



## SHEDDING LIGHT ON THE FUTURE: FLUORESCENT SENSORS

**Fluorescence emission is a well-known physicochemical phenomenon that has been used in research for decades. New methods based on fluorescence emission have been developed by CRA-W. A brilliant prospect!**

Hydrogen peroxide is known in phytopathology as a harbinger of the infection signal in infected plant cells. The cells alter their metabolism to fight the infection and ward off the disease. To investigate the spatiotemporal development of the early stages of the infection and devise strategies for optimising means of control, a fluorescent sensor that detects hydrogen peroxide was synthesized in cooperation with the University of Louvain. The CRA-W teams tested, validated and applied this innovative sensor to fluorescence microscopy. This research opens up new routes to understanding the mechanisms of fungal, parasitic or microbial infections in our crops and agricultural ecosystems.

On the back of this, other work carried on at CRA-W also aims to use fluorescence in response to a scien-

tific challenge. In the context of feed quality control, animal proteins are detected by microscopic observation of particles such as bone, cartilage and muscles and PCR analysis to detect the DNA of species or groups of species, like ruminants. Although complementary, these two methods cannot answer all the questions, especially when it matters which tissue or cell is the source of the DNA detected. Hence the idea of “getting bones to talk” by using the fluorescence in situ hybridization method – FISH for short. This involves linking (or hybridization) of a fluorescent DNA sensor with its complementary DNA sequence (the target). The pair is then observed by fluorescence microscopy. This method enables DNA to be detected in tissue or cells and accurately located. Based on that, a promising method has been developed within the framework of EURL-

AP for detecting animal protein in animal feed. The FISH method can also be applied to a great many microstructures of animal, fungal or plant origin occurring in agricultural produce, feed and food. This is a complementary new analytical tool for quality control, authentication and detecting contaminants of different origins.

CRA-W has thus gained new scientific expertise underpinned by advances in its equipment, namely the acquisition of LED technology and a new, fully automatic inverted microscopy platform. Fluorescence microscopy is thus demonstrating its potential for innovative research. It will undoubtedly be a major asset to CRA-W in future and to the services the Centre provides on the basis of its work. We see a bright future ahead!

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## CRA-W'S EXPERIMENTAL PIGGERY SERVES INDUSTRIAL RESEARCH

Whereas CRA-W's experimental farm is known for its zotechnical approaches with animals, it is also involved in more specific cooperative links that contribute to industrial research. Supplying piglet skin samples for pharmaceutical and cosmetic ingredient cutaneous penetration studies is one of several examples.

Dow Corning, at the Seneffe Business Park, is the world leader for innovation and silicone-based technologies used in many applications in areas ranging from the automotive industry to aviation, construction, electronics, solar energy, cosmetics, medicine, textiles and many more. The Beauty and Health Care Department assesses the performance of silicone technologies in releasing active cosmetic and pharmaceutical agents through the skin. To that end, the department studies the skins of animals that were neither reared nor kept to be used in animal experiments but that died on the farm. Although pigs' ears from

the slaughterhouse are most commonly used, they are not the ideal model for cutaneous penetration studies. The Beauty and Health Care Department therefore switched its attention to newly-dead piglet flanks, as a larger skin area is available and the thickness and quality of the cutaneous tissue are more constant.

A cooperative link with CRA-W was initiated in 2012 in order to use skin samples from piglets that died in the first few days of life. The skins are removed very soon after the animals die, to preserve the integrity of the skin. The skins are kept in low-temperature storage and treated as

soon as they reach the laboratory. The experimental farm's rearing systems ensure traceability and enable a strict timetable to be adhered to. Obviously, Dow Corning is authorised to use animal by-products for research purposes and CRA-W has implemented all the operating conditions. As at the end of 2014, more than 115 excisions had contributed to Dow Corning's industrial research with the aim of developing good methods, significantly improving the reproducibility of the results and increasing the scope of work done by the laboratory and the company overall.

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## VALINE REQUIREMENT OF PIGLETS FED LOW-PROTEIN DIET

Economic and environmental constraints are pushing down piglets' dietary protein content and several amino acids (AA) tend to limit performance. Various synthetic AA are often used to overcome this problem.

Apart from lysine (Lys), methionine, tryptophan and threonine, the use of which is common and well documented, valine (Val) is proving to be restrictive in piglets receiving low-protein diet. However, the data in the literature are still scant and need to be corroborated.

To that end, trials have been carried out at the CRA-W's experimental piggery, in cooperation with ORFFA Belgium, to validate growth-limiting lysine and valine levels in weaned piglets fed low-protein diets. The first step was to verify that a level of 0.95% standard-

ised ileal digestible (SID) lysine and a Val/SID Lys ratio of 60% were limiting in post-weaning piglets (Phase 1). Then, a valine dose-response test on post-weaning piglets was carried out to establish the Val/SID Lys ratio that optimizes expression of the growth potential (Phase 2).

The Phase 1 tests showed that a low-protein diet deficient in lysine and valine restricted animal performance. Performance was improved when the diet was supplemented with those amino acids. A level of 0.95% SID lysine in the feed in combination with a Val/SID Lys ratio

of 60% was therefore found to limit the performance of piglets on a low-protein diet. The Phase 2 valine dose-response test established the optimum Val/SID Lys ratio for expression of the animals' growth potential in these conditions. By applying a curvilinear-plateau statistical model to the performance data (weight gain and consumption index) the Val/SID Lys ratio was established as 69.2%, which is close to that quoted in the literature (70%).

The study has validated the valine level needed to optimise growth in piglets fed a low-protein diet.

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## THE SPORE SENSOR: A FOREST HEALTH BAROMETER

Many of the diseases affecting our forest species are caused by fungi that spread via wind-borne spores, sometimes over long distances.



Some spores, such as those associated with rust, can travel thousands of kilometres and still remain viable. Others originate from wooden packing or young plants from infected nurseries from distant parts of the world. If the environment is favourable (in terms of weather conditions and host plants in situ), these “exotic diseases” can become established and possibly

spread from the original seats of infection, causing considerable damage to susceptible woody species. This probably accounts for the introduction of *Chalara fraxinea*, the causal fungus of ash dieback in Europe, and *Cryphonectria parasitica*, the agent of chestnut blight; both these fungi come from Asia.

The proposed response to these threats to plant health involves using early detection tools and in particular, spore sensors. However, sensors used in environments like forests, parks, ports or nurseries must fulfil various criteria. They must be (1) inexpensive (to enable large-scale use and to keep down the replacement cost in the event of vandalism), (2) easy to transport and install and (3) robust (operational at all times). The CRA-W Mycology Laboratory is developing such sensors in the context of the European RESIPATH project (<http://www.slu.se/resipath>).

Comparative tests involving other capture systems were conducted in Wallonia in 2014, looking at three diseases occurring in our forests (ash dieback, oak powdery mildew and Fomes root rot in conifers). These tests showed that the sensors developed by CRA-W met the above three criteria and were able to detect the lowest spore concentrations compared with passive sensors (paper filters) or impact sensors (Burkard sampler). In the next stage of the research the sensor will be combined with new techniques for detecting a large number of fungi at once. This should give plant protection authorities a useful tool for rapid response in controlling emerging diseases. It should also lead to a better understanding of the epidemiology of fungal diseases affecting our forest ecosystems in a context of climate change.

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## TOWARDS COORDINATED INTEGRATED PEST MANAGEMENT IN EUROPE?

Integrated pest management has gained a new European tool: the IC-IPM Era-net consortium, in which CRA-W represents Wallonia. The consortium comprises 21 countries from within and outside the Community.

In many European countries integrated production, which is considered to be more environmentally sound and more beneficial to human health, has been progressively developing for a number of years. Some integrated production sectors are already well established and have more or less supplanted traditional production methods. In many other sectors, due to a lack of adequate scientific knowledge, support and advice for producers, training or perhaps outlets for the products, integrated production systems are struggling to become established.

To promote the development of integrated production research and services, 21 European countries have decided to combine forces within a

consortium called ERA-Net, funded by the EU and the partners, in a quest for synergies and collaborative solutions to common problems. CRA-W currently represents Wallonia in this consortium.

One of the consortium's main activities is analysing the status quo of the various research programmes being carried on in the different countries, drawing up an inventory of the resources and means available, and determining which results achieved and/or which system developed by one or more of the consortium member countries could readily be transferred to others. This analysis will also help to set the direction of future national and European research programmes by identifying priority research topics.

The consortium's other main line of work is initiating cooperative links between countries in order to fund research into common topics, drawing on each contributor's expertise, experience and facilities. As diseases, pests and weeds know no borders, many of the partners are indeed faced with the same kinds of problems. Also, solutions trialled in certain crops could possibly be applied to other sectors. Various project invitations combining national and European funding are scheduled for 2015 and 2016.

In these straitened times for research, pooling the partners' knowledge, means, resources and ideas ought to facilitate the setting up of research programmes that will promote the development of integrated pest management.

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## THE PAMESEB NETWORK: AUTOMATIC AGRICULTURAL WEATHER STATIONS FOR INTEGRATED PEST MANAGEMENT IN WALLONIA

With its 29 automatic agricultural weather stations, the Pameseb network records and distributes weather data that have been validated for agricultural warning systems. At a time when environmental considerations are increasingly guiding choices in terms of agricultural practices, this network is set to play a vital part in establishing Wallonia's agricultural sector in a sustainable process, in line with the targets set by Wallonia and Europe.



precipitation, soil temperature at the surface and at a depth of 20 cm, wind speed and direction, sunshine and leaf wetness. The data recorded are automatically sent to a central system for validation before being supplied to various decision support systems.

This system currently provides hourly measurements every day to the Late Blight and Septoria warning services set up for integrated pest management of potato late blight and wheat septoria leaf blotch. In addition to these long-standing partnerships, fresh requests are coming in from the industry to use the data produced by the Pameseb network to support existing tools or as input for new decision support systems in areas like market gardening or horticulture.

Integrated pest management, long advocated by CRA-W, became a legal obligation in Wallonia on 1 January 2014, pursuant to a European Directive. Within this new legal framework a number of measures are recommended in order to limit the use of chemical plant protection products. Warnings for farmers figure prominently on the list. Recommendations are based in particular on pest development forecasting models, with the ultimate aim of applying plant protection treatments judiciously rather than systematically, to avoid unnecessary

treatments. To ensure that the recommendations made to farmers are relevant it is essential for these models to be supplied with appropriate, reliable meteorological data.

The Pameseb network was established twenty-five years ago and **became part of CRA-W in January 2015**. It has extensive experience of recording, transmitting, validating and distributing meteorological data. The 29 automatic agricultural weather stations scattered throughout Wallonia measure air temperature and humidity,

A meteorological observation network also has other potential applications in fertilisation management, irrigation advice, parasitism management on livestock farms and precision agriculture, all of which are research fields for CRA-W. Integration of the Pameseb network has given CRA-W an essential tool to support its research and help to establish Wallonia's agricultural sector in a sustainable process

For further information: [www.pameseb.be](http://www.pameseb.be)

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## AGENDA



**24-27 July 2015**

**LIBRAMONT AGRICULTURAL FAIR**

Mechanic Show - Demonstration of agricultural machinery - Highlight of the fair.

Conference "The Farm of the Future".

Visit the CRA-W stand and its many activities.

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