

Waterdrinker: Bee Friendly plants

Neonicotinoids: bee killers

- Decreased lifespan
- contraceptive for bees
- significantly damage bumblebee populations
- 2013: ban on imidacloprid, thiamethoxam of clothianidin
- Outside use
- Crops that attract bees
- Exception:
    - Greenhouse use
    - Harvest before flowering
    - Application after flowering
    - Winter crops
- Seeds coated with neonicotinoids treated seeds forbidden

Total ban on neonicotinoids?

theguardian

Pesticide manufacturers' own tests reveal serious harm to honeybees

Bayer and Syngenta criticised for secrecy after unpublished research obtained under freedom of information law linked high doses of their products to damage to the health of bee colonies

The newly revealed studies show Syngenta's thiamethoxam and Bayer's clothianidin seriously harmed bee colonies at high doses. Photograph: Farooq Khan/EPA

Unpublished field trials by pesticide manufacturers show their products cause serious harm to honeybees at high levels, leading to calls from senior scientists for the companies to end the secrecy which cloaks much of their research.

The research, conducted by Syngenta and Bayer on their neonicotinoid insecticides, were submitted to the US Environmental Protection Agency and obtained by Greenpeace after a freedom of information request.

Neonicotinoids are the world's most widely used insecticides and there is clear scientific evidence that they harm bees at the levels found in fields, though only a little to date showing the pesticides harm the overall performance of colonies.

September 2016

EU criticised for 'emergency authorisations' of banned bee-harming pesticide

Just under half of requests for exceptions to the neonicotinoids ban were filed by industry not farmers, legal analysis shows

'Limited and controlled' exceptions to the pesticide ban are permitted, where pest outbreaks pose an economic danger that can't be treated any other way. Photograph: Amelia Collins/Friends of the Ea/PA

The EU has been criticised after a new legal analysis showed it had allowed scores of "emergency authorisations" of banned pesticides that threaten bee colonies.

The research emerged as the EU's general court began hearing a case by Syngenta and Bayer to overturn the pesticides ban. A ruling is expected shortly.

Three neonicotinoid pesticides were outlawed in Europe in 2013, following analysis - since confirmed by the pesticide industry's own research - that they posed a grave risk to bee populations.

"Limited and controlled" exceptions to the ban were permitted for emergencies, where pest outbreaks posed an imminent economic danger that could not be treated any other way.

February 2017

Europe poised for total ban on bee-harming pesticides

Exclusive: Draft regulations seen by the Guardian reveal the European commission wants to prohibit the insecticides that cause 'acute risks to bees'

A Carniolan honey bee (*Apis mellifera carnica*) is collecting nectar at a yellow rapeseed blossom. Bees and other vital food crop pollinators have been declining for decades. Photograph: Frank Bienewald/LightRocket/Getty Images

The world's most widely used insecticides would be banned from all fields across Europe under draft regulations from the European commission, seen by the Guardian.

The documents are the first indication that the powerful commission wants a complete ban and cite "high acute risks to bees". A ban could be in place this year if the proposals are approved by a majority of EU member states.

March 2017

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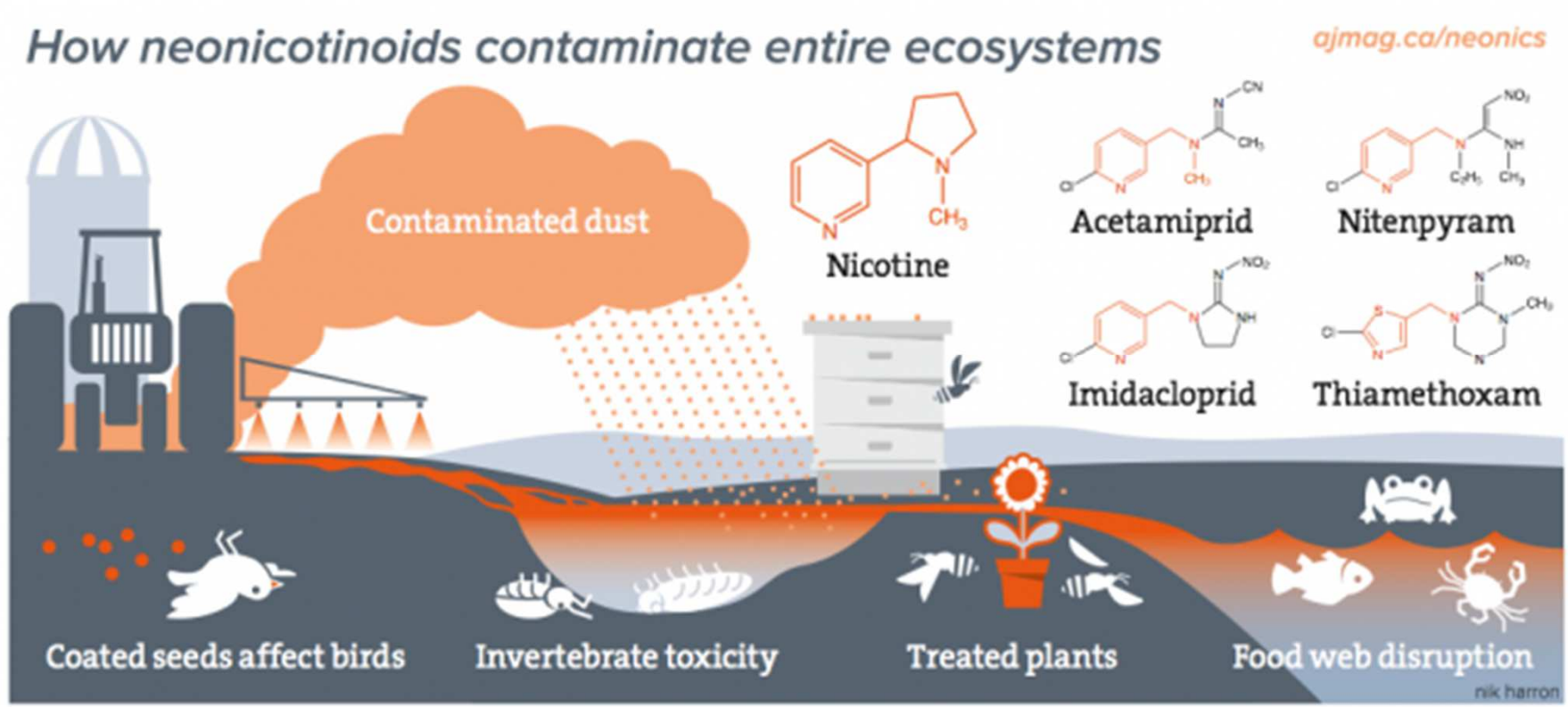
Ecosystem


How neonicotinoids contaminate entire ecosystems
ajmag.ca/neonics
Contaminated dust
Nicotine
Acetamiprid
Nitenpyram
Imidacloprid
Thiamethoxam
Coated seeds affect birds
Invertebrate toxicity
Treated plants
Food web disruption
nik harron

Exploration of the strip test (Wageningen)

Homogenize a mix of flowers, leaves and roots using liquid nitrogen

Exploration of the strip test (Wageningen)

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Exploration of the strip test (Wageningen)

After cooling down, add 2 drops to each channel of the striptest. Read result after 15 minutes.

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Spiking of control orchid extract

No imidacloprid and no acetamiprid

10 ng/ml imidacloprid and acetamiprid

50 ng/ml imidacloprid and acetamiprid

Good results for spiked orchid matrix samples, looks promising!!!

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Strip test analysis of “positive” plants

Plant material prepared, extracted and analysed at RIKLT using a multi-pesticide LC-MS/MS method.

blad bellis	IMIDACLOPRID 0.67 MG/KG.
blad skimmia	IMIDACLOPRID 0.034 MG/KG.
zantedeschia's	IMIDACLOPRID 0.030 MG/KG.
mandevilla	IMIDACLOPRID 1.9 MG/KG.
gewas van geraniums	IMIDACLOPRID 3.2 MG/KG.
vegetatie van perkgoed (geranium)	IMIDACLOPRID 0.052 MG/KG.
blad van howea forsteriana (kentia)	IMIDACLOPRID 6.4 MG/KG.
blad van lelie	IMIDACLOPRID 0.54 MG/KG.
blad van snijlelie	IMIDACLOPRID 0.14 MG/KG.
gewas anthuriums	IMIDACLOPRID 0.14 MG/KG.
blad van zantedeschia	IMIDACLOPRID 0.056 MG/KG.
chrysantenbladeren	IMIDACLOPRID 0.092 MG/KG.
blad rozen	IMIDACLOPRID 0.050 MG/KG.
rozenbladeren	IMIDACLOPRID 0.19 MG/KG.
blad van sierkool	IMIDACLOPRID 0.10 MG/KG.
blad chrysanten	IMIDACLOPRID 0.28 MG/KG.
blad skimmia	THIACLOPRID 1.1 MG/KG.
blad van violier	THIACLOPRID 0.025 MG/KG.
gewas van appelen	THIACLOPRID 0.14 MG/KG.
blad van lelie	THIACLOPRID 0.60 MG/KG.
gewas	THIACLOPRID 1.3 MG/KG.
onkruidvegetatie	ACETAMIPRID 0.27 MG/KG.

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Weeds
0.27 mg/kg
acetamiprid

Mandevilla
1.29 mg/kg
imidacloprid

Zantedeschia
0.03 mg/kg
imidacloprid

Leaves of lilly
0.14 mg/kg
imidacloprid

WAGENINGEN
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Striptest analysis of “positive” plants

Weeds
0.27 mg/kg
acetamiprid

Mandevilla
1.29 mg/kg
imidacloprid

Zantedeschia
0.03 mg/kg
imidacloprid

Blad van snijlelie
0.14 mg/kg
imidacloprid

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Towards a rapid and easy on-site screening for neonicotinoid pesticides

Jeroen Peters[1], Yirong Guo[2], Ying Zhao[2], Ines van der Boon[3], Koen Druijf[3], Eric Poot[4], Theo de Rijk[1], Piet Stouten[1], Guonian Zhu[2] and Willem Haasnoot[1]

Background

Worldwide, neonicotinoids are amongst the most used pesticides and are accounting for 25% of the total agrochemical market. Neonicotinoids bind strongly to the nicotinic acetylcholine receptor affecting the central nervous system of insects. Recently neonicotinoids made headlines because of their supposed negative environmental effects.

Neonicotinoids

Controversy

In 2013, the European Union partially restricted the use of 3 neonicotinoid pesticides because they presumably caused mortality in bees. After application on plants the neonicotinoids can be found in the pollen and nectar of plants.

Neonicotinoids are soluble in water and therefore spread easily in the environment. Especially imidacloprid and clothianidin are very stable in soil and can remain there for several years. Therefore they can accumulate and easily be taken-up by non-treated plants.

Cooperation

Waterdrinker Aalsmeer is a market leader in the field of wholesale indoor and garden plants. To ensure high quality and safe products, they are interested in a rapid on-site screening tool for self-control on neonicotinoids.

The Institute of Pesticide and Environmental Toxicology in Zhejiang University, works on the production and application of antibodies against pesticides. They developed a dual striptest for the detection of imidacloprid and acetamiprid in tea samples. The imidacloprid striptest also detects imidaclothiz and clothianidin, as such, the 2 tests together detect 4 neonicotinoids.

Method

- Select RIKILT LC-MS/MS analysed plant samples
- Add 50 ml of boiling water to 5 grams of homogenized material
- After cooling down add 2 drops to each channel of the striptest
- Read results visually after 15 minutes

Results

Figure 1. Extraction of ornamental plants and the detection of imidacloprid (I) and acetamiprid (A) using dual strip tests. The presence of the Control line (C) indicates that the strip test worked succesfully. For positive samples the Test line (T) should not be visible, or less intense, compared to a blank sample.

Conclusions

- User friendly striptest with visual read-out
- Easy extraction of neonicotinoids from plants
- All LC-MS/MS confirmed contaminations tested as positive
- Cross-reaction at high concentration of imidacloprid in the acetamiprid striptest

Acknowledgements

This work was funded by the RIKILT Small Innovative Project "NOBODIES" and a specific research grant ("knipkaart"of Waterdrinker Aalsmeer) from the "Club of 100"Wageningen UR Greenhouse Horticulture.

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WATERDRINKER aalsmeer

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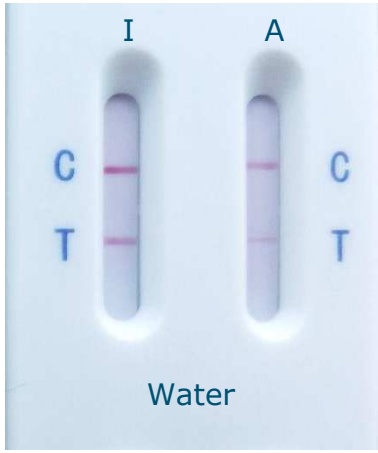
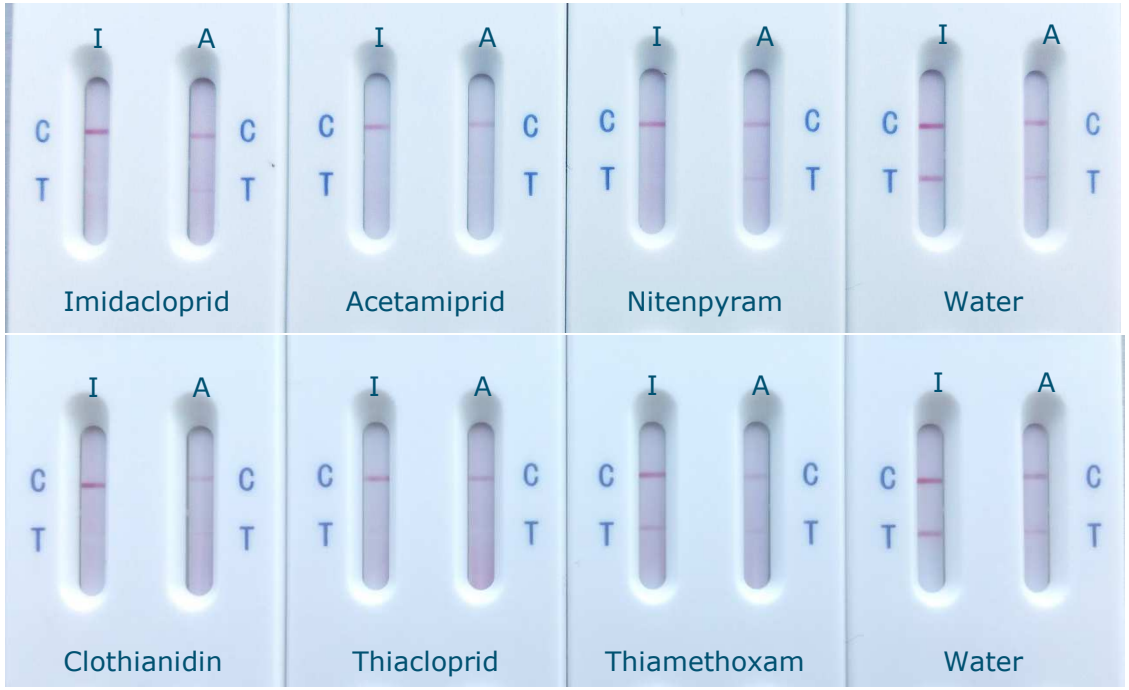
[3] Waterdrinker Aalsmeer, Sierteeltstraat 25, 1431 GM Aalsmeer, The Netherlands
[4] Wageningen University & Research Greenhouse Horticulture, Violierenweg 1, 2665MV Bleiswijk, The Netherlands

Cross-reactivity at 10 ng/ml

New batch of neonics strip tests



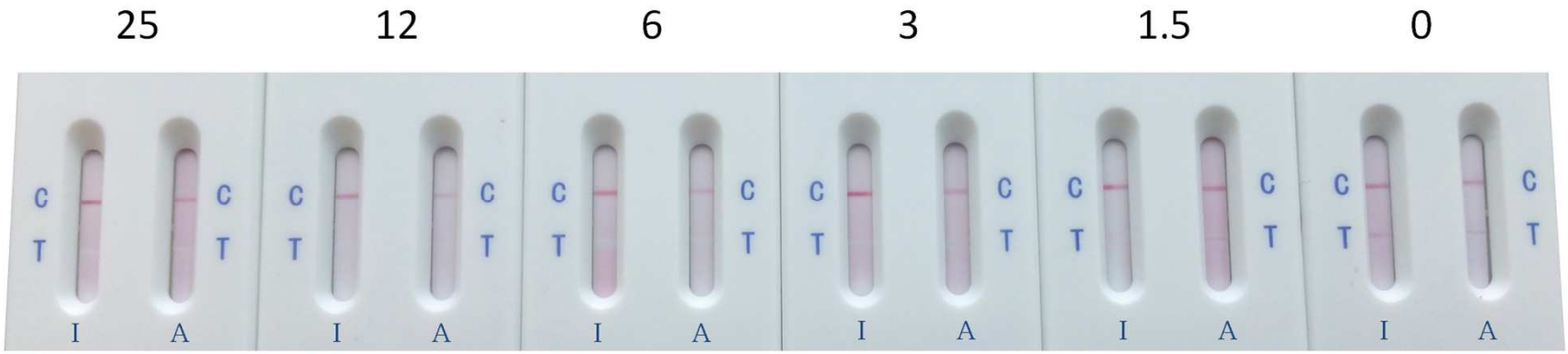
Cross-reactivity at 10 ng/ml



Neonicotinoid	Imidacloprid strip	Acetamiprid strip
Imidacloprid	++	-
Acetamiprid	++	++
Clothianidin	++	-
Thiacloprid	++	++
Nitenpyram	++	-
Thiamethoxam	+	-

Sensitivity

Dose response curves for imidacloprid and acetamiprid in ng/ml

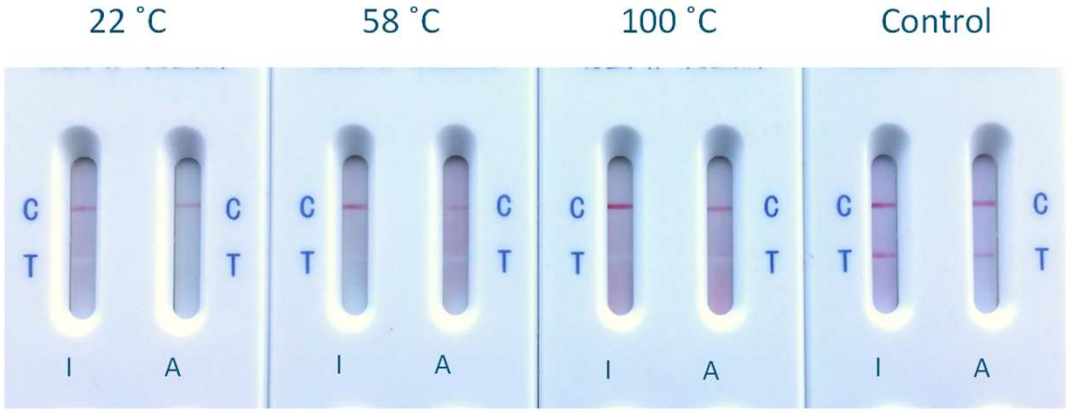


Extractio

- Suitable o
- Cold tap w
- Hot tap wa
- Boiling (ta
- Contamina
- 3 different

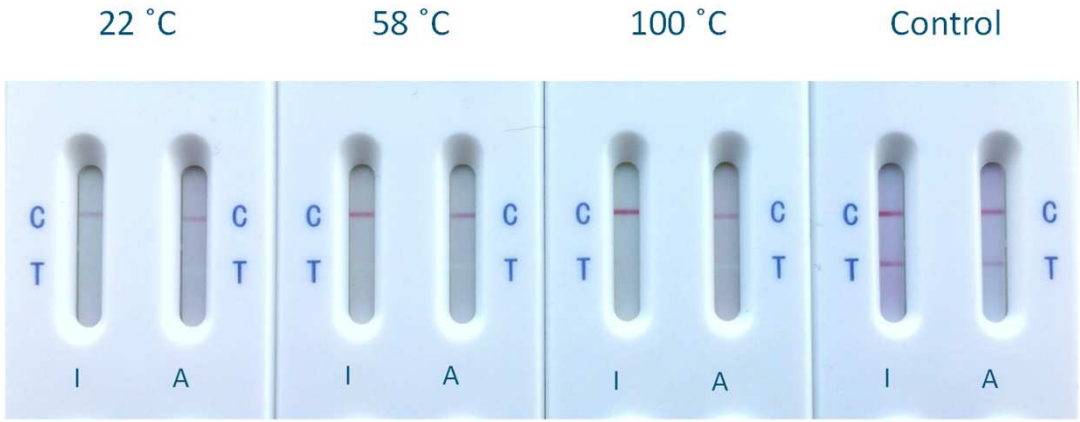
Skimmia

Thiacloprid 1.1 mg/kg



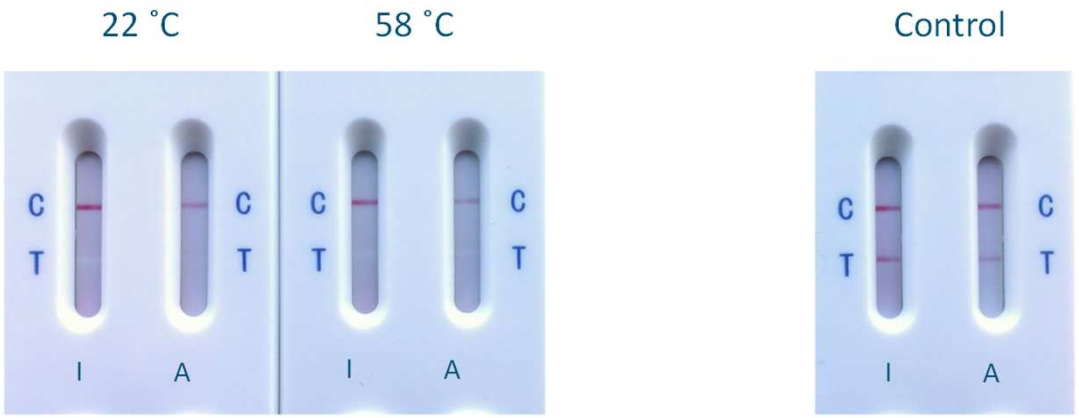
Mandevilla

Imidacloprid 1.9 mg/kg



Weeds

Acetamiprid 0.27 mg/kg



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Screening of cut flowers

Cut flowers samples: 4 supermarkets and 2 florists

Screening of cut flowers for neonicotinoids

Screening of cut flowers for neonicotinoids

Species	Source	Imidacloprid	Acetamiprid
Aster	Grower 1	-	+
Phloxes	Supermarket 1	--	++
Chrysanthemum	Supermarket 1	--	--
Germini	Supermarket 1	--	+
Charmelia	Supermarket 1	--	--
Carnation	Supermarket 1	++	--
Lily	Supermarket 2	++	-
Alstromeria	Supermarket 2	--	--
Carnation	Supermarket 2	+	-
Rose	Supermarket 2	--	--

Species	Source	Imidacloprid	Acetamiprid
Gladiolus	Supermarket 2	--	--
Lily	Supermarket 3	++	++
Carnation	Supermarket 3	-	++
Chrysanthemum	Supermarket 3	+	--
Rose	Supermarket 3	--	-
Germini	Supermarket 3	--	--
Sunflower	Supermarket 4	--	-
Roses	Supermarket 4	+	++
Gladiolus	Supermarket 4	--	-
Chrysanthemum	Florist 1	++	-

Species	Source	Imidacloprid	Acetamiprid
Fresia	Florist 1	+	-
Alstromeria	Florist 1	--	--
Carnations	Florist 1	-	+
Chrysanthemum	Florist 1	--	--
Phloxes	Florist 2	++	--
Lily	Florist 2	--	--
Chrysanthemum	Florist 2	++	-
Carnation	Florist 2	+	--
Delphinium	Florist 2	++	+
Tanacium	Florist 2	++	+
Chrysanthemum	RIKILT Canteen	++	--

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Conclusion

- Dual LFD allows easy and fast detection of neonics
- Detects all neonics at 10 ng/ml
- Less sensitive for thiamethoxam
- Sensitivity below 10
- Differences between batches
- Latest batch of LFDs perfect!
- Detection of neonics in confirmed plant material (LC-MS/MS)
- Sensitive quantification on xMAP platform

Future planning

- LC-MS/MS analysis of all cut flower samples
- Repeat screening with a new batch of LFDs
- Repeat screening with tap water extraction
- Further validation of the strip test
- Use greenhouse controlled plants
    - True positive
    - True negative
- Extend collaboration with Zhejiang University

Showing 1 to 1 of 1 entries 50 records per page

« ‹ 1 › »

Pesticide residues and maximum residue levels (mg/kg)

Code number	Products to which MRLs apply (Part A of Annex I to Reg. 396/2005)	Acetamiprid (R)	Thiacloprid	Imidacloprid	Thiametoxam	Clothianidin
0631000	a) bloemen	0.05*	0.05*	0.05*	0.05*	0.05*

Showing 1 to 1 of 1 entries

« ‹ 1 › »

Table legend

Category | Group | Subgroup | Main product | Others | Clickable Footnotes | N/A Not published yet | * Indicates lower limit of analytical determination

Science

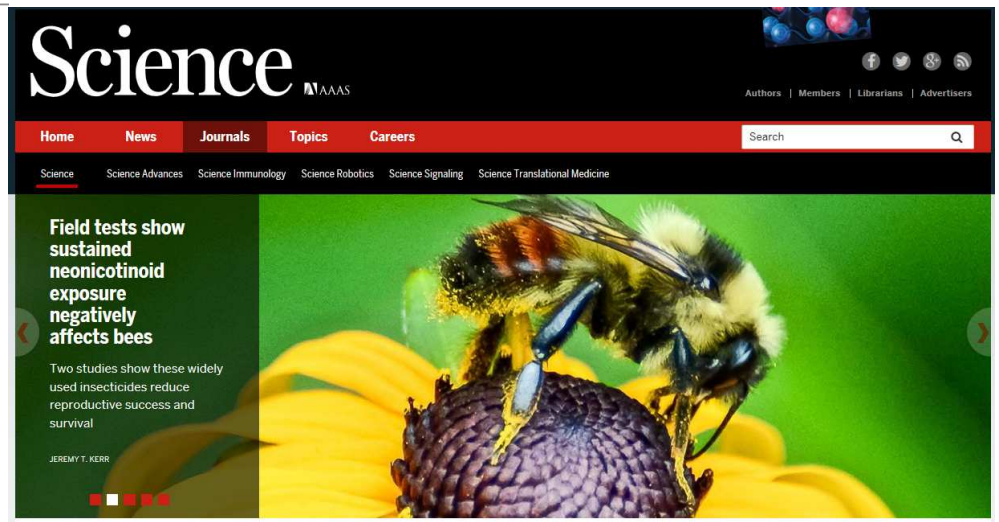
RESEARCH

NEONICOTINOIDS

Country-specific effects of neonicotinoid pesticides on honey bees and wild bees

B. A. Woodcock,[1]* J. M. Bullock,[1] R. F. Shore,[2] M. S. Heard,[1] M. G. Pereira,[2] J. Redhead,[1] L. Ridding,[1] H. Dean,[1] D. Sleep,[2] P. Henrys,[2] J. Peyton,[1] S. Hulmes,[1] L. Hulmes,[1] M. Sárospataki,[3] C. Saure,[4] M. Edwards,[5] E. Genersch,[6] S. Knäbe,[7] R. F. Pywell[1]

Neonicotinoid seed dressings have caused concern world-wide. We use large field experiments to assess the effects of neonicotinoid-treated crops on three bee species across three countries (Hungary, Germany, and the United Kingdom). Winter-sown oilseed rape was grown commercially with either seed coatings containing neonicotinoids (clothianidin or thiamethoxam) or no seed treatment (control). For honey bees, we found both negative (Hungary and United Kingdom) and positive (Germany) effects during crop flowering. In Hungary, negative effects on honey bees (associated with clothianidin) persisted over winter and resulted in smaller colonies in the following spring (24% declines). In wild bees (*Bombus terrestris* and *Osmia bicornis*), reproduction was negatively correlated with neonicotinoid residues. These findings point to neonicotinoids causing a reduced capacity of bee species to establish new populations in the year following exposure.



30th June 2017

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Science

PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Straub L *et al*. 2016 Neonicotinoid insecticides can serve as inadvertent insect contraceptives. *Proc. R. Soc. B* **283**: 20160506.
http://dx.doi.org/10.1098/rspb.2016.0506

Received: 4 March 2016
Accepted: 29 June 2016

Subject Areas:
ecology, environmental science, health and disease and epidemiology

Keywords:
Apis mellifera, insecticide, pollination, reproduction, sperm, sub-lethal

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Neonicotinoid insecticides can serve as inadvertent insect contraceptives

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There is clear evidence for sublethal effects of neonicotinoid insecticides on non-target ecosystem service-providing insects. However, their possible impact on male insect reproduction is currently unknown, despite the key role of sex. Here, we show that two neonicotinoids (4.5 ppb thiamethoxam and 1.5 ppb clothianidin) significantly reduce the reproductive capacity of male honeybees (drones), *Apis mellifera*. Drones were obtained from colonies exposed to the neonicotinoid insecticides or controls, and subsequently maintained in laboratory cages until they reached sexual maturity. While no significant effects were observed for male teneral (newly emerged adult) body mass and sperm quantity, the data clearly showed reduced drone lifespan, as well as reduced sperm viability (percentage living versus dead) and living sperm quantity by 39%. Our results demonstrate for the first time that neonicotinoid insecticides can negatively affect male insect reproductive capacity, and provide a possible mechanistic explanation for managed honeybee queen failure and wild insect pollinator decline. The widespread prophylactic use of neonicotinoids may have previously overlooked inadvertent contraceptive effects on non-target insects, thereby limiting conservation efforts.

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