



Ministry of the Flemish Community



Agricultural Research Centre



ACTIVITY REPORT 2005 Flemish Agricultural and Fishery Research

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Ministry of the Flemish Community

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Introduction

Dear reader,

This activity report of the Agricultural Research Centre (CLO) is the last in its series as CLO has come to an end. CLO was founded as an umbrella organisation for what were once the Governmental Research Stations. From 1 January 2006 CLO will be part of the Institute for Agriculture and Fisheries Research (ILVO) together with the scientific section of the Centre for Agricultural Economics. ILVO will function as an internal liberalised agency with a corporate personality consisting of four knowledge units (Plant, Animal, Technology and Food, Socio-Economics), each with a number of competency domains. A management contract with the authorities will outline the framework of the applied scientific agricultural research in Flanders; its performance indicators will yield an insight into the results that are to be obtained.

With the closure of the activities of CLO, I want to highlight a number of remarkable results and research themes that have been achieved during the era of the then governmental research stations and the current departments, such as:

- the varieties developed with grasses, clovers, feed crops, inulin chicory, roses, azaleas and tree nurseries in the framework
- of a sustainable agriculture and horticulture
- · determination of the diversity among plants including genetically modified plants
- research into pasture management
- the use of mineral balances as an instrument for controlling NPK surpluses on animal farms
- the microbiological and chemical quality of food
- · experimental dairy technological work with a pilot plant for cheese, milk powder and UHT-milk
- labour rationalisation in agriculture
- · slatted floor animal houses, cubicles, mats and mattresses
- · the advisory and knowledge centre for spray technology
- the electronic potato for determining harvest damage
- plant-pathogen interactions
- diagnosis centre for plant diseases
- · nutritional research with dairy cattle, pigs and poultry
- determination of feed value
- · the development of an objective mesh gauge OMEGA for fishing nets
- the cultivation of sole under controlled conditions and the cultivation of mussels in open sea.

These and other achievements are the results of the efforts of many. CLO may be proud to reflect on these achievements. The scientific quality of our research can also be deduced from various doctorates and A1 publications. Our efforts to introduce a fund for fellowships must contribute in increasing and maintaining this output.

A research establishment as CLO, financed by various sources forms an important link in the acquisition of knowledge for the authorities and the sector to guarantee objective and independent information. I dare say that CLO, within its limitations has been successful in reaching this goal.

During the past year CLO welcomed a number of prominent visitors led by Minister-President Yves Leterme who was introduced to our activities and promised his support for the future. The Flemish Parliamentary Commission for the Environment and Nature, Agriculture, Fisheries, Rural Policy, Town and Country Planning and Regional Heritage, and the European Commissioner for Fisheries, Mr Borg, were on a work visit to CLO. CLO-activities gained widespread recognition such as the inauguration of the new azalea 'Princess Claire' during the Ghent Flower Show, the presentation of the professional Prophyta Prize to the Knowledge and Advisory Centre 'Spray Technology', and the accreditation of various CLO laboratories. CLO was prominently present at Agriflanders, the Ghent Flower Show, the Agricultural

and Horticultural Machinery Days, Agribex and many demonstrations and workshops, not to forget the successful Flanders' Day at the Sea Fisheries Department.

On this occasion I would like to thank the staff of CLO and all those who have supported us ans directly or indirectly been instrumental in making all these achievements possible. I would like to congratulate the staff of CLO all for their daily efforts, dedication, support and trust. I am also much indebted to the supervisory, management and advisory boards, which were an important support for CLO.

> Erik Van Bockstaele General director of the Agricultural Research Centre (CLO)





Flanders' Day at CLO-DVZ-Oostende



Inauguration of the azalea ' Princess Claire' during the Ghent Flower Show



Visit of the Minister-President Leterme







Visit of the Parliamentary Commission



CLO-rape seed on Agribex



EU-Commissioner Borg at CLO-DVZ



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Mission CLO:

CLO's mission consists of the execution and coordination of policy-supporting scientific research and the accompanying public service with a view to sustainable agriculture and fisheries in an economic, ecological and social perspective.

Based on scientific disciplines CLO will build up the necessary knowledge for improving products and production methods, for monitoring the quality and safety of the end products and for improving the policy instruments as a basis for the development of the sector and rural policy. CLO will inform the authorities, the various sectors and society at regular intervals.

The Staff at CLO Headquarters consists of:

Prof. dr. ir. Erik Van Bockstaele General Director ir. Frank Lagaisse Safety, environment and welfare ing. Steven Cools IT ing. Sabine Nelis

- IT
- ing. Ellen Claeys
- André Roelandts Coordination





Staff of CLO

Some figures about the staff of CLO on 31 December 2005

		VINISTRY OF THE FLEMISH (Community				
	Male/FTU	Female/FTU	Total/FTU	Average age			
A-level	48/48,0	19/16,8	67/64,8	44			
B-level	10/10,0	11/9,6	21/19,6	36			
C/D-level	72/67,9	74/53,5	146/121,4	46			
Total	130/125,9	104/79,9	234/205,8	45			
	Male/FTU	Female/FTU	Total/FTU	Average age			
A-level	45/44,0	50/46,5	95/90,5	31			
B-level	24/23,1	38/34,6	62/57,7	29			
C/D-level	59/58,8	32/27,1	91/85,9	38			
Total	128/125,9	120/108,2	248/234,1	33			
	GENERAL TOTAL						
	Male/FTU	Female/FTU	Total/FTU	Average age			
A-level	93/92,0	69/63,3	162/155,3	37			
B-level	35/33,1	49/44,2	84/77,3	31			
C/D-level	130/126,7	106/80,6	236/207,3	43			
ſotal	258/151,8	224/188,1	482/439,9	39			



1. ONE CLO... SEVEN DEPARTMENTS / THREE UNITS

1.1 Department of Plant Genetics and Breeding (CLO-DVP)

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The keys research tasks of the department focus on the generation of new knowledge and supporting policy in the field of plant genetics and breeding. A multidisciplinary approach and the integration of knowledge from different research domains are essential to achieve the proposed goals.

The breeding activities focus on the development of pre-breeding material. Improving resistance to diseases and plagues is a priority in this respect. A better use of nitrogen and quality are important points of attention in the creation of new varieties of fodder grasses and vegetables. In ornamentals, interspecific hybridisation techniques are used to look for new shapes and colours.

One of the tasks of the department is to search for alternative applications of crops. We investigate the heredity of the inulin chain length in chicory, the production pathway of bioactive prenylflavenoïds in hops and the fodder quality of grasses and clovers. In addition we aim to optimise the function of crops for the production of bio-energy.

Maintaining the genetic heritage is an important aspect of international agreements. For this purpose, gene banks were created for important Flemish agricultural and horticultural crops with the aim of maintaining genetic biodiversity on the one hand and for the exploitation of interesting characteristics in the development of sustainable agriculture and horticulture on the other hand.

The application of new developments in biotechnology and bio informatics in combination with field and glasshouse trials requires a multidisciplinary approach. A close collaboration with other institutes guarantees optimal results will be obtained. The department is involved in projects of the 5th and 6th framework programme of the EU, R&D-projects and the 'KMO'programme of the Flemish Government.

Besides the research activities focused on product improvement and innovation, the department carries out public services for the government and the agricultural and horticultural sectors with regard to authenticity control, GMO characterisation and detection, post control and seed certification. In addition, the European Commission (DG-JRC) and the European Food Safety Agency (EFSA) rely on our expertise for advice and specific research. Finally the department ensures that research results can be directly implemented in agriculture and horticulture by delivering high quality starting material for the concerned sectors. The department also contributes to socio-economic progress, through publications and seminars.

DVP – Research themes 2005

Selection towards quality parameters in plants

- Approving disease-, pest-, and stress-resistance in agricultural and horticultural crops (for example grasses, clover, green manure, leek, roses, azalea)
- Approving nutritional and ecological aspects of grassland
- Development of markers for assisted breeding and s ustainable agriculture
- Molecular genetic research towards production of bio-active components in plants like hop

Valorisation of genetic diversity and breeding technology

- Genetic diversity of agricultural and horticultural crops and analysis of the potential use of wild types
- Study of the biodiversity of populations of wild species
- Pre-breeding and interspecific crosses in agricultural and horticultural crops (green fodder, open field vegetables, green manure crops, nursery stock, azalea)
- Asymmetric somatic hybridisation and alternative induction of polyploidy in horticultural crops
- Research towards optimising seed production techniques

GMO research

- Characterisation of GMO's
- Detection and quantification of GMO's in agricultural products and co-existence

Services

- Tracing, authenticity control and ploidy analysis
- Development of cultivars, production and delivery of starting material
- Technological advice and services for the horticultural sector
- Collection and maintenance of the genetic patrimony of fodder crops, open field vegetables and horticultural crops





1.2 Department of Crop Husbandry and Ecophysiology (CLO-DFE)

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The mission of the department is to explore research activities in the area of crop husbandry and ecophysiology with respect to sustainable agriculture and horticulture. The CLO-DFE examines the impact of agricultural activities on yield, quality and on the environment. Quality is a major concern and the accreditation of some chemical analyses according to ISO 17052 are under preparation.

On the social level, there is a permanent need for good communication between all players: the Government, the practice and the consumer. Yearly, the descriptive and recommended list for agricultural species is updated. The cooperation with the activities of the Agricultural Centre Forage Crops is very intensive, with advice and support given. There is extensive networking on national and international levels, covering different EU actions, COST activities, federal and Flemish cooperation programmes with countries in Central and Eastern-Europe.

The basic research applied in crop husbandry gives special attention to the investigation of C storage capacity of grasslands and urban ecosystems, the influence of tillage activities on erosion, nitrate leaching, the use of farm-made compost and the influence of a lower input of external production means on the yield and quality of agricultural productions and their environment. The further development of organic farming and co-existence with classic and GMO farming is under investigation.

In the framework of the EU Regulation 53/2003, the Valve for Cultivation and Use (VCU) trials of all agricultural species and the Distinctness Uniformity and Stability (DUS) tests of some species for the updating of the national catalogue for agricultural species are carried out as a mandated activity for the Administration ABKL. For DG SANCO visits to the "comparative trials" for grasses will be organised in 2006-2007. The fundamental ecophysiological research is focused on the photosynthesis of crops (grasses, maize, chicory, ...) in stress situations. Techniques are developed for the morphological characterisation of crops and varieties by image analysis.

DFE – Research themes 2005

Sustainable crop production systems

- Grass/clover, production and feeding value
- N-efficiency of forage crops
- Maize as an energy crop
- Co-existence (GMO, non-GMO, organic farming)
- Minimum tillage, soil food web and use of compost

Evaluation of plant species and varieties

Updating new criteria for example in the frame of changed legislation

Plant characteristics: morphology, physiology and developing processes

- Morphological characterisation of plant parts via image analyses
- Developing physiologically based screening techniques for cold stress

Plant quality, water and soil management

- Optimisation and quantification of C-storage under grassland
- Research of chemical, physico and physicochemical and non-destructive analyse techniques for the evaluation of plant material, soil, substrate, manure and water

Services

- DUS and VCU research (national and EU variety catalogue for agricultural crops), descriptive and recommended v ariety list and support of seed control
- Technologic Advisory Service FarmCOMPOST
- Laboratory: quality/composition plant, soil, substrate and water
- Cooperation of Flanders with Central- and Eastern-Europe





1.3 Department of Crop Protection (CLO-DGB)

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With its innovative research, the Crop Protection Department participates in the development of durable plant production. The research into acarology, bacteriology, entomology, mycology, and nematology focuses on (i) improved identification of plant pathogens, (ii) a better knowledge of the plant-pathogen relationship, and (iii) the development of alternatives to the chemical control of pathogens in different cropping systems.

The services of the Crop Protection Department are mainly provided via the Flemish Diagnostic Centre for Plants and support durable plant production in Flanders. With the correct detection of plant pathogens and a rapid diagnosis of abnormal plant growth, the department responds to requests coming from diverse authorities, extension specialist, research stations and private enterprises.

DGB – Research themes 2005

Development of methods for the detection of plant pathogens

- Development of molecular diagnostic methods for plant parasitic nematodes, fungi, and bacteria
- Development and validation of tests for the detection and identification of plant pathogenic bacteria
- Identification and quantification of beet cyst nematodes (*Heterodera schachtii* and *H. betae*) in soil samples.

Research on specific pests and diseases

- Bacterial diseases of leafy vegetables
- Phytophthora ramorum on Rododendron
 - White rust of chrysanthemum
- Ecology of *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus*
- Ecology and control of important plant parasitic nematodes
- Registration and cataloging of natural enemies of spider mites

Disease control

- Recirculation of nutrient solutions in soilless cultures through effective sterilisation techniques
- Enhancement of disease resilience through the use of composts
- Use of natural enemies in the control of insect pests of Belgian endive

Explorative basic research into the interface of agriculture, nature, and living environment

- Watermark disease of willows in agricultural areas
- Bleeding disease of horse chestnut
- Interaction between a plant parasitic nematode (*Pratylenchus* spp.) and helm grass in a dune environment

Service

- The Centre for Plant Disease Diagnosis
- Assignment of critical use permits for methyl bromide as a soil disinfestant





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CLO-DVV carries out applied scientific research in animal production aiming at more efficient and therefore environmentally friendly animal nutrition and sustainable, consumer oriented and socially acceptable animal husbandry. This primarily implicates research in nutrition physiology in cattle, pigs, poultry and rabbits. *In vivo* evaluation of the nutritive value of feedstuffs provides a reference databank for prediction based on *in vitro* parameters and contributes to a better nutrient supply of the target animal. *In vitro* methods for feed evaluation are developed and improved so that analytical labs are able to estimate the nutritional value of feeds in a more accurate and fast way.

Society is concerned about the environment and asks for more diversity in the countryside. Therefore, efforts are being made to reduce the excretion of nutrients and to use alternative feeds in animal nutrition. To increase social acceptability of livestock production, research tries to improve animal welfare through dietary as well as housing techniques. Examples are ad libitium feeding of pregnant sows with fibre rich feeds, group housing of rabbits and feeding measurements to prevent frequent occurring digestion disorders in rabbits.

Due to the growing awareness for healthy human nutrition, research is increasingly focused on the healthy value of animal products. Through "functional animal nutrition" possibilities are studied to enrich milk, meat and eggs with nutrients important for the health of the consumer. Examples are an increased level of polyunsaturated fatty acids in animal products as well as the incorporation of natural antioxidants in poultry products.

Service research is mainly focused on support for products such as newly developed feed additives, e.g. enzymes, organic acids, probiotics, prebiotics and immune stimulating products that improve either feed efficiency or animal health and by consequence are an alternative for antibiotics, which are contested by the consumer.

DVV – Research themes 2005

Animal welfare

- Effect of fibre sources on spontaneous feed intake of pregnant sows and breeding calves
- Gnawing blocks as cage enrichment for rabbits

Sustainable animal husbandry

- Indicators of N-excretion in dairy cattle
- N- and P-emission reduction in animal husbandry

Efficient and functional animal nutrition

- Optimisation of animal nutrition integrating zootechnical performances, health status, housing, feedstuff availability and end product quality
- Incorporation of polyunsaturated fatty acids and natural antioxidants in animal products
- Effect of feed additives and byproducts on nutrient availability, zootechnical performances, meat quality and health of livestock
- Reduction of boar taint in non-castrated male pigs

Nutrient evaluation and -analysis

- Digestibility and N balance trials with livestock
- Implementation of a laboratory quality control system for the analysis of nutrients and end products
- Determination of the chemical composition of animal feeds and prediction of the nutritional value
- Residue evaluation in animal end products





1.5 Department of Animal Product Quality and Transformation Technology (CLO-DVK)

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CLO-DVK performs research aimed at the improvement of the microbiological and chemical safety and functional quality, as well as the control of the authenticity of food of animal origin, in order to better protect the consumer and to improve the market position of the Flemish producer in the frame of durable production and processing methods.

Food safety is very important for the consumer. A lot of attention goes into research concerning microbiological safety. Here, molecular identification and typing techniques play an important role. Animal experiments are to a certain extent replaced by the use of in vitro techniques. Research is carried out on the behaviour of zoonotic microorganisms in the food production chain and the virulence for human and animal as well as on the combating of pathogens and harmful bacteria on the farm via adapted management. The effect of remediation measures is quantified by risk assessment.

Chemical safety is another important research item. Detection methods for tracing of contaminants and residues of veterinary drugs are developed. New screening methods are tested and chromatographic methods developed in the laboratory are used for confirmation. Herewith the contamination source is traced and remediation suggestions are formulated. The reduction of problems with veterinary drugs at the farm level receives a lot of attention and the influence of the housing of poultry on chemical contaminants is also studied.



In the frame of fraud control techniques are tested to determine the species and treatment authenticity. Chemical-physical and technological research are carried out for innovative functional food and quality improvements connected with stability, composition, taste, processing and shelf live of dairy products. The product development on the level of the farm dairy producer is another important aspect.

In the context of service, reference work is mainly focused on the scientific guidance of laboratories, ring trials for the dairy industry and accredited analyses.

Moreover, CLO-DVK has a well equipped test factory with apparatuses on a semi-industrial scale for extrusion products, cheese, powder, drinks, desserts and ice cream for own technological research and scientific support of the food industry.

CLO-DVK is involved in many bilateral, national and international research projects and tries to reach good results by means of a multidisciplinary approach and close cooperation with other institutes. The obtained knowledge is made useful for the community by the publication of results and the organisation of seminars and workshops.

CLO-DVK – Research themes 2005

Microbiological safety of food

- Methodology development: detection, identification and typing of new microbiological risks and in vitro techniques to replace animal experiments
- Virulence of zoonotic pathogens: differentiation of pathogens based on virulence towards animals and humans and influence of sublethal stress on virulence
- Influence of feed strategies and housing on the presence of pathogens (Salmonella and Campylobacter) and harmful aerobic spores on the farm

Chemical safety of food

- Methodology development: sample preparation by means of new technology as 'Molecularly Imprinted Polymers', pyramid structure for detection of coccidiostats in eggs, new screening methods for antibiotics in animal products
- Problematic veterinary drugs: reduction of the problems with residues; use of antibiotics for non-lactating cows, fecal recycling for laying hens and migration in honey

Authenticity

Methodology development for stability, shelf live and taste



1.6 Department of Mechanisation, Labour, Buildings, Animal Welfare and Environmental Protection (CLO-DVL)

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The mission of the department consists of carrying out applied scientific research into innovative animal and environmentfriendly agricultural and horticultural production systems acceptable for the sector and for society.

The applied scientific research in agricultural engineering within the department focuses specifically on the following research topics:

- Farm buildings: design and optimisation of the construction and equipment of animal houses, applying new technologies. Special attention goes into the reduction of emissions towards the environment (ammonia, dust, etc.) and to the well-being of man and animal.
- Animal welfare: research on measuring, monitoring and improving the welfare of farm animals and evaluation of animal-friendly production systems.
- Environmental Protection: research on the impact of agricultural activities on the environment and research on environment-friendly production systems (animal housing, manure injection, etc.).
- Agromechanisation: the development of measuring techniques, procedures and equipment to determine the technical characteristics of agricultural and horticultural machinery. New technologies are applied for the optimisation of the performance of these machines, for improving the quality of the products and for the protection of the environment.
- Harvesting, post-harvesting, storage and processing: through interaction with the research into field mechanisation and farm buildings new harvesting and post-harvesting techniques are developed, evaluated and optimised. The main target is to maximise the quality of the harvested, stored and processed products.
- Labour organisation and farm management: improvement of production systems to increase the efficacy and efficiency of the labour organisation and farm management.



Besides applied scientific research, the department possesses a knowledge centre for technological advice that provides in the working areas mentioned active services to different organisations: government, federations, small enterprises, farmers, etc.

DVL – Research themes 2005

Techniques for sustainable animal husbandry

- Continuous monitoring of (milking) systems
- Development of measuring techniques and models for the automatic determination of group and animal-related indices of farmed animals
- Research into the sustainability of elements for construction, equipment and enrichment of animal houses

Evaluation and improvement of the welfare of farmed animals

- Research into indicators for animal welfare (fluctuating asymmetry amongst others)
- Evaluation of housing systems, housing environment and management regarding animal welfare
- Cooperation with the development of a European standard for monitoring animal welfare
- Animal-friendly alternatives for castrating piglets and other mutilations

Environmental technology

- Reduction of dust and ammonia emissions
- · Spreading techniques for fertilisers

Sustainable agricultural mechanisation and post-harvest technology

- Optimisation of spraying technology with field, orchard and lance sprayers
- Harvest, post-harvest and conservation techniques of crops and vegetables
- Agrotextiles and membranes
- · Soil improvement and erosion control

Public service

- Technological advisory centres Agriconstruct and Agromech
- CONTROL: quality control project in connection with the maintenance of milking installations
- Spray technology: Laboratory and Inspection of spraying machines

1.7 Sea Fisheries Department (CLO-DVZ)

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The core tasks of the Sea Fisheries Department are situated in the research of fisheries biology, aquaculture and restocking, technical fisheries, and the quality of the marine habitat and its biological resources. This mission complies with the provision of scientifically sound policy advice on fisheries, mariculture and environment, as requested by national and international governmental bodies and professional sectors.

The Sea Fisheries Department anticipated the rising unfavourable economical circumstances (high fuel costs, more rigorous fishery policy measures, increasing exploitation of the sea ...) and has taken initiatives to develop feasible alternatives in collaboration with the primary and secondary sector. These alternatives focus especially on energy saving adaptations, reduced by-catches, alternative fishing methods, new target species, quality aspects of landed fish and re-conversion. Furthermore, an integrated biological, technical and economical research approach has been installed to develop an optimal rational and sustainable exploitation model for the Belgian fleet.

There is a growing awareness of the fact that besides fisheries pressure, other anthropogenic activities have a worldwide effect on the marine habitat and especially on the marine biodiversity. It is therefore essential that the deleterious effects are considered on an ecosystem based approach in a cumulative way, in order to achieve a sustainable management plan for the natural resources of the seas. Within this integrated coastal zone management it is important that the Sea Fisheries Department remains closely involved in programmes that examine the biological and chemical quality at several trophic levels of the marine habitat, not in the least to ameliorate the intrinsic capacity of the marine ecosystem.



For the correct scientific support of all aspects of the research and management, the Sea Fisheries Department is developing optimised sampling protocols and analytical methods, which should lead to an integral quality assurance.

DVZ – Research themes 2005

Fisheries biology

- National data collection programme in support of the EU Common Fisheries Policy
 Population dynamics, exploitation patterns and stock size of commercial fish and crustaceans
- Predation pressure and discards in the sea fisheries

Aquaculture, mariculture and re-stocking

- Development of land-based fish culture (sole, turbot, sea bass) and regeneration of wild fish stocks
- Development of open-sea shellfish culture (blue mussel)
- Genetical identification of rotifers as a food source for reared fish larvae

Technical fisheries research

- Restoration of the North Sea cod stock through technical measures
- Development of new fishing gear and methods, netting materials and testing methods
- Sustainable and ecologically sound long-term strategy for the Belgian sea fisheries, in co-operation with the fishing industry

Environmental quality

- Antropogenic effects, other than fisheries, on the seabed and the benthos of the BCS
- Biodiversity of marine organisms (macrobenthos, epibenthos and fish)
- Chemical contaminants (PCB, OCP, PAH, OT, HM) in marine sediments and biota
- Fish diseases, imposex in marine snails and biochemical stress-indicators in fish
- Quality of fishery products
- Pollutant levels (PCB and pesticides) in fish from the Flemish freshwater basins
- Biochemical and genetic authenticity control of fish and shellfish
- Freshness, food safety aspects and nutritive properties of marine food products

Customer-oriented services

- Management advice on fish quota (multi-species approach) and evaluation of the EU fisheries advisory system
- Advice in support of sustainable and ecologically sound exploitation of both living and non-living marine resources
- Accreditation and training courses for (bio)-chemical analyses and the evaluation of fish quality and authenticity



2. PLANT UNIT

2.1 Strategies to circumvent crossing barriers in plants

Plant breeding aims to obtain certain gene combinations by performing specific crosses and selections. In some crosses, crossing barriers present in nature can form obstructions to obtaining offspring. Therefore, a lot of research is done at CLO-DVP to develop strategies to circumvent barriers present at the species level or at the level of the individual plant. Some examples below illustrate this. performed between different species within each of the proposed genera. Protocols for rescue of immature hybrid embryo's, including optimisation of the *in vitro* medium and retrieving the best maturation state of the embryo for *in vitro* initiation, were adapted for the four genera. Some combinations of species give complete or partial albinism of the seedlings. Retarded growth can also be a limitation in certain crosses. Performing the reciprocal crosses can form a solution to these problems. Finally, the obtained hybrid offspring are characterised morphologically and molecularly, using DNA markers. GISH (Genomic *In Situ* Hybridisation) is used to visualise introgression of the DNA.

Until now, for *Hibiscus* and *Buddleia* a large F_1 -population has been constructed and crosses were performed to obtain the F_2 generation. The highly reduced fertility in the hybrid offspring

Product renewal and improvement is of great importance for

nursery stock, especially for the 'visually attractive' plants in this category. Some pursued characteristics are: disease resistance, winter hardiness, compact growth, flower colour, leaf variations and fragrance. Mostly these characteristics are present within the genus, but not always, particularly in species or cultivars of commercial interest. In some cases, interspecific crossings (crossings between species) can be a solution to exploit these gene pools. As a consequence a lot of new variation becomes available. Within the framework of this research, the genera *Hibiscus, Hydrangea*, *Ligustrum* and *Buddleia* have been proposed by the growers as important model plants.

This research started with mapping all possible barriers for these four genera. Testcrosses were





of *Buddleia* is an important obstacle. Chromosome doubling can probably restore the fertility. For *Ligustrum* early abortion and the formation of empty seeds is an important problem in certain species combinations. So far, for *Hydrangea* only crosses between closely related species successfully produced offspring.



Interspecific hybridisation is also an important topic for the breeding programmes of azalea and certain pot plants. For pot plants, CLO-DVP frequently acts as a research partner in different KMO programs. In this way, the knowledge obtained from research becomes available for Flemish companies.

Another point of interest in some species is how individual plants can discriminate between their own and foreign pollen and can circumvent crossing barriers. The majority of flowering plants are hermaphrodites, having both female (pistils) and male (anthers) organs in the same flower structure. These features promote self-pollination and inbreeding, leading to a decrease in genetic variability. Flowering plants have developed numerous morphological and physiological mechanisms to reduce or prevent self-fertilisation. One of the physiological (genetical) mechanisms is self-incompatibility (SI). Selfincompatibility prevents inbreeding in flowering plants and promotes outcrossing. The basis for this mechanism is the cell-cell recognition between pollen and stigma. SI is not due to physical barriers in the style, but the stigma can distinguish between self (related) or foreign (unrelated) pollen. Only nonself pollen of the same species are able to germinate and result in sucessful fertilisation. Own, self-incompatible, pollen on the other hand is prevented from producing a fully grown pollen tube. The inhibition of the pollen tube growth happens in several steps, involving several genes. Two genes, S and Z, are the most important genes for recognition between the pistil and pollen.

The working of this SI-mechanism has important consequences for ryegrass breeding as it prevents the efficient production of inbred lines and hybrids. However, the production of hybrids would have several advantages, namely, exploiting the heterosis effect. Therefore, the temporarily breakdown of the SI-response can help to produce inbred lines.

At CLO-DVP, a research topic includes the identification of genes which are directly or indirectly involved in the regulation of the SI-response in ryegrass (*Lolium perenne*). Several strategies have been followed for this. We first investigated whether homologous genes involved in the SI-response of other plant families and grass species are also present in *L. perenne*. Another objective was to identify genes involved in the signalling cascade triggered by a SI-response, using several differential expression techniques. Using both strategies, we were able to identify several genes putatively involved in the SI-response of *L. perenne*. Confirmation of their role in the SI-response is the subject for further research.

2.2 Plant breeding and genetics for functional food and biopharmaceuticals

Flanders is too small for the large-scale cultivation of arable crops in order to produce bulk raw materials. Developing crops for the production of raw materials with a large added value may contribute to the re-orientation of Flemish agriculture. In this context, CLO-DVP runs two projects: the industrial breeding of chicory (*Cichorium intybus* L.) for the production of inulin and a study into the genetics of hop (*Humulus lupulus* L.) for the production of bio-active prenylflavonoids.

The chicory root contains inulin as a storage carbohydrate. Inulin

is a chain of fructose molecules ending in a glucose molecule. Fructose is obtained by hydrolysis of inulin. Fructose has the same calorie content as the sugar in a sugar beet, but a larger sweetening power. Due to the reform of the European sugar market, the production of fructose from chicory has become less profitable and hence the chicory industry now focuses on the production of inulin. Inulin has numerous good properties. It is neutral of taste and acts like a dietary fibre. The long inulin chains are not digested in the stomach but fermented in the intestines and as a result are low in calories. Inulin promotes the growth of the Bifidus bacteria, improving the function of the colon and inulin also improves calcium absorption.

The quality of inulin is determined by its chain length. Short chains have less good fibre properties, are higher in calories and are sweeter than long chains. The chain length is strongly affected by the harvest time. From mid October

onwards and especially after a period of cold weather the chains become shorter. At an early harvest date, the inulin chain length is larger but the root yield is lower. Early sowing increases yield significantly but enhances the risk of bolting. Therefore bolting resistant genotypes with a good field emergence and early growth at low temperatures are desired. At CLO-DVP bolting resistant genotypes are selected in very early (from the beginning of February onwards) sown trials.

Bolting resistance is inherited in a strongly additive way, just like the inulin content and the inulin chain length. Root yield is more determined by heterotic effects. For this reason we try to develop hybrid varieties based on vegetatively multiplied male sterile plants and self-incompatible clones or pseudo-self-incompatible lines.

Diploid and tetraploid roots, selected for their shape, root weight, inulin content and chain length are the starting material for chicory breeding. We clone these roots and select the clones on bolting sensitivity and leaf diseases. We combine the best clones in polycrosses and test their offspring (families) in yield



and bolting trials. With the roots of the best families, selected in bolting trials, we compose candidate varieties. The best mother clones are self-pollinated to produce inbred lines. Lines with good specific combining ability are the basis of hybrid varieties. Some bolting resistant CLO-DVP varieties with high inulin content and chain length are already on the market.

The hop plant (*Humulus lupulus* L.) is an essential ingredient for the brewing industry, as hops accounts for the characteristic bitter beer taste and for the formation of a stable foam head. In Belgium, hops are mainly cultivated in the Poperinge



region, but the total acreage gradually decreased over the last decennia to 189 ha in 2005. However, recent advances have opened new perspectives for diversified applications of hops. It was found that hop-derived prenylflavonoids possess highly interesting biological activities. Comparison with well-known phytoestrogens, e.g., genistein and daidzein (from soy), identified that 8-prenylnaringenin (hopeïn) is currently the most potent phytoestrogen known in the plant kingdom. Moreover, studies showed that the estrogenic potency of hopeïn is only ten-fold less pronounced than that of β -estradiol, the endogenous female hormone. Based on this finding, a food supplement (MenoHop®) was introduced for alleviation of menopausal symptoms. Research is also focussing on another hop-derived component, xanthohumol, which shows an exceptionally broad spectrum of inhibitory mechanisms at all stages of carcinogenesis.

Research is being carried out at CLO-DVP aimed at the characterisation of genes involved in the biosynthesis of prenylflavonoids in hops. Three hop varieties, Whitbread Golding Variety, Wye Challenger, and Admiral, with contrasting capacities for the accumulation of desmethylxanthohumol (precursor of hopeïn) and xanthohumol were selected for this purpose. Studies were performed on RNA extracted from leaves and from different flowering stages. Most interestingly, some of the isolated DNA fragments showed high homologies with plant genes known to be involved in the biosynthesis of secondary metabolites. Thus, pivotal genes in the pathway leading to prenylflavonoids were identified in hops. More specifically, these were genes for phenylalanine ammonia-lyase, cinnamic acid 4-hydroxyase, 4-coumarate:CoA ligase, isopentenyltransferase and O-methyltransferase. Future work includes functional characterisation of the genes of interest using expression analysis and reverse genetics approaches.

2.3 Breeding outdoor vegetables

The research on outdoor vegetables at CLO-DVP follows the policy to 'support the economic sustainability of the Flemish agriculture' and to 'enhance the quality of the production methods in an environmental and social context'. For example, the introduction of hybrid cultivars in leek allows better mechanisation and therefore will be labour-saving.

Leek (*Allium porrum* L.) is an important outdoor vegetable in Flanders (4.500 ha) and Western Europe (30.000 ha). For many Flemish farmers, leek is the backbone off their crop rotation.



Until recently most of the cultivars used were open-pollinated (non-hybrid). In these cultivars, the lack of uniformity in the field asks for more labour during transplanting and harvesting and reduces productivity. Much of the variation within open-pollinated cultivars is explained by strong inbreeding depression that occurs in selfed plants (up to 20% self-fertilisation in this outbreeding species). Research at CLO-DVP shows a wide variation in yield reduction by inbreeding depression (from 15% to 75%).



Complete elimination of selfed seed by sieving the seed lot is impossible. Many years of research shows that methods based on family selection, line selection or clone selection can not deliver the so wanted uniformity in this crop. Only hybrid selection methods based on male sterility can deliver uniformity in the leek crop, by eliminating self-fertilisation. Until now there is no cytoplasmic male sterility (cms) found in leek. Therefore research is based on plants showing genetic male sterility (GMS). This includes the vegetative propagation of the female lines. Other research at CLO-DVP aims to create populations that are enriched with male sterile plants. This allows much more selection in female lines towards disease resistance, plant shape, firmness of transplants, storability and of course seed yield. Research on leek at CLO-DVP also involves the detection and characterisation of genes that are linked to resistance for leek rust (Puccinia allii) using DNA markers and genetic maps. This research revealed that resistance for leek rust depends on several genes which are distributed over several chromosomes. The tetraploid character of leek complicates the research towards genetic mapping. Nevertheless, a first map is available at CLO-DVP, with indications to the genome regions involved. Adding new DNA markers will improve the genetic map. Ultimately, this will result in a marker-assisted selection programme for leek rust.

The research program on celery comprises celeriac (root celery) and leaf celery (green and yellow types). A predominant topic in celery research is the problem of late blight caused by *Septoria apiicola*. We started a recurrent selection program in green leaf celery with *septoria* tolerant plants. For celeriac, the inner bulb quality parameters are improved, for the leaf celery uniformity, colour and canopy are important characteristics. Breeding strategies employed here are family and line selection programmes, followed by recombination testing.

The CLO-DVP research programme on parsley aims to improve the uniformity, vigour and dark green colour of the finely curled leaves. The breeding strategy employed here is family selection. Topics for research in scorsonera are improving the root quality and disease resistance on the leaves. Clone and family selection are the most suited selection techniques for scorsonera.

The breeding research on leek, celery, parsley and scorsonera at CLO-DVP has resulted in numerous candivars and commercially available cultivars.

2.4 Laboratory for plant-, substrate- and water analyses

The determination of the quality of end products is one of the most important phases in the production process. This is the same for the evaluation of plants, especially for forage crops. To support the research within the unit PLANT, forage quality is determined at CLO-DFE on the basis of the Weende and the Van Soest scheme. According to the Weende system, crude ash, crude protein, crude fat and crude fibre are determined. The Van Soest analysis determines NDF (neutral detergent fibre or cell wall), ADF (acid detergent fibre or cellulose + lignin) and ADL (acid detergent lignin). Furthermore, digestibility, soluble carbohydrates, starch content and the macro minerals K, Na, Ca, Mg and P can be determined. The sugar content and degree



of polymerisation in chicory are analysed after hydrolysis of shredded roots by HPLC equipped with ELSD (Evaporative Light Scattering Detection). Chlorophyll concentrations in plants are determined with UV-VIS spectroscopy.

The NIRS technique is used for the evaluation of varieties (variety testing and breeding purposes). The calibrations are updated and validated regularly with new samples.

The chemical analyses are validated by participating in the interlaboratory comparison studies (ring tests) of the 'International Analytical Group (IAG)' and the 'Bureau InterProfessionnel d'Etudes Analytiques (BIPEA)'.



The accreditation procedure is running for the most frequently determined parameters (moisture content, crude ash, crude protein, crude fibre, starch, NDF, ADF and ADL). The pre-audit was organised in September 2005 and the real audit will be held in the spring of 2006.

In the context of the project "Carbon sequestration in terrestrial ecosystems", soil samples of all agricultural zones of Belgium were collected and analysed for total organic carbon content (TOC), according to the method of Walkley and Black. In addition, all the samples were scanned by NIRS and a global calibration for the TOC was calculated. The samples were divided into two groups according to their practice: agricultural and natural grassland. Moreover, the samples were divided into three texture groups: sand, silt and clay. Calibrations for these different classes were developed. The prediction accuracy of the TOC was improved compared to the global calibration.

The Haldrup grass harvesting machine was equipped with a NIRS Diode-Array spectrometer to do on-line measurements at harvest. Part of the cut grass is transported by a conveyor belt below the spectrometer. In this way, the grass is scanned continuously between 960-1690 nm. A strong signal for water is detected around 1450 nm. Two samples of the scanned grass are collected: one for the determination of the dry matter content, using classical oven drying and one for quality analysis after freeze drying. For perennial ryegrass and timothy NIRS calibrations for dry matter, crude protein and crude fibre were developed on the basis of 580 samples of different cuts taken over the years 2004 and 2005. The R² (determination coefficient) and SECV (standard error of cross-validation) were respectively 0,95 % and 0,89 % for dry matter content, 0,90 % and 1,10 % for crude protein and 0,88 % and 0,94 % for crude fibre content.

The other laboratory of the department, Agrolab, is active in the area of water, soil and substrate quality. The support for the azalea and begonia growers in the region of Ghent is



very well appreciated. Upon request by the Belgian potting compost federation, a charter for the determination of quality characteristics of substrates has been edited and finalised. UPOV guidelines (International Union for the Protection of New Varieties of Plants, TG/172/3 and TG/107/3). For each crop, different parameters, mostly morphological, are judged. Due to the international recognition of the CLO-DFE, foreign institutes request to be supported and trained to perform analogous tasks.

The following table gives an overview of the analyses of potting compost in the last three years and their percentage norm overrun.

	% norm overrun												
period	mg/kg dry matter				weeds	bag potting	pН	EC	Na	Cl	dry	organic	
	Cd	Cu	Pb	Ni	Zn		compost volume		microS	mg/l	mg/l	matter %	%
spring	0,0	0,0	0,0	0,0	3,6	0,0	28,6	3,6	7,1	3,6	7,1	0,0	14,3
autumn	6,5	0,0	0,0	28,3	6,5	0,0	26,1	0,0	6,5	6,5	0,0	0,0	21,7

Furthermore the laboratory supports the campaign of the determination of nitrate residue in the soil after harvest in the context of "Mestactieplan MAP II" of the Flemish Government (implementation of the EU nitrate Directive).



2.5 Measuring objectively with image analysis

Industrial chicory and tuberous begonia are crops that are traditionally cultivated in Belgium and for which active breeding is still being done here. For the protection of new varieties testing of breeders right needs to be preformed. New varieties are being tested for **D**istinctness, Uniformity and Stability (DUS) and are compared with a reference collection.

The research for the Community Plant Variety Right for the above mentioned crops is executed at the CLO-DFE, which is internationally recognised by the Community Plant Variety Office (CPVO, Angers, France). This research is done following Up till now, the judgment of morphological parameters of new varieties is done by (i) manual measuring, and (ii) scoring for non measurable parameters. Colour is evaluated by using reference colour charts (RHS Colour Chart, Royal Horticultural Society, 1995).

In the case of manual measured parameters, such as length or width, the measurement is done precisely. However, errors can be made when writing down the data or typing the data into Excel[©]. We can say that the errors are relatively limited, but this way of working is labor intensive; certainly if several parameters of the same object have to be measured.

Scoring is done using a scale from 1 to 9, and is used for evaluating, for example, the shape of the shoulder of roots of industrial chicory. The evaluator uses a reference with a description and image of the parameter. By comparing the reference with the object, a score is given. This method is less time consuming, except if many parameters need to be scored on individual plants. The subjectivity is greater compared to the manual measurements and can be dependent on the evaluator.

To assess colours, of for example flowers, colour charts are used. This assessment is difficult due to dependency on the light environment at the time of evaluation. It is recommended to perform the evaluation in diffuse light. Also background colour plays an important role, as is the case with flower petals, which can be transparent. As such we can conclude that colour assessments are difficult and very subjective. Often flowers have multiple colours, complicating the above. Furthermore, colour codes are obtained and consequently it is impossible to do statistics on such data.


We can conclude that manual measurements and visual scores are often time consuming, partly subjective and subjected to unwanted errors. Measuring morphological parameters using image analysis leads to a more exact measurement. Furthermore, all measurements are done the same way irrespective of the evaluator or year. Finally, the object (leaf, root, flower,...) can be evaluated on more parameters (shape or colour) and with less time needed. For leaves it is easy to determine in addition to length and width, center of mass (or its relative position), number of leaf lobes, start of the leaf stalk, leaf area,.... to enumerate a few.

In 2004, at the CLO-DFE four image analysis programmes were developed for the evaluation of morphological characteristics of the leaves and roots of industrial chicory, the leaves of tuberous begonia and for the evaluation of the flower colour of tuberous begonia.

About 7400 images were evaluated during the first growing season (2004). Each plant part was evaluated manually and visually in combination with the image analysis system.

Measurements were compared and we concluded that measured parameters could be substituted by parameters determined by image analysis.

In 2005, 6400 new images were taken. New programmes are being developed using shape describing methods such as elliptic Fourrier descriptors. For the colour determination of flowers, measuring in different colour spaces is being tested. A further growing season will also enable us to evaluate the year effect on the parameters determined by means of image analysis.

From the research we can conclude that manual measurements, at this moment described in the UPOV guidelines for industrial chicory and tuberous begonia, can be partially replaced by measurements done by image analysis. The image analysis measurements are more accurate and more parameters can be determined. Further research is needed to determine a sufficient number of parameters with a discriminating power, which is as large as possible.



The interest for white clover in permanent grassland has significantly increased because of a stronger limitation in the use of N and P in Flanders. White clover can establish in the sward and persist for many years if the application of fertiliser N is below 200 kg ha⁻¹.

Research focused on the effect of decreasing fertiliser N in combination with the use of a grass/clover mixture on dry matter (DM) yield, feeding value and botanical composition, started in 1996 and finished in 2005. The annual rate as well as the distribution of N in the season is different for the grass and the grass/clover treatment. Before and after grazing, strips are mown in each treatment to calculate the net grass yield. Preliminary calculations result in a small difference of 0.9 ton DM ha⁻¹ in yield in favour of the grass (275-300 N ha⁻¹). In the grass/clover treatment, receiving 150-170 N ha⁻¹, the clover persists very well. The clover content is evaluated regularly during the growing season and exceeds the optimum of 30-50% of the dry matter in midsummer. A high clover presence in the sward results in a more open sward and an increase of *Stellaria media* L. and *Poa annua* L. in spring.

The white clover presence in mixed swards with a high clover content (29) and intensive managed grass swards (12) were followed-up in the period 2003-2005. Two systems are used: estimation of the area covered by white clover (%) and the frequency method using squares of 10 cm x 10 cm. Yearly, in the period 1-15 October, soil samples (0-90 cm) are taken to measure the nitrate content as a parameter for the risk of nitrogen leaching during the winter. Results from the first two years show that the nitrate content in the soil profile varies greatly between the years and that there is no relationship between the clover content in the sward and the nitrate content in the soil profile.

Perennial ryegrass is characterised by a successful installation and a dense sward. So, white clover has a big problem to establish and to survive in these conditions. Maybe there are more opportunities for white clover in a mixture of perennial ryegrass and timothy. In an experiment, white clover was sown in monoculture and mixtures of perennial ryegrass and timothy (60/40 and 15/75). The first results indicate that the presence of white clover was not influenced by the use of a mixture of the two grass species. Only a monoculture of timothy favoured the clover establishment. The research on the behaviour of timothy and white clover in these swards was continued in 2005.

Subsidies in support of the cultivation of protein crops on-farm



has offered new opportunities for lucerne and red clover in particular. These crops are able to fix substantial amounts of N in association with Rhizobium bacteria, they enlarge the crop rotation, however to receive the subsidies no grazing is allowed. The farmers are no longer familiar with the cultivation of these crops and they need information about sowing period, choice of the mixtures, N-fertilisation, cutting regime and conservation. The experiments started in 2003-2004 and will continue until 2006. In one location sowing in autumn is compared to sowing in early spring. In another experiment, red clover and lucerne were sown in monoculture and in mixtures with Italian and perennial ryegrass on a sandy and a sandy-loam soil in spring 2004. Some of these mixtures also contained white clover. The influence on weed development, dry matter and protein yield, feeding value and botanical composition is studied. These parameters were also examined in an experiment with three N levels 0, 150 and 265 kg N ha-1 in one location (sandy loam). Some preliminary results are: August is the best sowing period; weeds invade easily in a red clover monoculture, so there is a considerable amount of weeds in the first cut in the first year, as well as in the second year. The combination legume/perennial ryegrass is optimal for several reasons, legumes have a high yield potential without N fertilisation and N fertilisation has no influence on the DM yield.



Leaf loss by shatter, due to the rapid loss of moisture from leaves compared with stems during drying is a major hazard. Prewilting to 35% DM and no further is optimal to minimise this leaf loss. Red clover and lucerne are traditionally regarded as difficult crops for silage making, on account of low water-soluble carbohydrates (WSC) content and high protein content. In 2005 the application of additives was investigated in collaboration with the Department of Biotechnology, Landscape Architecture and Agriculture (Hogeschool Ghent). Additives were applied in monocultures of red clover and lucerne and in mixtures with perennial ryegrass at a DM level of 35%. Results will be available in 2006.

It is not always possible to sow forage legumes in August because the previous crop is not harvested yet. Maybe it is interesting to sow the grass before the winter and to sow the white clover in the young sward in the spring? Oversowing can be done early in the season (1 April) or after the first cut (< 1 May). Such an experiment was set up at three locations in 2005 in collaboration with the Agricultural Centre of Fodder Crops.

2.7 White rust of chrysanthemum

Chrysanthemum white rust, caused by the fungus *Puccinia horiana*, is one of the most important problems in the culture of potted chryanthemums. A large proportion of the commercial cultivars are sensitive to this disease and the symptoms seem to appear suddenly and intensively. Hence, regular preventive spraying is common practice. There are also reports that chemical control treatments with triazole and strobilurin fungicides are becoming less effective due to the development of resistant strains. There are several nurseries in Flanders that produce propagating material. *P. horiana* is a quarantine pathogen on propagating material so especially for those nurseries this pathogen is an important problem.

The research program on chrysanthemum white rust at the Department of Crop Protection is a prototype of research towards the use of disease detection and disease warning systems that should lead to more targeted fungicide applications and to the use of resistant cultivars. This fits in the government strategy for more durable use of pesticides. The research goals are grouped in four work packages of which the first was recently completed.

• The first research goal was the development of methods for fast detection of the pathogen, even when it is still in a latent stage. Two strategies were explored. The first one was the detection of infections in an early stage through the use of chlorophyll fluorescence image analysis. An infecting pathogen causes suboptimal photosynthesis which results in increased chlorophyll fluorescence. This fluorescence can be detected with specific illumination and imaging systems. Such an analysis was carried out on artificially inoculated chrysanthemum leaves in a collaboration with CLO-DFE. Results showed that the chlorophyll fluorescence due to *P. horiana* infection could be visualised no more than two days before the appearance of visual symptoms (after 12 days). This is too short for practical applications.

The second strategy for fast detection was a molecular method based on specific PCR amplification of the internal transcribed spacer region of the ribosomal RNA genes (rDNA-ITS). After sequence analysis, specific rDNA-ITS sequences of the target organism are used for the development of specific PCR primers. Before we could reach that stage, we developed an easy method to maintain this obligate parasite in culture. Using this method, a collection of 28 isolates was established. To determine the DNA sequence of interest, it became apparent that we had to start from pure fungal spores and not from pustules: the dissected pustules contained a small amount of plant material that was preferentially PCR-amplified. To prevent this problem, we





developed a method to collect pure basidiospores from infected plant tissue. Using pure fungal DNA from these spores we were able to PCR amplify and sequence the DNA areas of interest. Several isolates were analysed in order to verify the identity and uniformity of the ITS sequence within and between the isolates. Apart from a few repetitive sequences, it appeared that the area of interest was sufficiently conserved. The entire ITS sequence of P.horiana was aligned with a selection of rDNA-ITS sequences from other fungi (among which there were several other rust species) in Genbank. Based on unique differences in base pair sequences, several candidate primers were developed and tested in PCR and real-time PCR. A few primer combinations reliably PCR-amplified the DNA from P. horiana up to 10 fg (nested PCR) and 100 fg (real-time PCR). The selectivity of the primers was tested on a collection of (rust) fungi. In order to do this, the rDNA-ITS regions of several of those rust fungi had to be cloned. A few primer combinations were selective for P. horiana,

even when high levels of non-target DNA were present. The primers allow detection of *P. horiana* in latently infected plant material, as soon as a few hours after inoculation. This technique can be used directly on nursery samples, for example during the screening of propagating material. The idea is also to combine this molecular detection system in combination with a spore sampler so that quantitative detection of the fungus in the air can be accomplished. A spore sampler is a machine that collects aerial spores on a piece of tape. Early detection of the spores in air could serve as an early warning system for targeted fungicide applications.

• The second goal of this project is to obtain insight into the latent survival and spread of the fungus. How does the fungus enter a nursery? Through the air, through infected cuttings, or does it survive latently on mother plants? This entry process will be studied through the monitoring of air and plant samples

from nurseries with the real-time PCR technique. Based on the results we hope we can inform the growers on how to be more successful in keeping this fungus off their nursery.

When symptoms do occur on a nursery, they tend to appear suddenly and rather intensively. There are indications that the fungus develops slowly when the temperature rises, thus we suspect that latent presence may occur frequently, and that the fungus expresses itself quickly once the conditions become favorable again. There is little knowledge about this possible latent presence of the fungus inside the plants. Using the quantitative molecular detection technique and inoculation and incubation trials under different temperature regimes we plan to analyse the fungal behaviour as a function of temperature. This knowledge can then be integrated into the warning system.

• The third goal is to determine the diversity in fungicide resistance within the Flemish population of *P. horiana*. Is it true that fungal strains with reduced fungicide sensitivity have emerged? Isolates collected in 2003 and 2005 will be screened for their sensitivity against two strobilurin and two triazole fungicides. In the case where a reduced sensitivity is observed, those isolates will be checked for specific DNA mutations in the gene sequences of the target molecules of these fungicides. If we observe mutations that are correlated with fungicide resistance, a PCR technique will be developed that will allow detection of this mutation in suspect new isolates.

• The fourth and last goal of this research is to propagate the identification and development of resistant cultivars. A test was developed that allows the screening of candidate new cultivars for their sensitivity to *P. horiana*. This test allows screening of a substantial number of cultivars in a relatively short period of time. In the long term we also hope to use this test to determine the genetic basis for resistance to this pathogen in several cultivars. We hope to do this in collaboration with CLO-DVP and a few chrysanthemum breeders. This will not only reveal which cultivars can be good donors of resistance but also what kind of resistance they can deliver. This should lead to more durable long term disease resistance.

With these four goals we aim to develop control strategies based on effective warning systems, prevention of the introduction and spread of the disease in nurseries, and the development of durable disease resistance.

2.8 Bacteria causing midrib rot of butterhead lettuce

Bacterial soft rot and necrosis of leafy vegetables occurs, in the field, in glasshouses and is also a leading cause of postharvest losses of vegetables in the marketplace worldwide. Bacterial rot is caused by a group of plant pathogens that includes *Erwinia carotovora* and pectolytic pseudomonads, such as *Pseudomonas viridiflava*, *P. cichorii*, *P. marginalis*, *P. fluorescens* and *P. putida*. Their mode of infection and their tissue macerating ability through the production of pectic enzymes implies little specificity, hence their broad host range and wide ecological distribution. These plant pathogens easily proliferate once infection is initiated and disease build-up can be rapid resulting in severe financial losses for the grower. Also, pectolytic



breakdown of affected tissues results in softening, liquefaction and exudates that can spread bacteria over commodities in bulk storage. The incidence of bacterial soft rot caused by *Pseudomonas* has increased from a rather sporadic to a chronic problem that now represents a serious threat for the production sector. Pectolytic pseudomonads are generally considered as opportunistic bacteria with low efficiency to attack actively growing plants but can cause disease on weakened plants or when all conditions for infection are optimal. A specific disease problem is midrib rot of glasshouse-grown

butterhead lettuce. Over the last ten years, damage by midrib rot has emerged as an important economical threat for the lettuce production sector in Flanders. Symptoms are a brownish rot along the midrib of one or more inner wrapper leaves, often accompanied by soft rot of the leaf blade. Two types of midrib rot can be distinguished and often are found in combination. The first type, referred to as soft rot, usually affects the outermost



leaves of the head and symptoms appear as slimy light brown to reddish brown discolorations of the midrib commonly accompanied by a slimy decay of the leaf blades. The second type, referred to as typical midrib rot, is characterised by dark brown to greenish black discolourations on the midrib of one or more inner wrapper leaves. The symptoms are always inside the head, while the outermost leaves of the head are usually not affected. Crop damage by midrib rot is most common during autumn and winter seasons but can also occur throughout the year. Midrib rot symptoms are consistently associated with



nearly mature plants. The high humidity between the inner wrapper leaves of the head facilitates the proliferation of bacteria resulting in rapid disease development, often in less than 24 hours. Since the symptoms appear typically inside the head, the disease can easily remain unnoticed until harvest. If symptoms develop on several inner leaves, damage can be extensive and usually results in partial or total loss of crops. Midrib rot of lettuce is studied as a model system at CLO-DGB, in collaboration with POVLT (Provincial Research and Advisory

Centre for Agriculture and Horticulture) and the Laboratories of Microbiology and Phytopathology at University Ghent. Forty-three symptomatic lettuce samples were collected from commercial growers in Flanders. Isolations of bacteria, from macerated diseased tissue extracts, on dilution plates yielded more than 400 isolates. All isolates were examined for morphological, physiological and phytopathological characteristics. More than 60% of isolates from midrib rot symptoms produced colonies on pseudomonas agar F medium that were green to blue fluorescent under UV light, and were identified as Pseudomonas species. Their tissue macerating ability was evaluated by injection of an aqueous bacterial suspension into a chicory leaf, and in combination with the potato rot test a distinction was made between weak or strong pectolytic ability. Their ability to induce midrib rot symptoms was evaluated in pathogenicity tests on lettuce by injection into the leaf midrib and by spray-inoculation of mature plants. The results indicate a clear relation between lettuce midrib rot symptoms and the presence of fluorescent pseudomonads,



which suggests that Erwinia spp are not directly associated with the disease problem. The isolated fluorescent pseudomonads can be differentiated into four phenotypic groups based on two pathological tests, (i) the nonhost hypersensitive reaction (HR) on tobacco for presence of virulence factors, and (ii) the potato rot test for pectolytic ability (P). The group HR⁻P⁻ comprises pseudomonads that do not cause any damage on inoculated plants and are considered saprophytic bacteria. The group HR⁻P⁺ comprises pseudomonads that cause various degrees of rotting upon injection into the leaf midrib, but not upon spray-inoculation of healthy plants, and hence are considered opportunistic soft-rotting bacteria. They are commonly found throughout the lettuce samples and represent a highly heterogenous group based on colony morphology and DNA fingerprinting profiles. It is still unclear whether these HR⁻P⁺ pseudomonads on their own can be responsible for inducing rotting on healthy plants. Their disease potential results mainly from their ability to produce pectic enzymes, but is probably also determined by factors associated with environmental conditions and complexities of the plant-bacteria interactions. The group HR⁺ comprises pseudomonads that cause symptoms upon injection into the leaf midrib and also reproduce the typical midrib rot symptoms upon spray-inoculation of healthy lettuce plants, and hence are considered primary midrib rot pathogens. They represent a homogeneous group based on colony morphology, DNA fingerprinting profiles and partial sequence analysis of 16S rDNA. On the basis of the potato rot test, however, these primary pathogens can be differentiated into isolates with and without pectolytic ability (P⁺ and P⁻). The primary P⁺ pathogens are the most aggressive ones in greenhouse pathogenicity tests. The fact that these primary pathogens are consistently found in association with typical midrib rot symptoms and until now not have been isolated from healthy plants, suggests that they are introduced by an external inoculum source, such as irrigation water or plant material. In which case, identification of the inoculum source will be required before appropriate control strategies can be devised. Efforts to develop quantitative PCR detection probes for these primary pathogens

PCR-DGGE profiling of bacterial communities on glasshouse butterhead lettuce revealed profiles that are dominated by bands identified as belonging to diverse *Pseudomonas* spp. It is clear from these results that abundant *Pseudomonas* populations can be found on glasshouse lettuce. Further analysis of the DGGE profiles also revealed that the opportunistic soft-rotting pseudomonads seem to be part of the natural bacterial flora of lettuce. Therefore, control strategies for this group of soft-rotting pseudomonads will have to be directed at devising appropriate cultural practices, which suppress their pectolytic ability and increase resistance of the host plant, rather than detection of

are currently ongoing.

inoculum sources.

In conclusion, evidence has been provided that fluorescent pseudomonads are the causal agents of midrib rot of glasshouse lettuce. This is based on the isolation of fluorescent pseudomonads from symptomatic tissue, the identification of *Pseudomonas* strains as primary pathogens that reproduce the typical midrib rot symptoms upon spray-inoculation of healthy lettuce, and fulfilment of Koch's postulates.

2.9 Plant-parasitic nematodes in organic and conventional vegetable cultures under glass

The cultivation of vegetables under glass is the most intensive form of agriculture. For economic reasons, crop rotation is much narrower than in open-air cultivation. Vegetable crops especially grown in glasshouses include tomato, pepper, cucumber and lettuce. Possible alternative cultures, such as eggplant and zucchini, belong to the same botanic family as tomato and pepper (solanaceous plants). Moreover, crops under glass last much longer than outdoor crops. The soil is planted with the same species for ten to eleven months. The temperature of the soil is high during the entire year due to heating. Soil fauna and flora thus have ideal developing conditions for almost the entire year.

For these reasons, it is not surprising that nematodes are one of the largest threats in the cultivation of vegetables under glass, and especially in organic cultivation. Nematodes are a big problem for vegetables growen under glass, much more than above-ground pests. A sufficient suppression strategy for nematodes has not yet been developed. Resistant root stocks of tomato are resistant to three species of root-knot nematodes (Meloidogyne incognita, M. arenaria and M. javanica), however this resistance is broken at higher temperatures. The cucumber root stock Harry is tolerant, but not resistant and allows a buildup of the nematode population for the next crop. Most of the organic growers steam sterilise the soil when the nematode problem becomes inbearable. For conventional growers, chemical resources can be deployed, although the products do not result in 100% kill-off. Since 2005, the most effective means of chemical control, methyl bromide, can only be applied in certain crops and only after it is shown that the nematodes are present. In all cases the control measures are not cheap.

In cooperation with the Provincial Test Centre for Vegetables (Provinciaal Proefcentrum voor de Groenteteelt, PCG) an investigation was conducted on vegetables grown under glass, to examine which nematodes occur most frequently in order to develop an adapted control strategy. It was also investigated if



natural antagonists (enemies) of nematodes were present the soil. Five organic companies and five conventional companies were used in the study so that a comparison between these two cultivation methods could be made.

Soil samples were taken at each of the ten companies. The number of samples per company varied between three and eleven, depending on the number of crops and glasshouse compartments and on the presence of areas with visible growth reduction. A total of 63 samples were taken, 40 in organic companies and 23 in companies using the conventional cultivation system. In the laboratory both migratory type (freeliving) nematodes and cyst nematodes were extracted from the soil. Also, for most of the soil samples a bio-assay was carried out, i.e. a tomato seedling was planted in the soil remaining after

taking soil for extraction of the nematodes. The bioassay allows the nematodes and/or antagonists that might be present to multiply and thus increases the chance of their detection. Plant-parasitic nematodes were identified morphologically using a microscope, or by molecular techniques. In addition to plant-parasitic nematodes saprophytic nematodes were also counted. The presence of fungi that parasitise the nematodes (nematophagous fungi) and of Pasteuria spp., a

nematophagous bacterium, were also examined microscopically. In addition, a diluted solution of each soil sample was spread onto culturing medium in Petri-dishes and bait nematodes were added to discover antagonistic fungi. The presence of the wellknown fungus *Pochonia chlamydosporia* was examined with a special culture medium.

Plant-parasitic nematodes were found in nine out of the ten companies. The most frequently found nematodes were rootknot nematodes, *Meloidogyne* spp., which were found in eight companies, equally divided among the two cultivation systems. *M. javanica* and *M. arenaria* are thermophile and cannot survive winter outside in our regions. It is striking that not only thermophile root-knot nematodes were found. Nematodes typical for our regions assumed not to survive well in warm environments, such as *M. hapla*, were found in the glasshouses. However this species, *M. hapla*, was present especially in organic companies, where there was no heating in the winter. The quarantine nematode, *M. fallax*, was found



at one company. This indicates that this nematode is more naturally spread than assumed or that it is distributed by means of planting material. Root-knot nematodes were found in crops of tomato, cucumber, pepper, eggplant and lettuce. Also potato cyst nematodes (*Globodera* sp.) were found in three companies with conventional cultivation. In addition to potato, tomato and eggplant are hosts for this well-known nematode. Finally, less damaging plant-parasitic nematodes were found (*Tylenchus* sp. and *Paralongidorus* sp.), but only in organic companies. In soils of organic companies, the number of saprophageous nematodes was three times higher than in the soil of companies with conventional cultivation. These nematodes indicate an active soil life. Since the saprophagous nematodes are a food source for these fungi the number of non-specific nematophagous fungi could increase by their presence. However, has not yet been

demonstrated.

Few nematodes were found parasitised by nematode antagonists (less than 1% of the nematodes examined). *Pasteuria* spp or *P. chlamydosporia* were not discovered in any of the samples. Using the method of the soil-bait nematodes, only some types of fungi were found. This is most likely due to the small fraction of soil that is tested for the presence of antagonists (0.5 g soil) in this method. In total, eight

types of fungi were found in soils of four organic companies and of one company with conventional cultivation. The specimens found belong, among others, to the nematode-trapping fungi *Arthrobotrys*, *Dactylella* and *Dactylaria*, and also to plantparasitic fungi *Verticillium* and *Aspergillus*. The company where no nematodes were found applied horse manure to the soil, but regularly disinfested with methyl bromide.

The nematode problems cannot be denied; on almost each company sampled (both conventional and organic) plantpathogenic nematodes were found. In contrast to what was expected, antagonists were only present in a small percent. In the organic companies few damaged nematodes were retrieved, even though, more antagonists were found in the organic companies than in the conventional companies. There is still no clear explanation for the cause of the presence of nematodes. It is not yet known if nematodes are brought in by contaminated shoes, machines, planting material or other means. What is important is that the nematode populations can build very rapidly, once they are present because of the narrow crop rotation and the long cultivation in a warm soil.

An effective control measure is not currently available. Methyl bromide was during many years the most chosen option in the conventional growing system, but this chemical is now being phased out. Other chemicals are less effective and are not an option in organic cultivation. In the mean time, organic growers use steam to sterilise the soil, although the results are only moderately effective. The disinfecting action of the steam does not penetrate the soil deep enough and careless application can enable some of the nematode population to survive. After one or a limited number of years the population becomes again too large and economically significant losses are seen. Biological control is not yet available. There is at present (to a limited extent) investigations into biofumigation (the incorporation of organic material into the soil and coverage with plastic foil) and into the nematode suppressive action of a number of fungi. However, a useful product will not be available soon. Most hope is expected from resistant - in most cases tolerant - root stocks. Further research is recommended to support the growers of vegetables under glass, especially the organic growers, in their choice for a specific, efficient treatment of the nematode problems.

	BIOTEELTEN				GANGBARE TEELTEN			
	aantal monsters				aantal monsters			
gewas	totaal	besmet	nematode	aantal/100 g grond*	totaal	besmet	nematode	aantal**/100 g grond
tomaat	18	8	Meloidogyne hapla	1 tot 97	16	3	M. hapla + G. rostochiensis	15 tot 63 + 4 tot 27
		6	M. arenaria	1 tot 154		1	M. arenaria	361
		2	Tylenchus sp.	15 tot 81		1	M. arenaria + G.rostochiensis	130 + 2
				-		2	M. javanica	43-487
						2	M. javanica + G.rostochiensis	1 tot 12 + 15 tot 29
						2	Globodera spp.	1 tot 2
komkommer	9	4	M. hapla	10 tot 60	1	1	M. arenaria	
	-	1	Meloidogyne sp.	541				
		3	Tylenchus sp.	16 tot 70				
paprika	4	2	M. hapla	12 tot 173				
aubergine	1	1	M. hapla + Meloidogyne sp.	91				
courgette	2	1	M. hapla	697				
-		1	Tylenchus sp.	19				
sla	5		· ·					
spinazie	1	1	Paralongidorus sp.	60				
boon			- .		1	1	Globodera spp.	0,3
geen (lege kas)					2	1	Globodera spp.	0,7
totaal	40	30			20	14		

* de schadedrempel voor Meloidogyne spp. varieert ngl. gewas, variëteit en onderstam, maar kan algemeen als 100 nematoden/100 g grond aangenomen worden ** aantal vrijlevende nematoden + aantal cysten

3. ANIMAL SCIENCE UNIT

3.1 Animal welfare

3.1.1 *Ad libitum* feeding of fibre rich diets to group-housed pregnant sows

Group housing of sows is nowadays compulsory for production units that are constructed or rebuilt. Moreover, from 2013 onwards, all pregnant sows will need to be housed in groups. Apart from problems concerning the behaviour of grouphoused sows, their nutrition also deserves specific attention. Currently, almost all pregnant sows are restrictedly fed. The application of restricted feeding for group-housed sows implies high investment costs (adaptation of the infrastructure, animal recognition, ...). Moreover, the restricted feeding system affects animal welfare negatively. *Ad libitum* feeding would be a cheaper and more animal-friendly solution. Unfortunately, with the classical pregnant sow diets this may lead to sows that are too fat and produce inferior reproduction results.

Most of the animal feed manufacturers have tried, with variable success, to introduce a modified *ad libitum* diet on the market. At first the use of high quantities of sugar beet pulp seemed to be an evident solution. However, the dependency on one specific feedstuff with regard to price, quality and availability urges the search for alternatives. Therefore, seven vegetal feedstuffs, plus sugar beet pulp as a reference, were investigated for their energyintake-limiting properties with ad libitum fed pregnant sows. With the exception of rapeseed meal, every incorporated fibre source decreased the spontaneous net energy-intake significantly. This decrease was most pronounced for sugar beet pulp, chicory pulp, maize gluten feed, alfalfa and oat husks. Wheat bran, rapeseed meal and sunflower meal also led to a decrease, albeit to an insufficient extent to realise an acceptable net energyintake. Furthermore, combinations of several fibre sources (e.g. chicory pulp with alfalfa and maize gluten feed) in the pregnant sow diet reduced the individual animal variation for net energyintake. In addition, it was demonstrated that 70-80% of the variation in the average spontaneous net energy-intake of ad libitum provided gestation diets can be predicted by means of the non-sterch carbohydrate (NSP)-content or the swelling capacity of the diets.

Digestibility trials revealed that not all fibre sources yielded more net energy for pregnant sows compared with growing pigs. However this was the case for alfalfa, maize gluten feed and wheat bran, but not for oat husks, sunflower meal, chicory pulp and sugar beet pulp. Dietary incorporation of 25% of these fibre sources had no significant effect on total manure production, when feeding restrictedly and iso-energetically at a similar water provision. The dry matter content of the manure, on the contrary was, for six out of seven fibre sources tested, significantly higher for the fibre rich diets in comparison with the control diet, which holds implications for emptying the manure pits. All tested fibre sources, with the exception of maize gluten feed, increased faecal nitrogen losses. This can be attributed to increased endogenous secretions, a lower protein digestibility or an increase in microbial activity in the hindgut, leading to a higher faecal excretion of nitrogen incorporated into microbial protein. Some of the fibre sources, i.e. sugar beet pulp, alfalfa and chicory pulp, showed a shift in nitrogen excretion from urine to faeces, which implies lower ammonia emissions.

In conclusion, combinations of chicory pulp, alfalfa and maize gluten feed offer perspectives for *ad libitum* feeding of pregnant sows. However, further research is necessary to predict the longterm (health, reproduction, welfare, environment) effects and suitability for the application of *ad libitum* feeding in practice.

3.1.2 Environmental enrichment for caged rabbits

Apart from the restricted space allowances, the barren environment is considered a main factor strongly limiting the welfare of caged animals. As for other meat-producing animals, regulations for rabbits can be expected in the near future, encouraging at the least environmental enrichment. In collaboration with a French company specialised in the production of lick blocks (Nutrilac) and Nutreco France, three different compositions of a rabbit gnawing block were examined. The conic blocks all had the same shape and were attached to the wall of the cages with a wire thread. Control blocks contained 10% of wood pulp whereas the experimental blocks contained 15% of inulins or 15% of chicory pulp. Both the females with offspring and the meat rabbits gnawed intensively on the different blocks, however a large difference was found between the three compositions. For the "soft" blocks with wood pulp, the consumption was on average three times higher than for the inulin blocks. However, a high variability was observed between cages, ranging between one and nine times higher consumption. Due to the high intake of material with negligible feeding value (wood) a tendency to decrease performance was observed with



these blocks. On the contrary, blocks with chicory pulp were more gradually consumed and the highest performances were obtained with these blocks both before and after weaning.

Behavioural observations were recorded on a limited number of females to verify if the presence of gnawing blocks led to a decrease in stereotypic behaviour. However, this kind of behaviour is limited in rabbits and moreover strongly animaldependent. As a consequence, no clear positive impact of cage enrichment using growing blocks on behaviour could be found.

3.1.3 Laying hens: furnished cages versus non-cage systems

The EU directive 1999/74 prohibits the housing of laving hens in battery cages from 2012 onwards. The battery cages may be replaced by either furnished cages (cages furnished with a nestbox, a litter area and a perch and provide a larger space allowance per bird as compared with battery cages) or noncage systems (aviaries or single level systems). In Belgium the translation of this directive into national legislation (KB 17/10/2005) was delayed until 2005 after a conviction by the European Court of Justice. The Belgian government contemplated for a long time whether or not the national legislation should be more stringent than the EU directive by banning furnished cages or by imposing additional requirements for these cage-systems. Partly due to the lack of relevant objective data, the EU directive was translated literally but with the provision of a possible revision following the completion of a scientific study.

This study is being carried out by CLO-DVL in collaboration with CLO-DVK and the Provincial Centre for Applied Poultry Research and consists of several workpackages. Workpackage 1 is an international observational study in which animal welfare and sanitary statuses are compared between farms with furnished cages versus farms with aviaries. Workpackage 2 concerns a survey among international poultry experts concerning animal welfare and health, sanitary status, labour conditions, and economic results in the different types of housing systems. Workpackage 3 is a comparative study of furnished cages versus aviaries on the experimental farm regarding (1) bacterial restcontamination after cleansing and desinfection, (2) the vaginal and caecal flora of laying hens, and (3) parameters of stress and immunity in laying hens. This study will be completed in the summer of 2006.



3.1.4 Group-housing of sows

Although the management may be more complex, keeping sows in groups and the associated greater freedom of movement are considered to be preferable for the welfare of the sows as compared with housing them individually. Recent EU legislation therefore stipulates that sows should be kept in groups during a period starting from four weeks after service to one week before the expected time of farrowing. As sow are individually confined on 90% of the farms in Flanders, this new regulation poses a huge challenge to our pig industry.

This project is a collaboration between CLO-DVL, CLO-DVK and the Universities of Ghent, Leuven and Antwerp and will help the pig industry in their transition to group-housing systems. The aims of this study are to quantify the importance of various animal, management or environment-related factors that influence the behaviour and welfare of group-housed sows and to evaluate the consequences of group-housing on sanitary and hygienic aspects. The first part of this research project concerns a cross-sectional observational study, on ca. 40 pig farms with a group-housing system, in order to identify factors associated with sow welfare, health and zootechnical performance of the sows as well as the concentrations of dust and ammonia, and the sanitary status on the farms. As the observational study allows finding associations but not causal relations, the second part of the study is tightly linked to a series of experimental trials. The aim of these trials is to reduce aggression among group-housed sows. This study will be completed in 2009.



3.1.5 Reduction and detection of boar taint in intact boars as an alternative solution to surgical castration

The surgical castration of male piglets is a controversial practice that is being hotly debated in Belgium as well as in many other European countries. Across Europe, male piglets are routinely castrated in order to prevent boar taint. This practice, however, has some serious drawbacks: castrating is a time-consuming and an unpleasant duty for many pig farmers, the zootechnical performance of boars is superior to that of barrows, and boars produce relatively smaller amounts of manure and fewer pollutants than barrows. Additional drawbacks include: the reduced welfare of the pigs by the stress and pain before, during and after surgery, the effect on the immune system, and the assault on the integrity of the animal. By signing a declaration of principle of the working group on piglet castration of the national animal welfare council, Belgium has committed itself by 2006 to allow surgical castration only when anaesthesia is used and by 2009 to forbid surgical castration even with the use of anaesthesia.

In this project, we will investigate the feasibility of an alternative production method that would allow a complete ban on surgical castration. The project is a collaboration between CLO-DVL and CLO-DVV and aims to solve the boar taint problem without castrating pigs and thus enabling entire males to be reared and marketed. The strategy to achieve this ambitious goal consists of three steps. In order to limit slaughter losses, we will attempt to reduce the occurrence of boar taint via management alterations related to a combination of diet, hygiene, breed and slaughter age (Step 1). As these alterations are unlikely to eliminate the occurrence of boar taint there is also a need for detection. Therefore, possibilities will be investigated to detect boar taint in young boars so that specific actions (immunocastration or early slaughter) can be taken for these "high risk" individuals (Step 2). It will be investigated whether the amount of boar taint at slaugther age can be predicted on the basis of the animals' physiological development, their socio-sexual behaviour, or other measures (skin lesions, cleanliness). In order to prevent boar-tainted meat being retailed, post-mortem detection is necessary too (Step 3). Although various detection systems are available, none can readily be implemented at the slaughter-line. The various detection systems (laboratory analyses of skatol and androstenone, electronic noses, sniffer pigs trained to detect boar taint) will be evaluated against the results from a taste-and-smell test conducted on a panel of Belgian consumers.



3.2 Sustainable animal husbandry: Combined culture of grass and clover in dairy cattle nutrition

During the past few years clover has regained attention in ruminant nutrition. By using clover, more specifically its ability to fixate nitrogen from the air in combination with *Rhizobium*, N-fertilisation can be reduced. This is very important in organic farming and in areas where N-fertilisation is strongly restricted (e.g. due to environmental legislation). Moreover, the combination grass/clover is promoted as a possible way to reduce the dominance of maize in our regions, thereby reducing soil erosion and improving soil fertility, the control of diseases and plagues and the diversity of the landscape. Financially, introducing grass/clover is also interesting, since from January 2004 a premium is given for each acre of maize that is turned into grass/clover.

White clover has a higher digestibility and contains more protein as compared to red clover and generally also as compared to grass. Unfortunately, white clover cannot be cultivated solely. Red clover, however, can be cultivated solely, but the ensiling process is very difficult and it has a relatively low energy and protein value compared to grass. On the other hand the ingestibility of red clover is higher than that of grass silage at a comparable crude fibre content. By combining red clover and grass, the advantages of both cultures can be combined (grass: easy ensiling and high feeding value; clover: intake, N-fixation capacity). Trials are currently being performed to determine which of both combined cultures is the most promising for dairy cattle from a feed technical point of view.

In a first trial, a combined culture of grass/white clover, with about 40% white clover was compared with a combined culture of grass/red clover with about 65% clover (on DM-basis). The results indicated that at a comparable energy and protein



provision, no specific influence on milk production or on milk composition (fat, protein) is to be expected. Digestibility trials confirmed the expected higher energy value of grass/white clover (5.53 MJ net energy/kg DM) as compared to grass/red clover (5.18 MJ net energy/kg DM). The protein value (digestible protein in the small intestine) of both silages was very similar: about 72 g/kg DM. The rumen degradable protein balance of grass/white clover silage was 77 g/kg DM, while for grass/red clover it was only 50 g/kg DM.

3.3 Efficient and functional animal nutrition

3.3.1 Evolution of muscle gain in slaughter pigs during fattening

Multiple phase feeding is gaining importance in slaughter pig husbandry for economic and environmental reasons. This implies that pigs are more accurately fed according to their requirements for nutrients and energy, which change with age. In this way the excretion of nutrients decreases. In fact, each weight category or phase is fed a different diet. To predict the requirements at each live weight a factorial model can be used, in which a range of characteristics of the pig type have to be known, such as live weight, sex, expected feed intake, dietary energy content, space per pig, ambient temperature and lean meat or muscle gain. It is evident that in a period of high potential protein gain the daily protein intake has to rise as well. Muscle gain is unknown for the slaughter pig Piétrain x hybrid, frequently raised in Belgium.

Therefore, a series of experiments were conducted in which body composition was determined for Belgian slaughter pigs at different weights until commercial slaughter weight. The diets were formulated in order to achieve optimal animal performances. In barrows daily muscle gain increases from 8 to 50 kg; between 50 and 70 kg there is a dip and then muscle gain rises again until 110 kg live weight is reached. The necessary daily protein intake will hence increase between 8 and 50 kg, decrease between 50 and 70 kg and rise again from 70 kg on. In gilts muscle gain continues to increase with live weight and the necessary protein intake will therefore have to rise accordingly. Until 50 kg barrows have a higher muscle gain, from 50 kg on and until slaughter weight (110 kg) the roles reverse and the gilts have a higher muscle gain. When considering the whole trajectory from piglet till slaughter pig, gilts gain 20-30 g more muscle per day than barrows. Moreover, barrows have at any moment a higher fat gain than gilts. Consequently, separate fattening of barrows and gilts can be considered as an interesting option.

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3.3.2The bio-efficacy of the 25(OH) vitamin $\rm D_{_3}$ metabolite in laying hens

The profitability in layer husbandry depends mainly on the zootechnical performance (egg production and feed efficiency) but also on egg quality such as the percentage of sellable eggs. The egg is a complex biological and chemical entity, the internal quality of which is related to the characteristics of both the albumen and yolk (e.g. the nutritional, health and safety properties) and the external quality related to the shell (for protection of the content against bacterial contamination). Consumer studies have indicated that the shell quality is the second most important parameter after yolk colour.

Both laying performance and egg quality depend on a lot of factors, such as genetics, age, stress and health, environmental factors and nutrition (a balanced supply of several nutrients). In this way vitamin D₃ has an important function because of its specific impact on the mineral metabolism (skeleton) and the formation of the eggshell. The absorption of vitamin D, depends mainly on the intestinal conditions (e.g. emulsification). Vitamin D₃ is converted into 25-OH-D₃, which in turn is converted in the kidneys into (1) 1,25-(OH),-D, at low levels of Ca and P in the blood (also dependent on parathyroid hormone) or (2) one or two other metabolites, namely 24,25-(OH),-D, or 1,24,25-(OH),-D, at normal levels of Ca and P in the blood. Moreover, it is clear that the efficacy of vitamin D, also depends on the dietary Ca and P levels: their respective concentrations and mutual ratios, phase feeding and particle size of the Ca-source. The diet can also be directly supplied with 25-OH-D₃, with a two-fold benefit of better absorption from the intestine and a by-pass of the liver metabolism



At the CLO-DVV a multifactorial trial was done to test the efficacy of 25-OH-D₃ in combination with three different dietary Ca:P-ratios. Ca and P had a significant effect on the egg production, eggshell quality and skeleton mineralisation. The introduction of 25-OH-D₃ in the diet resulted in a significant improvement in eggshell quality and a tendency for better zootechnical performance. There was no interaction between 25-OH-D₃ and both Ca and P for the main response parameters. In general, it can be concluded that 25-OH-D₃ has (1) a clearly favourable effect on eggshell quality and zootechnical performance, (2) a compensating effect for the differences in Ca and P levels in the diet and (3) a favourable impact on the economic profitability of layer husbandry.

3.3.3 Research for alternatives to antimicrobial feed additives

Until now, antimicrobial feed additives have been used intensively all over the world. They have been used therapeutically as well as because of their growth promoting effects. However, due to the fear that their intensive use in the feed of livestock leads to resistance against bacteria, pathogenic for humans, the European Commission has decided to ban the four remaining feed antibiotics starting from January 2006. From this moment on, antimicrobial feed additives can only be used therapeutically.

The total ban of these additives will surely have negative effects for animal production. Indeed, it will lead to more health problems, worse feed conversion ratios and lower uniformity in performance. In other words, there is a strong need for alternatives. There are a number of additives, including enzymes, (in)organic acids, probiotics, prebiotics, herbs and essential oils, immunostimulants and other management practices, which have been proposed as alternatives for antibiotics. However, research at the Department for Animal Nutrition and Husbandry with broilers and slaughter pigs shows that, at this time, no available alternative is as efficient and consistent as the antimicrobial feed additives. Moreover, there are still many questions concerning the mechanisms of action and what circumstances are required to maximise their effectiveness. More research is needed to establish the effect of the combination of pro- and prebiotics as 'synbiotics', as prebiotics can improve the colonisation in the gut with the probiotics used. Moreover, a combination of pro- and prebiotics with organic acids seems to be one of the next steps that should be considered.



3.4 Sustainable and ecological fisheries

Sea fisheries are economically important for the coastal communities of Flanders and Europe. Moreover, their importance from a socio-cultural point of view should not be underestimated. The Flemish government opts for the conservation of an independent and cost-effective Flemish sea fishery, using the Common Fisheries Policy (CFP) as a conditional framework. However, the CFP failed in providing sustainable management of the complex whole of quota species, fishing methods and vessel types - as clearly stated in the Green Book of the European Commission (EC). The catch capacity largely exceeds the catch potential. As a result many fish stocks are heavily pressured and many species are or will be included in a recovery plan. A partial reduction of the fishing fleets is an essential step towards sustainable fisheries, however other measures should also be taken. The so called *roadmap* of the EC clearly states that radical measures will follow, not only to preserve the fish stocks, but also to protect the environment and biodiversity. This ecosystem approach is determinative for European fisheries and in consequence also for the Flemish fishery.

Due to the fleet structure and the applied fishing method the consequences of this new trend in the CFP are considerable for the Flemish fishery sector. More than 90% of Flemish fishermen use a beam trawl, a fishing method which is (rightly) criticised because of its environmental impact, mainly on the natural marine resources (but also due to the CO_2 -emmission). A high discard and mortality rate of undersized commercial fish and non-commercial organisms, the direct disturbance (mortality) of the benthos, the physical change of the marine ecosystem and a drastic alteration in seabird populations are important effects of trawling, and beam trawling in particular.

As such, the Flemish fishery scores badly according to the new CFP approach, and the problems get even worse taking into account the unfavourable business-economical situation linked to the fishery method. The fishing gear technology section of Sea Fisheries Department investigates both the economic and ecological problems and tries to define structural solutions. On the study day of 17 March 2005 "In the eye of the storm: the Flemish fishery entering the 21th century", a vision on the future of the Flemish fishery sector was formulated. This vision is primarily based on many years of experience in technical adaptations to reduce the environmental impact of the beam



The vast experience of the fishing gear technology section demonstrates that in the short term technical adaptations to the beam trawl could and should form the onset to make the beam trawl more species and length selective. Within the framework of the projects NECESSITY and RECOVERY, adaptations to the beam trawl were sought to reduce the by-catch of cod. The Flemish project Innovation Centre Sustainable and Ecological Fisheries (IDEV) explored the possibilities to reduce the by-catch of benthos and undersized fish species. During 2005 several campaigns at sea were scheduled to test these technical adaptations on board of commercial vessels and the oceanographic research ship BELGICA. The results are positive and can help Flemish fishermen to react quickly on the CFP. However, these adaptations are insufficient to convert beam trawling into an environment-friendly and profitable fishery method. On the medium to long term scale, a partial switch to different sustainable fishery methods is necessary. The Flemish fishery sector is highly vulnerable due to its specialisation towards single target-species and the applied fishery method. A switch to less energy-consuming and more environmental-friendly fisheries could be a solution for Flemish fishermen. Alternative trawl net fisheries should be considered in the medium term, taking into account restrictions for vessel characteristics, target species and fishing grounds. In the long term, the introduction of passive fishery methods is a possibility. Due to their typical capture mechanism, these fisheries consume less fuel and it is anticipated better that they offer protection of the environment.

At present several economical analyses are performed to examine to which extent the business-economical problems can be solved by diversification and the use of other fishery methods. In parallel and closely linked to this economic study, an ecological and technical study is being carried out. The combination of these three studies forms the crucial onset to transform the Flemish fishery into an innovative, durable activity.

3.5 Research on molecular markers for the authentication of fish, crustaceans and molluscs

The government and the public, as well as the industry, need reliable analytical methods for the authentication of fisheries and aquaculture products. These methods help to correctly identify species and origin, which make it possible to detect fraudulent substitution of expensive species by cheaper ones and can assist the industry in the application of an eco-label. They also assist the government in upholding fish quota and in determining taxes on imported fisheries and aquaculture products.



The research at the Sea Fisheries Department focuses on the development of molecular markers for the authentication of fish, crustaceans and molluscs. The choice for using molecular markers is based on the fact that DNA is a more stable molecule compared to proteins, which are more prone to denaturation. Thus, it is possible to authenticate not only fresh or frozen products that have lost their morphological properties, but also processed foods.

Currently, two markers, a fragment of the mtDNA cytochrome b gene (CYTB) and a fragment of the mtDNA cytochrome oxidase I gene (COI) are being used to identify a number of commercial flatfish and roundfish. Genetic polymorphisms within the markers are detected through sequencing, Restriction Fragment Length Polymorphism (RFLP), Single Strand Conformational Polymorphisms (SSCP) and Denaturating Gradient Gel Electrophoresis (DGGE). The obtained DNA patterns are added to the existing database with protein profiles based on iso-electric focusing (IEF). Optimisation of the PCR reaction conditions used for the amplification of the CYTB and COI markers in fish has allowed the use of the same primer pairs for the identification of crustaceans. For molluscs, new primer pairs for CYTB and COI have been successfully developed and tested on 12 Chilean bivalve species.

In addition to authenticity determination, molecular markers are also used for genotyping. In this way, eight microsatellite markers were developed for the determination of the genetic diversity of turbot fry, reared for restocking purposes. They are being studied further with the aid of PCR-seq and PCR-SSCP. Serious efforts are also being made in the field of the genetic characterisation of *Brachionus* sp. and *Artemia* sp., which are used in large quantities in the aquaculture industry as live feed. The research on the biodiversity within these species is based on PCR-RFLP, PCR-DGGE and PCR-seq of the amplified HSP26 (heat shock protein 26), ITS (nuclear DNA rDNA, internally transcribed spacer) and mitochondrial 12S-16S rDNA markers.

3.6 Post-extraction study of the macrobenthos on the Kwintebank

Sand extraction is a very intensive industrial activity on the Kwintebank, due to the relatively short distance to the coast and the suitability of the sand for various construction purposes. After 30 years of exploitation, a depression has been created in the 'central part' of the Kwintebank. The ministry of Economic affairs decided to cease extraction in this part of the exploitation zone for a period of three years (from February 2003 onwards) to allow geomorphological recovery of the area.

The project SPEEK (Study of Post-Extraction Ecological effects in the Kwintebank sand dredging area) is a co-operation between three Belgian laboratories (UGent/Marine Biology; UGent/ Renard Centre for Marine Geology and CLO/Sea Fisheries

Department) and one Spanish institute (AZTI, Department Oceanography and Marine Environment). This co-operation aims at joining the expertise present in these groups to gain insight into the possible restoration of the benthic life in the central part of the Kwintebank. Changes in the geomorphology of the sandbank can have repercussions on the composition of the benthic community. The findings of this ecological project will contribute to a sustainable management of the sand extraction areas on the



The "Central Depression" on the Kwintebank

Belgian Continental Shelf. SPEEK focuses on several benthic compartments: the meiobenthos (comprising all metazoans between 38μ m and 1mm) are studied by the UGent/Marine Biology (Nematoda) and AZTI (Harpacticoida) and the macrobenthos (all organisms > 1mm living in the bottom, e.g. Polychaeta, Crustacea and Mollusca) are studied by the Sea Fisheries Department. All biological data are supported by geological data, collected by UGent/RCMG.



Macrobenthos density

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Macrobenthos - Fltr Scoloplos armiger, Urothoe brevicornis, interstitial bristle worm Hesionura elongata

In order to study the recovery of macrobenthic life in the central area of the Kwintebank, six locations (ZG05 - ZG10) were sampled seven times between 2003 and 2005 with a Van Veen grab from the RV Belgica. Density and diversity of the macrobenthos were lower in spring 2003, one month after cessation of the dredging activities, compared to the subsequent periods. Density was higher in the most central part of the depression (ZG06 and ZG09), mainly because the amphipod Urothoe brevicornis and the carnivore bristle worm Nephtys cirrosa were already present in high numbers shortly after cessation of the dredging activities. However, density and diversity indices were lower compared to other areas with a similar environment. Density and diversity did increase from 2003 to 2004, but except for ZG09 lower values were recorded again in spring 2005. From autumn 2003 onwards, species such as the amphipod Urothoe brevicornis and the polychaetes Hesionura elongata, Polygordius appendiculatus, Spiophanes bombyx, Scoloplos armiger and Nephtys cirrosa (both juvenile and adult species) were (again) the most important species in the central depression area of the Kwintebank.

The interpretation of the data is hampered by the lack of a base line study (data of the pristine area before any extraction activities took place, as well as data from a comparable reference area). The positive evolution in density and diversity values may be a consequence of the cessation of sand extraction but can also be a result of natural variability. As such, the question of whether the current macrobenthos community composition is comparable to the original community or has it evolved to a new stable community different from the original, cannot be answered. However, it can be concluded that the poor macrobenthic community that was found after cessation of the sand extraction in the central depression of the Kwintebank, has evolved quite rapidly (in one year) to a community typically found on other sandbanks of the Belgian Continental Shelf.



4. TECHNOLOGY AND FOOD UNIT

4.1 Food safety

4.1.1 Residues of coccidiostats in eggs

In the research project concerning coccidiostats, the presence of residues of nine coccidiostats in eggs were monitored. 320 different egg samples were gathered in eight European countries. On the basis of the code indicated on the eggs, the origin of the eggs could be traced. The first figure of the code indicates the manner of the housing of the laying hens (code 0: organic eggs; code 1: free range eggs; code 2: barn eggs; code 3: eggs from caged hens). The two letters refer to the country of origin (e.g. BE: Belgium), while the last figures refer to the registration code of the producer. Figure 1 and 2 show respectively the number of analysed samples according to the country of purchase and the country of origin. This shows that a lot of eggs sold in Belgium are not from Belgian origin.

Of the samples analysed, 35.6% contained one or more coccidiostat(s) with concentrations ranging from 0.1 to 63 μ g/kg. Almost 90% of all positive samples contained a concentration less than 2 μ g/kg. Moreover, 77% contained even less than 1 μ g/kg. At this moment the Belgian action limit, proposed by the scientific committee of the Federal Food Agency (FAVV), is 10 μ g/kg.

By also analysing eggs from other Euopean countries, it was possible to evaluate the Belgian situation in relation to those other countries. Significant differences were found between eggs originating from Belgium, The Netherlands and France. In eggs from Belgium and the Netherlands, low concentrations of salinomycin were mainly found, while in the French eggs high concentrations of lasalocid were mainly encountered. Lasalocid and salinomycin were clearly the most frequently found compounds (figure 3). On the other hand, both compounds were rarely found in barn eggs. The analyses of eggs from private breeders revealed in some cases rather high concentrations of coccidiostats. When possible an inquiry was done concerning the use of feed, housing etc. The main cause of the presence of the residues seemed to be ignorance of the private breeder, e.g. the use of wrong feed intended for broilers or other animal species.



Figure 1 : Distribution of the samples according to the country of purchase



Figure 2 : Distribution of the samples according to the country of origin



Figure 3 : Distribution of the positive samples according to the compounds found

4.1.2 Validation of the Copan Milk Test for the detection of antimicrobials in milk

The Copan Milk Test (Copan Italia S.p.A., Brescia, Italy) in microplate format was validated by means of natural as well as artificial contaminated raw milk samples. This test is a microbiological inhibitor test for the detection of antimicrobials in milk. All β -lactam antibiotics mentioned in Council Regulation (EEC) No 2377/90 (15 compounds), were detected with a test sensitivity below the respective minimum nsk level (MRL), excepted for cefquinome and cefoperazone.



Additionally, 20 non- β -lactam antibiotics and chemotherapeutics were tested. Except for sulfadiazine these compounds could be detected, below MRL level.

Dairy cows are mainly treated with penicillins and cefalosporins. As a consequence mainly residues of β -lactam antibiotics are found in milk by monitoring programs and in the framework of the official determination of the quality of raw milk. So, the Copan Milk Test is meeting the expectations as screening test with respect to test spectrum and test sensitivity.

The test is rather robust and not often disturbed by natural animal-related inhibiting substances. The incubation period is fixed at three hours. The differences in test sensitivity between different batches are limited and the test remains stable during a minimum of one year.

An automatic colour reading is possible by means of a flatbed scanner. With the special C-Scan program the colour of the agar in the cups is measured and expressed as a Colour Impact Factor (CIF) with values ranging from 0.1 (yellow) to 10.0 (purple). In a comparative study with more than 6000 milk samples the best correspondence with the visual reading was obtained for a cut-off CIF value \geq 4.5. The repeatability of the colour reading of two independent calibrated scanners was good.

Test sensitivity of the Copan Milk Scan with C-Scan reading (cut-off CIF = 4.5) expressed relative to the respective MRL (mentioned in between brackets).

The Copan Milk Test was integrated as a screening test in the monitoring program of Belgian milk. No false positive and no false negative results were obtained. Also the results from participation in an international ring trial were very favourable. A file was submitted to the scientific committee of the FAVV for recognition of the test as an inhibitor test in the framework of the official determination of the quality of raw milk by the Interprofessional Organisms. Since October 1st 2005 the Copan Milk Test is in use by the Interprofessional Organisms as the official inhibitor test.



Non-betaeta-lactam compounds



Inner circle = 10 MRL, circle 2 = 2 MRL circle 3 = 0,5 MRL, outer circle = 0,25 MRL

4.1.3 Screening of residues of sulfonamides in honey and migration from beeswax

Quality control of Flemish honey samples revealed contamination with sulfonamides. The high concentrations were caused by an illegal addition of sulfonamides to the winter feed of bees for the prevention of nosemosis, a bee disease caused by the intestinal parasite *Nosema apis*. In some honey samples, however, only weak contamination from an unknown origin was determined. Since beeswax used for the production of wax foundations can be contaminated with sulfonamides, *in vivo* tests were set up to study the migration of residues from contaminated beeswax to the honey stored in the honeycombs. However, the migration noticed so far is too small to consider contaminated beeswax as the direct origin of the contamination.

For this study an extraction method was optimised for the determination of sulfonamides in honey and beeswax by LC-MS/MS. The method for honey was validated according to Decision 2005/657 on residue analysis; it has a detection limit of 2 μ g/kg. This sensitivity is more then sufficient for decision making in the framework of the action limit for sulfonamides in honey of 20 μ g/kg as set by the Scientific Committee of the FAVV. The results of this validation will be used for the accreditation of the analysis method.



In vitro inhibition of Salmonella

4.1.4 Control of *Salmonella* Enteritidis in laying hens by use of lactobacilli as probiotics

In Belgium, the majority of the salmonellosis cases is caused by *Salmonella* Enteritidis, with contaminated eggs being the major infection source. A possible route for egg contamination is an ascending infection from the cloaca to the vagina and lower regions of the oviduct. To control the contamination of laying hens with *Salmonella*, specific actions are necessary. The presence of lactobacilli in the vagina and cloaca is important to maintain a microbial ecosystem that prevents the growth and invasion of pathogens such as *Salmonella* spp. The use of lactobacilli as probiotics for laying hens seems an interesting option to reduce *Salmonella* Enteritidis infection. The aim of the current study was to isolate a large number of lactobacilli and to select from this collection strains for potential use as a probiotic to prevent *Salmonella* Enteritidis infection in laying hens.

About 200 lactobacilli strains were isolated from the vagina and the cloaca of 35 laying hens. These were typed and identified at the species level by a polyphasic approach. Ten different species were isolated, all belonging to the *Lactobacillus acidophilus*, *Lactobacillus reuteri* or *Lactobacillus salivarius* phylogenetic groups. A rational selection of about 50 strains was analysed *in vitro* for functional properties related to the use as probiotics. Strains were tested for acid and bile tolerance, both important properties for survival and colonisation within the gastrointestinal tract. Furthermore, strains were tested for inhibitory activity against 20 different *Salmonella* strains under anaerobic conditions. A large variation in inhibition was observed within the different *Lactobacillus* strains. Analysis of the supernatant of the growing strains indicates that organic acids such as lactic and acetic acid are involved in the observed inhibition.

Based on the results of the in vitro assays, a selection was made of Lactobacillus strains with properties that indicate a high survival capacity and growth within the gastro-intestinal tract as well as a strong Salmonella inhibition. These strains were tested in vivo in experimentally infected one-day-old chicks. One day before Salmonella infection, chicks were orally treated with lactobacilli. Colonisation of the lactobacilli in the gastro-intestinal tract was studied by analysis of cloacal swabs and caeca by selective plating and analysis of individual colonies by rep-PCR. All lactobacilli-treated groups showed clear colonisation of the gastrointestinal tract. After six days, caeca and internal organs (liver and spleen) were analysed for Salmonella infection. Two of the tested groups showed a ten-fold reduction of Salmonella counts in caeca as well as in liver and spleen. Colonisation of the gastro-intestinal tract of laying hens after a single oral dose of these strains seemed less efficient, probably due to the endogenous microbiota.



4.1.5 Development of detection methods for non-O157 pathogenic *E. coli* in food: first step in prevention (in cooperation with Prof. Dr. L. De Zutter, UGent)

The verocytotoxin producing E. coli strains (VTEC) are forming an important group of human pathogenic zoonotic E. coli. Human pathogenic strains are characterised by the presence of verocytotoxins mostly together with additional virulence factors as the outer membrane protein intimin. VTEC are forming the fourth major group of foodborne pathogens in Belgium in respect to amount of cases. VTEC cause a spectrum of clinical symptoms ranging from asymptomatic carriage to diarrhoea, bloody diarrhoea and the life-threatening haemolytic uraemic syndrome (HUS), which affects especially children and the eldery. In Belgium the most frequent isolated serotype is O157: H7 (VTEC O157), accounting for 20% of all VTEC cases diagnosed in the hospitals. The most important non-O157 serotypes are O26, O103, O111 and O145. Opposite to sorbitol negative O157, no standard detection methods are available for these serotypes.

The development of a microbiological detection method is based on extensive knowledge of the bacterial species. To achieve this, a collection of strains was compiled by isolation from clinical samples. This collection of strains was both genotypically (RAPD, virulence factors) and phenotypically (carbohydrate metabolism, antibiotic resistance, acid tolerance) characterised to map the differences between the serotypes in order to develop serotype-specific isolation methods. Four protocols for selective isolation of VTEC were developed. These protocols are composed of several optimised stages namely a pre-enrichment stage to allow resuscitation of stressed or sublethally injured cells, a selective enrichment stage to obtain a selective inhibition of background bacteria followed by spreading of the enriched culture on a selective agar medium. A novel selective isolation medium was developed not only to inhibit growth of background bacteria but also to allow discrimination of different serotypes. This discrimination is based on the presence of ß-galactosidase present in all coliforms, and variations in carbohydrate metabolism between pathogenic and commensal E. coli strains.

During a first and extended evaluation phase several types of samples were artificially contaminated: raw milk, raw milk cheeses, cheese made from pasteurised milk, minced meat, dry sausage and cattle facees. Two isolation protocols were retained the end of this evaluation phase. These two protocols will be validated by means of detection of VTEC in naturally contaminated samples.





4.2 Functional food

4.2.1 Optimisation of chocolate preparation

Via IWT-R&D-projects CLO-DVK collaborated for more than eight years with Barry-Callebaut NV for the optimisation of the production process of milk chocolate and white chocolate. Dark chocolate is prepared from cocoa mass (roasted and grinded cocoa nibs), cocoa butter and sugar to which lecithin and sometimes flavours are added. In the case of milk chocolate and white chocolate, milk powders are also added; white chocolate, however, contains no (dark colouring) solid cocoa constituents. Special attention goes to the so-called conching process. During this process the batter is processed intensively for hours at temperatures varying from 50°C to 95°C, while several biochemical, chemical and physical processes, which are of considerable importance for the quality and properties of the final product take place. The quality and characteristics of the milk powders used are important for the properties of milk chocolate and white chocolate.

In a first phase, several parameters for the evaluation of the raw materials and the conching process conditions were identified. In addition, the conching process was also studied by confocal and electron microscopy. Knowledge of these parameters and of alterations occurring during this process could lead to a better predictability of the taste and rheological properties of the end product. This delivers numerous advantages. For instance, this enables the yield of the cocoa butter to be increased; this means that the quantity of cocoa butter, which is the most expensive ingredient and which has to be added to obtain the desired viscosity and yield value of the end product, could be reduced. Also the desires of the customer can be met more specifically and the duration of the conching process can be better evaluated. Further this will allow a more efficient use of the available conches by an optimised usage of raw materials, energy, production machinery and operators. Finally, in the future this knowledge should contribute to the development of new conching systems and innovative conching processes.

4.2.2 Improvement of the quality of milk drinks

The Flemish dairy company INZA CVBA is part of the Milcobel group. The company is specialised in milk, milk products and functional drinks with long shelf life. The philosophy of the company is not only to stay competitive by producing high quality products, but it also invests a lot into the development of innovative products that follow new market trends. In this context there has been for many years cooperation between INZA and CLO-DVK through R&D-projects (partly financed by IWT). The objective of these projects is the development of methods to improve the quality of dairy products through optimisation of the process parameters or product composition, and the development of new innovative healthy functional dairy products. This second research item is based on the worldwide scientific investigation on, and isolation of, nutritional and health promoting food ingredients. Nowadays the consumers are more informed about and aware of the influence of food on health. Because of this, there is an increasing demand for healthy food and food with health promoting properties, called functional foods. The company wants to anticipate on this trend by the development of functional dairy products in cooperation with CLO-DVK.



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4.3 Environmental Protection

4.3.1 A technique for measuring ammonia emissions in naturally ventilated buildings

In a European context the ammonia emissions in Flanders have to be reduced by 40% by 2010. Since October 2003 the law prescribes that new pig and poultry houses must be built according to low ammonia emission building techniques. These techniques are described in a 'List of Building Techniques for the Reduction of Ammonia Emissions' and classified according to the different pig and poultry categories. However, at the moment only livestock buildings with a mechanical ventilation system are allowed on the list. Natural ventilation systems are not accepted because there is no applicable method for accurate, reliable and online ammonia emission measurements in naturally ventilated buildings. Therefore, the purpose of this study is to develop an affordable method for the determination of ammonia emissions from a naturally ventilated building. This research is in cooperation with K.U.Leuven who developed a measurement tool for ventilation rate in naturally ventilated buildings. CLO-DVL carried out the ammonia concentration measurements.

Ammonia concentration measurements were performed in the experimental cubicle house of Agrivet (University Ghent) during the winter period 2004-2005. Ammonia concentrations were measured with a photo-acoustic multigas monitor (Innova 1314) equipped with an eight channel multisampler. The ammonia concentration was measured every 19 minutes at each sample point. During the first experiments the eight sample points were spread over the dairy cow house. The results showed that the ammonia concentration varied markedly nearby the spaceboarding. Therefore, the ammonia concentration gradient in the vicinity of the space-boarding was measured in more detail (Figure 1).

Figure 2 shows the average concentration per wind direction in relation to the distance to the space-boarding. These measurements were performed on the southwestern side of the dairy cow house. For most of the wind directions there is a clear ammonia concentration gradient from the space-boarding up to 130 cm inside the building. Further inside (130 - 290 cm) the dairy cow house the ammonia concentration is more or less the same. The concentration profile of the southern wind direction is an exception. The higher wind speeds noticed during this measuring period for this wind direction can explain this.

In addition to the wind direction and wind speed, the ammonia concentration can be influenced by the defecating behaviour of the cows. Therefore, observations were done in the same cubicle house to determine the defecating and urinating behaviour of the cows. The cubicle house was divided in several imaginary zones and the number of times that cows defecated or urinated was registered. The results show that there are zones where the cows defecate or urinate more often and also that there is a variation in time. The following factors appear important: the positioning of the milking robot, feeding automat, feeding fence, water trough, and influence the travelling path of the cows and thus also their behaviour. The observations also demonstrated a large difference in defecating and urinating behaviour between the cows (Figure 3).

At the moment experiments are also being performed with a test installation where three little slurry pits are simulated (Figure 4). Above each slurry pit a wind tunnel is constructed in a way that the wind speed can be monitored and registered. With this test installation the relation between the ammonia emission and the following factors will be investigated:

- Wind speed over the slatted floors
- Height of the slurry in the pit
- Manure composition
- Type of slatted floors

The results of these measurements, combined with the experimental measurements, should give a better insight into the ammonia emission process in a naturally ventilated building.

4.3.2 Protecting the environment against spray drift – the importance of drift-reducing techniques

Over the last decade, drift of pesticides caused by spraying has been recognised as a major problem for the environment and there is a growing consciousness of the reduction of the emission of plant protection products to soil, (surface) water and air, and also the introduction of buffer zones. Recently, spray drift and its effects have also become an important aspect of risk assessment in the registration process of pesticides in Belgium. That is why there is still a need for drift research to enlarge the international drift database and to quantify the effect of spray application technique on the amount of spray drift. Spray drift is defined as the quantity of applied product (representing the active pesticide ingredient), which is

(representing the active pesticide ingredient), which is deflected out of the treated area by the action of air currents during the application process. Drift can cause crop protection chemicals to be deposited in undesirable areas with serious consequences, such as: (1) Damage to sensitive adjoining crops and other susceptible off-target areas, (2) Environmental contamination such as water contamination and illegal pesticide residues, (3) Health risks to animals and people, (4) A lower dose than intended on the target field which can reduce the effectiveness of the pesticide, wasting pesticide and money.





Figure 1. Eight sample points nearby the space-boarding



Figure 2. Average ammonia concentration per wind direction in relation to the distance to the space-boarding



Figure 3. Number of times that a cow defecated or urinated during an observation period of 24 hours



Figure 4. Test installation for ammonia emission measurements

Field drift measurements are carried out (according to ISO 22866) to measure and compare the amount of drift for different climatological conditions and spray application techniques under field conditions. Sedimenting and airborne spray drift are determined using a fluorescent tracer by sampling in a defined downwind area at different positions in a flat meadow with filter papers and pipecleaners. Meteorological conditions (wind speed and direction, temperature, relative humidity,...) are monitored during each experiment.

A reference spraying is used since comparative measurements are to be made under different weather conditions. Based on the Belgian and international agricultural practice, the reference spraying is defined as a standard horizontal spray boom with ISO 110 03 standard flat fan nozzles at 3 bar (1.2 l.min⁻¹), a spray boom height of 0.50 m above the meadow, a nozzle distance of 0.50 m and a driving speed of 8 km.h⁻¹ resulting in an application rate of 180 l.ha⁻¹. These drift prediction parameters are initially used to quantify the important effect of weather conditions (temperature, relative humidity and wind speed) on the amount of spray drift. A lower wind speed or a higher relative humidity decreases the amount of spray drift. Taking into account the correlation between temperature and relative humidity, a lower temperature will also result in lower drift values due to the cumulative effect of relative humidity.

These parameters are also used to perform spray drift risk assessments and to compare measurements using other spraying techniques under different weather conditions to the reference spraying. Drift measurements are performed for several combinations of nozzle type (flat fan, low-drift, air injection) and size (ISO 02, 03, 04 and 06), spray pressure (2, 3 and 4 bar), driving speed (4, 6, 8 and 10 km.h⁻¹), spray boom height (0.3,



0.5 and 0.75 m) and air support. Larger nozzle sizes, lower spray pressures and driving speeds, lower spray boom heights and using air support generally reduce spray drift. In regard to nozzle types, air injection nozzles have the highest drift reduction potential followed by the low-drift nozzles and the standard flat fan nozzles.

Characteristics of agricultural sprays belong to the most critical factors affecting spray drift. Furthermore, droplet size may influence the structure of the spray deposit and the biological efficacy of the pesticide applied. Thus a measuring set-up for the characterisation of spray nozzles using a Phase Doppler Particle Analyser (PDPA) was developed. This set-up is able to measure droplet sizes and velocities based on light-scattering principles. It is composed of different parts i.e. a climate room, a spray unit, a three-dimensional automated positioning system and an Aerometrics PDPA 1D system. Different nozzle pressure combinations have already been tested. From the results, the importance of the nozzle type and size on the droplet size and velocity spectra is clear. The results are linked with the drift potential of different nozzle pressure combinations (based on field measurements) and are used as the input for a Computational Fluid Dynamics (CFD) spray drift model for field spraying applications. This model will be able to predict drift for a wide range of variables and to enable the results to be presented in a visual way for ease of interpretation. The results of the drift field measurements are used to validate this model.

In future research, the crop effect and the effect of spray liquid properties on spray drift will be investigated. Finally, a protocol that helps the farmer or the pesticide user in general to apply pesticides in the best ecological and economical way will be developed, together with a scenario for the evaluation of new products and techniques.



Experimental set-up for the field drift measurements





PDPA laser measuring set-up



Drift curves for different nozzle types and sizes (at 3 bar) at standard weather conditions (relative humidity = 70 %, wind speed = 3 m.s^{-1} , temperature = 15 °C) and the corresponding droplet size spectra



Droplet size interval (?m)

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5. EXTRA HIGHLIGHTED

5.1 Belgian Blue Mussels

On the 26th of October, the first cultivated blue mussels *Mytilus edulis* from Belgian waters were proudly presented to the press. "Finally!" sighed several Belgians, while our northern neighbours were grinding their teeth, as the first commercial Belgian mussels became reality. This event, however, was only a small link in a long history. Let us go back in time...

In the mid '90s mariculture in Belgium was non-existing, although our country had a rich, but long foregone tradition in shellfish culture. At the end of the 18th century shellfish culture in Flanders was booming. Juvenile oysters were imported from Great Britain and cultured until they reached a marketable size. This activity reached a climax during the "Belle Epoque" period (1865–1914), with annual productions exceeding 35 million oysters and famous names such as "Ostendaises" and "Royales d'Ostende".

These oysters were mainly exported to France, Germany, Austria, the Balkans and Russia. However, during WW-I the oyster beds were damaged and an outbreak of the *Bonamia* disease in Great Britain resulted in a shortage in oyster seed. Consequently, the oyster culture in Belgium scarely recovered. In 1933, some experiments were carried out in the "Spuikom" in Oostende and the "Vlotkom" in Nieuwpoort. Until WW-II ten oyster farmers were active in Flanders, but only Halewyck & Co resumed after the war. However, this last company had to cease their culture activities in 1974 due to the bad quality of Belgian coastal waters and the development of food competitors, such as barnacles and slipper limpet (*Crepidula* sp.) in the "Spuikom". As the water quality improved during the '90s, an EU-funded project "Oyster culture in the Spuikom" was started in 1997, which led to the commercial culture of the European flat oyster (*Ostrea edulis*) and the Pacific oyster (*Crassostrea gigas*) in Belgium, and also, experiments with the culture of blue mussels along the Belgian coast.

However, during inspection dives at the end of the '90s, José Reynaert observed large quantities of mussels growing within a period of two years on offshore buoys and poles, allthough these constructions were treated with anti-fouling products to prevent the growing of shellfish. As the water quality in open sea exceeds that of the coastal zone, the idea formed to investigate the potential of an offshore mussel rope culture. In 1998, a project was started under the "5B-Objectives", where the Sea Fisheries Department scientifically monitored the seeding, growth rate and quality of the cultured mussels. As rope culture for mussel farming is normally used in protected areas, such as bays and fjords, a new concept had to be developed that could withstand the typical rough conditions of the North Sea. The installation was placed in a zone north of the sandbank "Buiten Ratel" and was beaconed by buoys. Unfortunately, during the experiment several fishing vessels trespassed the area and destroyed large parts of the rope culture. Still, the experiment showed that seeding of the ropes was successful, with a good growth rate and an excellent quality.







The experiment was extended under the EU-funded PESCA flag taken into account some important changes. In co-operation with "Loodswezen" and "Dienst voor Zeevisserij" the shellfish culture area was replaced towards the area north of the "Noordpas", in the vicinity of buoy D1 (Smal Bank). Also, the concept was changed: the culture ropes were protected by a metal frame that was kept afloat. Again, large parts of the so-called cage culture were damaged due to the rough weather conditions. Cages were pulled off and drifted or sunk. Research was therefore compelled to strengthen the cage constructions.

Despite this, the experiment proved that the biological and chemical conditions at the new location were equally favourable for the growth and quality of the mussels. More than 20 kg of mussels per running meter of culture rope could be obtained in one culture season (from April to July of the following year). Although the project ended in 2001, the cage culture was further improved with private funding and scientifically monitored by the Sea Fisheries Department. The constructions got larger and the harvest method was revised. However, the mussels could not be commercialised

due to the lack of a legal framework for the assignment of shellfish culture areas, licensing and quality control. With some instigation, the Ministry of the North Sea accelerated the open sea mussel culture, and in September 2005 a small company applied for an environmental license for shellfish production at four areas on the Belgian continental shelf. José Reynaert then applied for a concession for his rope culture of mussels from this company. Meanwhile, the Federal Agency for the Safety of the Foodweb assigned an A-label for shellfish production to the "D1 zone" area, which was necessary to bring the first cultivated Belgian mussels on the market in October 2005. Although the quantity was small, the event reached the news. Compared to the mussels from our neighboring countries (France, Netherlands), the Belgian mussels combined the two best qualities of the others: the intense taste of the mussels from the northern part of France and the size (meat content) of the "Zeeuwse" mussels.



In spring 2006, José Reynaert will place a large number of cages in the "D1 zone", which will bring the next load of Belgian mussels on the market in summer 2007. The total production will increase as more partners apply for concessions in offshore mussel farming. Also, the future windmill farms on the Thornton Bank create interesting perspectives for offshore cage culture. Within a new project in collaboration with "Stichting Duurzame Visserij Ontwikkeling (SDVO)" and Ghent University, several physical, chemical and biological parameters will be investigated in order to examine the overall quality conditions of these areas. Offshore shellfish culture opens up new ways towards diversification in the Flemish fishery and aquaculture sector, and delivers a typical regional product. This could mean a break through in the monopoly of the "Zeeuwse" mussel in Belgium. As such, mariculture can give a new élan to the Flemish coast, with profit for the distribution and fish processing sector, the seafood restaurants and the complete tourist sector.

5.2 Focus on the Phytofar Prize won by the Spray Technology Group CLO-DVL

Biennially, Phytofar, the Belgian interest group of manufacturers and/or importers of plant protection products, awards a scientific and a professional prize to people, enterprises or (research) institutes for their merits in various domains concerning the use of plant protection products. The professional prize for 2005 was awarded to the temporary consortium Center for Walloon Agronomic Research (CRA-W) Department of Agricultural Engineering & CLO Department of Mechanisation, Labour, Buildings, Animal Welfare and Environmental Protection' for the subject: "Knowledge and advice centres of spraying technology contribute towards a justified and sustainable use of plant protection products in Belgium".

The use of plant protection products in Western Europe and hence also in Belgium have increasingly been hampered in the past decade by extra regulations. These regulators are largely a result of the growing fear amongst governmental regulators and consumers about the risks of environmental pollution and food contamination. Not only have the conditions for the registration of pesticides become tougher, but also the conditions imposed on the application of plant protection products have continuously increased. Following up and appropriately reacting on the numerous conditions and regulations associated with a 'justified and sustainable use of pesticides' is a task whereby all those concerned i.e. the manufacturers and dealers of pesticides and spray machinery, the regulatory governmental bodies, the advisory services and especially the Belgian farmers and growers, can use all help available. The Center for Walloon Agronomic Research (CRA-W) – Department Agricultural Engineering and the Agricultural Research Centre (CLO) – Department Mechanisation, Labour, Buildings, Animal Welfare and Environmental Protection' play an increasingly important role as a 'Knowledge and Advisory Centre'. The former is the contact for Wallonia whereas the latter functions as the contact for Flanders. A close cooperation between both centres ensures a complimentary approach.

The activities developed by the 'Knowledge and Advisory Centre' can be summarised in three domains: research, policy-supporting and information. As research institutes, research on the use and optimisation of spray techniques is of course the core activity for both knowledge centres. For CLO-DVL this included, amongst others, the following issues in the past years:

- The importance of drift and associated techniques for the environment in Flanders;
- Evaluation of the mechanical stability of field sprayers;
- Optimisation of spraying techniques for biological plant protection products;
- Effect of spray technique on chemical haulm-killers in potatoes;
- Optimisation of application techniques for crop protection products in half-standard orchards;
- Evaluation and optimisation of sprayers with vertical spray booms;
- Study of the efficiency of lance sprayers in glasshouses.

There is a strong interaction between the policy-supporting activities on the one hand and the popularising advisory activities of CLO-DVL on the other hand. For the policy-supporting work there are contacts with both national and international government agencies (Flemish, Belgian, European governments) and interest groups (Agoria, ISO/CEN-standardisation, Phytofar, ECPA). The main contribution of CLO-DVL is the organisation of the three-yearly compulsory inspections of sprayers in Flanders (photographs).

For Flemish farmers and growers a final but nevertheless very important activity of the Knowledge and Advisory Centre for Spraying Techniques is the translation of research results and laws and regulations for daily use. CLO-DVL therefore possesses a Technological Advisory Service 'Agromech' what gives advice as one of its core tasks. Besides this, CLO-DVL biennially organises a course for 'Authorised Technician for Spraying Machines' (photograph) in cooperation with Fedagrim and the Practice Centre for Agriculture and Horticulture, and popularising articles are regularly published in the agricultural and horticultural press.

Throughout the years both departments have transformed themselves into a "Knowledge and Advisory Centre for Spray



Techniques" / "Réseau d'Excellence Technique de Pulvérisation" that closely follows, inventories, processes and explains the scientific and regulatory developments in connection with spray techniques and communicates with the Belgian "stakeholders". In this way both departments contribute to a sustainable and justified use of plant protection products.



International, national and regional regulations, laws, research and practical results concerning spray technology and the use of plant protection products.



Advice, information, support sustainable and justified use of plant protection products in Belgium











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6. PUBLIC SERVICE

6.1 PLANT

6.1.1 Technological advice and services in horticulture: SIETINET

Horticulture in Flanders is very innovative. Flemish horticultural products have an importance place on the world market. Nevertheless there is an important competition with low remuneration countries and countries profiting from a better climate. In Flanders, we have the advantage of technical capability. Flemish research institutes have access to scientific knowledge worldwide and perform a lot of prominent research. The bottleneck, however, is the flow of knowledge to the people in the field. Therefore, a project for cooperation between both was set up, called SIETINET. At first, 12 companies were involved in the project, however, by the end of 2005 SIETINET included 20 members. The participating companies are very diverse. In vitro companies, as well as production or young plant companies, either with or without breeding activities, are involved. Members differ also in plant species and company size. One-man companies as well as big international companies are participating in SIETINET.

The scientific partners of the project are: CLO-DVP, the Research Centre for Ornamental Plants (PCS), University Ghent, Hogeschool Gent and Flanders Interuniversity Institute for Biotechnology (VIB). Each of these partners is specialised in different topics within the broad scope of biotechnology research, which allows a broad presentation of questions from the companies. The cooperation is funded by IWT as a technological and advisory project. Twenty percent of the total cost is funded by the companies, the residual 80% is financed by IWT.

SIETINET employs a technical advisor. This advisor is responsible for the fluent information transfer from the research institutes to the companies. The information transfer concentrates on the topics:

- in vitro technology,
- breeding,
- DNA marker technology,
- genes and Genetically Modified Organisms (GMO's).

Yearly, SIETINET supplies more than 100 advices, most of them for free. Another service is performing research on specific questions from each company. The company pays for the research but IWT also contributes in the total cost. In addition to these individual services, activities are organised for the complete group of members. Workshops and symposia on themes like GMO's, breeding and contamination *in vitro* drew a lot of interest. Monthly, the members receive an overview of the most recent developments in scientific research and every three months they receive a newsletter. SIETINET accompanies the members with requests for financial support by funding organisations like IWT.

6.1.2 DUS and VCU research, Post control and Seed laboratory

In the context of a mandated assignment of the Administration for Quality of Agricultural Production, CLO-DFE (ABKL-NCPP, Flemish Region) gives scientific, technical and logistic support for the execution of the directives of the EU concerning the national variety catalogue of agricultural crops (directive 2002/53/EU). The protocols for the DUS (Distinctness, Uniformity and Stability) and VCU (Value for Cultivation and Use) trials (fodder beet and industrial chicory) are put forward by the Technical Interregional Working Group (TIW). By choosing better standard varieties and criteria based on sustainability (quality, disease resistance and harvest security), only the best varieties are admitted for inscription to the national catalogue. The reports are evaluated in four working groups, with a university professor as chairman.





The VCU-trials are executed for each agricultural crop (except for sugar beets), when there is a new requirement for registration. These trials are organised in all Flemish agricultural regions. The results of all trials (Flanders and Walloon region) are reported for all forage crops, green manure crops, rapeseed, flax and industrial chicory.

New VCU-testing cycles were set up in 2005 for silage and corn maize, grasses, industrial chicory, flax, forage beets, spring vetch, winter rye, winter barley, spring barley, triticale, spring wheat, and winter wheat. Most trials are done in collaboration with the CRA-DPV. In 2005, 342 varieties (14 crops) were tested at six to seven locations in different agricultural regions. On average, 10% of the new varieties were admitted to the catalogue. DUS research was done for industrial chicory and fodder beets in 2005. For other crops, there are bilateral agreements. Six new varieties of industrial chicory were tested for DUS research and compared with a reference collection (29 varieties) according to the UPOV guideline TG/172/3. For fodder beets two new varieties were tested for DUS research and compared with a reference collection (17 varieties) according to the UPOV guideline TG/150/3. New Begonia x tuberhybrida cultivars were tested for plant breeders right on request of the Community Plant Variety Office (CPVO, Angers) and the



Service for Intellectual Property – Ministry of Economical Affaires (national plant breeders right). CLO-DFE was also authorised as "Examination Office" for the research of industrial chicory and flax (oil and fibre).

In addition to the DUS and VCU trials, research was conducted to optimise the evaluation criteria. In 2005, the following criteria for variety testing were set up or reviewed:

- New criteria for spring vetch and winter rye (as green covering crops), white mustard, fodder radish, white clover and industrial chicory
- Review of the criteria for red clover (exploitation scheme and observation parameters) and silage maize (simulations for lodging)
- Research of fatty acid composition in grasses (species and varieties) and the possibility of incorporation of this characteristic in the criteria
- International collaboration for chicory: comparison of carbohydrate yield and other parameters coming from trials in Belgium, the Netherlands and France; working out a proposal for an international trials network

The following activities were set up for the executive control tasks, connected to the legal quality control of plant material (in the order of NCPP-ABKL) that concerns the whole chain, from testing a new variety to control of the seed production and post control of seed multiplications:

- Official pre and post control trials for grasses, flax and potatoes. In 2005, 948 samples from 21 grass species, 552 flax samples and 397 potato samples) were tested
- Supporting activities to the Seed Testing Laboratory

In the order of EU-DG SANCO (Health and Consumer Protection) comparative trials for grasses were set up in the spring of 2005. The following activities were established:

- Collecting all samples (293) coming from different certification services of EU countries
- Sowing these samples in the field next to the reference samples (in duplicate)
- Evaluating purity (off-types) and identity, based on the UPOV-description
- Preparation of an interim report

For distributing the results of the variety testing, a descriptive and recommended list is edited for forage crops and green covering crops (6^{th} edition) and industrial chicory (12^{th} edition).

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6.1.3 TAD FarmCOMPOST

The consultancy service FarmCOMPOST, located at CLO-DFE has got the green light to continue for a second period of two years, from May 2005 until April 2007, on the basis of a synthesis of its activities in the first two years and a planning for the second biennial period. With regard to the consultancy activities, FarmCOMPOST offers four different packages, including an enlargement of two packages.

Along with the advice as regards Controlled Microbial Composting (package 1), advice was given concerning the treatment of manure (package 3). It concerns the turning of the pile of stable manure on the field. The digested manure has a better fertilisation value. This treatment has been proven to be a good agricultural practice (CRA).

In the renewed FarmCOMPOST, package 4 is proposed. It concerns re-utilisation of organic waste products and composting

not previously considered. Possible practices are transforming the organic products using earthworms, conservation during the storage of the materials and surface decomposition on the land. The extension with package 3 and 4 means also an enlargement of package 2. There is not only an advice on utilisation of compost but also on application of treated manure, incorporation of harvest residues, etc. Advice concerning the application of organic fertilisers and adapted tillage were already included in this package in the first biennial period.

During the year 2005 there was an apparent need for support by brewing and the application of compost tea. FarmCOMPOST took up this job.

Again, an extention program of innovation stimulation was provided with the organisation of two seminars with respect to soil fertility (February and November 2005), some lectures, a demonstration on farm composting in cooperation with the Anchorage Environmental Care BOL and a demonstration


'preparing and application of compost tea' in the context of an ALT-project 'Soil Management in Organic Horticulture'. FarmCOMPOST was an exhibitor at Agriflanders and Agribex and at the "Werktuigdagen" in Oudenaarde.

Some examples of the actions of FarmCOMPOST as an advisory service in 2005 follow:

• Fresh application of horse manure on grassland inhibits grass growth and makes the grass unappetising for the animals. Composting of straw-rich manure resulted in a well digested product, free of ammonium. It is brought on some parcels at the end of the summer period after the cutting of the grass rests. FarmCOMPOST advised here for the fertilisation starting from the quality of the compost and the fertility state of the grassland parcels.

• With the supervision of FarmCOMPOST a tree nursery used compost tea on *Crataegus monogyna* (hawthorn) and *Acer campestre* (field maple), two species that are extremely sensitive to mildew. The disease was suppressed and the results where comparable with the fungicide application.

• On two farms that are cultivating *Begonia*, an experiment started in 2005 to investigate the possible advantage of the use of CMC-compost in comparison with the application of stable manure and/or mineral fertilisers. The question is if the adjusting of the soil biology by the fertilisation can induce the natural resistance against root rot and *Xanthomonas campestris* pv. *Begoniae*. The parcels with added CMC-compost after ploughing received an adapted organic fertiliser manuring. By field inspection of the crop, the plants in both fertilisation variants performed as well as eachother. The quality of the tubers is evaluated during the storing period.

• Different organic growers received consultation in composting and compost application, with a good result for their crops in 2005.

In cooperation with the University of Ghent, the Research Centres of the Provinces and growers (associations), the effects of the use of CMC-compost are investigated in different cultures/cultivation systems. Two students are being trained in an experiment investigating the addition of own-prepared compost to substrates for the culture of *Hedera helix* (ivy) in container.



6.1.4 Varieties developed by CLO-DVP

Cultivars on variety lists and commercialised

1. SEED MULTIPLIED

Perennial ryegrass – fodder	type:
a) diploid:	- Achat
, 1	- Isabel
	- Melland
	- Melpico
	- Melvina
	- Merbo
	- Merganda
	- Merks
	- Paddok
	- Plenty
	- Odessa
	- Rebecca
	- Ritz
	- Vigor
b) tetraploid:	- Ernesto
	- Floris
	- Graciosa
	- Meradonna
	- Merkator
	- Merkem
	- Merlinda
	- Pandora
	- Pomeroi
Bayannial magyage turthun	- KOY
rerenniui ryegruss – iurjiyp	Maruna
	Olano
	- Otalio
	- Oxialia
Italian maarass.	- Keloli
a) dinloid:	- Adin
	- Bellem
	- Belluna
	- Davinci
	- Lemtal
	- Melcasso
	- Melchior
	- Melclips
	- Meribel
	- Merode
	- Mertaki
	- Meryl
	- Nadine
	- Prospect
	- Romeldo
b) tetraploid:	- Gemini
	- Fedra
	- Melquatro
	- Meritra
	- Meroa
	- Racine
	- Salomé
Hybrid ryegrass:	
	- Hymer
	- Lemsor
	- Merini

• • • • • • • • • • • • • • • • • • • •			
D 11 (1)	11	117	
Perennial ryegrass – fo	aaertype:	westerwola ryegrass:	M.1. 11
a) diploid:	- Achat	a) diploid:	- Melworld
	- Isabel	b) 40411-	- Mendoza
	- Melland	b) tetraploid:	- Lemnos
	- Melpico		- Melmondo
	- Melvina	Meadow fescue:	
	- Merbo		- Merifest
	- Merganda	Timothy:	
	- Merks		- Comer
	- Paddok		- Dolina
	- Plenty		- Erecta
	- Odessa		- Tibor
	- Rebecca	Red fescue:	
	- Ritz		- Nevski
	- Vigor		- Rolf
b) tetraploid:	- Ernesto	Red clover.	
, r	- Floris	neu clorer.	- Global
	- Graciosa		- Lemmon
	- Meradonna		Mercury
	- Merkator		- Mericury
	- Merkem		- Merriet
	Merlinda		- Merviot
	- mornina Pandora		
	- ranuora Domorol		- Violetta
	- romeron Pov	White clover:	
Denomial manage	- NUY		- Merida
r erennuai ryegrass – tu	rjiype:		- Merwi
	- Mervue	Chicory:	
	- Olano		- Absylle
	- Oxiana		- Arancha
	- Relon		- Arioso
Italian ryegrass:			- Belcanto
a) diploid:	- Adin		- Crescendo
	- Bellem		- Hera
	- Belluna		- Melci
	- Davinci		- Vivace
	- Lemtal		
	- Melcasso	Stubble turnins:	
	- Melchior		- Durmelander
	- Melclips		- Dynamo
	- Meribel		- Leielander
	- Merode	Fadday basta.	- Deterander
	- Mertaki	rouder beets:	Adagia
	- Mervl		- Adagio
	- Nadine		- Apex
	- Prospect		- Bolero
	- Romeldo		- Dana
h) tetranloid.	- Gemini		- Gonda
oj teti apioiu.	- Gennin Eadra		- Ribondo
	- reula Molauotra		- Romeo
	- Meritar		- Uno
	- Meritra	White musterd:	
	- Meroa		- Chacha
	- Racine		- Flamenco
	- Salomé		- Meringue
			- Polka
Hybrid ryegrass:			
Hybrid ryegrass:	- Hymer		- Salsa
Hybrid ryegrass:	- Hymer - Lemsor		- Salsa - Solea



Fodder radish: - Brutus - Cassius - Sirius - Sixtus Forage rape: - Dino - Napoleon - Wilma

- Mersil
- Antonia
- Keukenfee
- Makostar

2. VEGETATIVELY MULTIPLIED

Roses:

	Cut roses:	
- Adolf Papeleu		- Païline
- André Brichet	Bromelia:	
- Annelies		- Cathy
- Anton Van Dijk		- Diabolo
- Balduinus		- Fernanda
- Benoit Friart		- Festival
- Celientje		- Regine de Ligne
- Cera		- Romero
- Dream	Hibiscus:	
- Floranje		- Melmauve
- Florizel		- Melroze
- Godelieve		- Melwhite
- Gold Cup	Malus:	
- Gomery		- DvP Obel/Red Obelisk®
- Graaf van Vlaanderen	Azalea.	
- Hertog van Brabant		- Cheons
- Jacky's Favorite		- Cupideau
- Joke		- Directeur Van Slycken
- Kanegem		- Flamenco
- Kasteel van Ooidonk		- Gilbert Mullie
- Koksijde- Liparfum		- Lara
- Lysa		- Laura Ashlev
- Margriet Hermans		- Lara Rood
- Marie Louise Velge		- Meyr André Heungens
- Melglory		- Meyr Jozef Heursel
- Melgold		- Meyr Marcel Vanbelle
- Melrose		- Meyr Roger De Loose
- Michelle d'Hoop		- Meyr Van Eetvelde
- Nele		- Mistral
- Pink Kanegem		
- Prinses Astrid		
- Prinses Mathilde		
- Professor Boesman		
- Rafael Braeckman		
- Rivierenhof		
- Rosarium Den Blakken		
- Sabine		
- Showy gold		
- Slot van Laarne		
- Toporanje		
- Ville du Roeulx		
- Wettra		

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- White Symphonie - Windekind



6.1.5 Centre for Plant Pest and Disease Diagnosis

In the past few years vegetable productions have increasingly been challenged by bacterial infections. The diseases are neither new nor unknown, but their presence has remarkably spread and economic losses have become general instead of occasional. In particular bacterial leek and cabbage diseases have been forwarded to the plant disease diagnostic service.

Bacterial blight infections caused by *Pseudomonas syringae* pv. *porri* have repeatedly been diagnosed on young leek plants as desiccated leaf tops and longitudinal lesions extending down from the leaf tips causing curled and split leaves. Various symptoms were found to be caused by the pathogen on production fields: greasy, yellow to orange warty leaf spots, elongated lesions extending as narrow strips at leaf margins, wilting and desiccation, rotting of the inner leaf in the sheath. Severely affected plants were stunted and misshapen. The cultures of *P.s.* pv. *porri* recovered from the leek samples had identical phenotypical, serological and pathological profiles.









From late fall until the end of winter another severe foliar disease of leek occurred on production fields during periods of relatively warm temperatures following a period of frost. Strong pectinolytic forms of Pseudomonas fluorescens were recovered from the rotting leaf tissues and their presence was generally associated with pathogenic fungi (Fusarium, Phytophthora porri, Stemphylium). Infections of P. fluorescens were apparently initiated during late fall and were arrested in frost periods as large white desiccated lesions extending in yellow stripes that were often mistaken for natural desiccation of the leaves. Affected plants were randomly distributed, contrary to infections of P.s. pv. Porri, which showed typical patch patterns. Occasionally, rotting or necrosis of leek leaves was caused by Pseudomonas viridiflava and Pseudomonas cichorii. Unlike P.s. pv. porri, pectinolytic Pseudomonads were not found on the seed bed and were shown not to be specifically pathogenic on leek or onion but also on Chinese cabbage, chicory lettuce, cauliflower and broccoli heads. Isolates of P. fluorescens were found to produce a substance for degradation of the waxy leek leaf surface.

In late summer cabbage productions suffered from black rot caused by *Xanthomonas campestris* pv. *campestris*. Hydathode infections resulted in chlorotic leaf margins with typical black vein pattern. Superinfection of *Pseudomonas viridiflava* was diagnosed on Brussels sprouts. Black rot management involves the use of a field diagnostic tool to eliminate the first diseased plants. In particular the Bacterial Express Test (Neogen Europe, UK) has been tried and tested for the past two years and showed good reliability for symptomatic plant tissue. A test protocol has been elaborated for efficient field use. The result of this antibody agglutination test provides the grower in as little as one minute with clear black rot verification.

It is known that both P.s. pv. porri and X.c. pv. campestris can be seed transmitted. Internationally validated methods for testing cabbage seed rely on selective isolation which detects one infected seed in 10000 seeds. Specific PCR primers (hrp gene or AFLP fragment) have become available recently. We also developed a dilution plating method for the analysis of leek seed. Samples from different varieties have been tested on a Pseudomonas medium modified to fit the selective isolation of the pathogen. Contamination in commercial seed lots has been monitored over a period of three years now. The presence of P.s. pv. porri was demonstrated in 30% of the lots tested in 2003. However, none of the lots submitted in 2005 tested positive. After the initial introduction of the pathogen, bacterial blight has become established on leek production fields by means of contaminated leaf debris and wash water from leek cleaning procedures.

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6.2.1 Quality control of fisheries products

The fisheries sector can no longer escape the increasing demands on freshness, quality and traceability of their products. To guarantee the quality of these products to purchasers and consumers, producers and the industry are under pressure to carry out objective testing in specialised laboratories. Several methods can be carried out at CLO-DVZ to determine the quality of fish with a range of tests for industry, including determination of physical, chemical and sensory properties of the fish.

The physical measurements include the determination of the number of fish-bones in fillets, the measurement of pH and electrical resistance and the determination of texture and ice layer around fishery products. To determine freshness quality, the oldest and most commonly used test is the chemical method based on total volatile basic nitrogen (TVB-N). TVB-N is derived mainly from trimethyl amine (TMA), dimethyl amine (DMA) and ammonia, all of which are associated with fish deterioration. The EU states maximum TVB-N values for certain fish types in directive EU/493/EEG. Furthermore, chemical testing for undesirable substances like sulphite, polyphosphates, histamine, polychlorinated biphenyls (PCB's) and polycyclic aromatic hydrocarbons (PAH's) can be carried out. Fish species identification may also be carried out by electrophoresis of water soluble proteins using either fresh or frozen fish. For processed products, where the proteins are denaturated, identification is done by DNA analysis.

Sensory assessment schemes are also used for determining fish freshness. A basic assessment system is specified by the European Commission. However, the freshness of fish can be determined more precisely by using the quality index method (QIM). This system uses several characteristics of chilled (and gutted) fish to determine the freshness. Different parameters (skin, eyes, gills...) are assessed on the basis of a point-scoring system from 0 to 3 for each characteristic. All attributes are equally important in the total score, to avoid a large amount of fish being rejected on the basis of a single characteristic.





6.2.2 Analysis of chlorinated pesticides and PCB's in yellow eel

Since 1994, the Chemical Monitoring Section of CLO-DVZ participates in the eel-pollutants monitoring network of the Institute for Forestry and Game Management (IBW). Yearly a number of samples are analysed (281 in 2005) on the following pesticides: dieldrin, endrin, transnonachlor, ß-hexachlorocyclohexane (B-HCH), lindane, hexa-chloorbenzene (HCB), p,p'-DDE, p,p'-DDD, p,p'-DDT. The analysed polychlorinated Biphenyls (PCB's) have IUPAC numbers 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. The analysis consists of a fat extraction, consecutive separation of the component from the fat on an alumina oxide and silica oxide column, and GC analysis with ECD detection. Integral Quality Assurance, including participation in international tests, is taken care of. Most measured PCB-concentrations exceed the norm, however, a number of sites do not exceed the more tolerant upper limits. However, an important number of sites are very contaminated by several substances, exceeding the upper limit by over a 100fold. Combined with the very high fat content of eel (up to more than 40% of the body weight), this causes a lot of concern for the consumption of wild Flemish yellow eel Anguilla anguilla.

The high degree of variation at specific locations is typical for size, body weight and fat content as well as for the substances of interest. The five most heavily contaminated waterways are the canal Dessel-Schoten, the canal Bocholt-Herentals, the canal of Beverlo, the Laan and the Maas. For the total of seven marker PCB's, only a few locations do not systematically exceed the Belgian norm for consumption of 75 ng/g fresh weight. These locations are mainly situated in the IJzer basin, the polder area, some old Scheldt meanders, and the Meetjesland region. For B-HCH and lindane, the IJzer basin is highly contaminated, as is the region of Tienen, because of the application of these products in the culture of sugar beets. For the sum of DDT's, some ten stations exceed the limits significantly, but the effects of the ban of DDT are unmistakeable. Transnonachlor is not of concern anymore for the major part of Flanders, but for the rare places where limits are exceeded, the problem is very serious. The same goes for endrin, dieldrin and HCB. Also, the ban of HCB has resulted in a very positive effect.

6.3 TECHNOLOGY AND FOOD





The Technological Advisory Service for Dairy Farmers and Dairy Farm Producers ("TAD

Hoevezuivel"), was developed by experts of CLO-DVK from an already existing individual services for dairy farm producers. The experts and the dairy farmers' organisations realised its foundation was necessary for two obvious reasons. First of all, the demands for product quality and prevention for the dairy farmer and the dairy farm producer are becoming stricter, for example, the increasing importance of antibiotics prevention. Furthermore, it is the wish of the organised dairy farmers that the advisory tasks of the former Ministry of Small Enterprises, Traders and Agriculture, which have not been taken over by the FAVV, are not be lost. This advisory service also has the aim to valorise the knowledge present at CLO-DVK.

The "TAD Hoevezuivel" primarily focuses on consultancy towards dairy farmers with problems or questions concerning the following three subjects: • Guidance for milk quality problems: Every dairy farmer who has problems with milk quality can ask for advice about bacteriological, chemical and sensory quality of milk, e.g. tracing of antibiotic residues in milk. Workshops will be organised in this field to enable producers to perform antibiotic tests and to understand the results of these tests. The workshops aim is to reduce the number of penalisations and delivery prohibitions due to the presence of antibiotic residues in the future.

• Assistance for dairy production on the farm: Dairy farm producers can receive individual consultation and guidance about technological and hygienic problems of dairy farm production. This consult can involve different items as pasteurisation problems, fermentation of cream or milk in the production of butter, cheese or yoghurt, yoghurt quality problems, problems with too dry or too humid cheese or a loosening cheese crust, starter cultures, temperature control, etc.





Help can also be provided in the development of a new product or in diversifying the current product variety. Additional to the consultation, analyses in the laboratories or testing in the pilot factory can be executed.

Another service provided to the dairy farm producer is to help with the autocontrol plan (compulsory by law since January 1st 2005 for all food producers) in cooperation with "Steunpunt Hoeveproducten" (*Policy Research Centre Dairy Farm Products*). In practice, "Steunpunt Hoeveproducten" aids with the design of the plan and the TAD adviser aids with the identification of the critical control points and their critical values.

• Special guidance to dairy farms with paratuberculosis: The "TAD Hoevezuivel" will also cooperate with "Dierengezondheidszorg Vlaanderen" (Animal Health Care Flanders) in the treatment of problems occurring at a farm infected with *Mycobacterium avium* subsp. *paratuberculosis*. Due to the implementation of procedures in the Belgian dairy husbandry and dairy industry to protect the dairy export in 2005, it is obvious that this consultancy is a topical.

6.3.2 Agromech

The Technological Advisory Service 'Agromech' belongs to the large group of technological advisory services which are mainly financed by IWT (Science and Innovation Administration of Flanders). This network groups a large number of experts in several disciplines who want to assist small enterprises with their innovation process by providing very specialised, technological advice. Agromech gives recommendations and information to farmers, contractors and developers of agricultural machinery around a number of topics: environmental protection, reduction of quality losses of agricultural and horticultural products, optimisation of the efficiency of the machines, reduction of the inputs and an improved safety for the operators of machinery. It is obvious that the subjects of the questions and the needs of the different target groups are diverse. Two collaborators operate the TAD Agromech and can use the collective expertise of CLO-DVL. The service is partly free. This was made possible by the co-financing of the 'Begrotingsfonds voor de Grondstoffen' (Fund for Raw Materials).

Farmers and contractors are mainly users of machines. The recommendations to this group are situated especially in the field of adjusting and improving the use of machines: fertiliser spreaders (spreading pattern), potato harvesters (prevention of discolouration), chicory harvesters, sprayers (including lance sprayers), etc. This kind of service necessitates on farm visits. After completing the necessary observations a report is prepared that it is provided to the people concerned.

Not all recommendations are of this size. Most questions can be answered with a short recommendation or by providing the necessary information. The questions from the constructors of agricultural machines are somewhat different. Agromech can assist this group in the design of new machines (e.g. how to guarantee the safety of the user, testing of sprayers, etc.) and with the acquisition of IWT-financing.





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6.3.3 AgriCONSTRUCT

The technological advice service AgriCONSTRUCT started its activities in 2002. Its main target is to advise farmers, contractors and manufacturers of building materials, on the use of building materials in the agricultural sector. The activities of this advisory service focus on the overall problem of the construction of animal houses: technical characteristics of the building materials, design of animal houses, climate regulation, animal welfare, ammonia emission and new animal housing designs. The manufacturers of building materials for the agricultural sector and the contractors are confronted with the new Belgian legislation concerning animal welfare and ammonia emissions. The farmer is also confronted with major challenges as a result of these changes.

With the support of different research institutions, like CLO-DVL and IWT, the technological advice service provides more than 330 individual advices each year. The main reasons for farmers to contact AgriCONSTRUCT are problems with ventilation, design and the possibilities for renovation of pig and cattle houses, concrete compositions and low-ammonia emission housing systems. A large part of the advices are given by telephone, but a visit to the farm is often necessary to visualise the problems and to suggest possible solutions. The questions posed by the contractors of animal houses and manufacturers of agricultural building materials are of a different nature and are mostly about new developments in the housing of animals and the construction of farm buildings.

Besides individual advice, much attention is paid to the quarterly periodical AgriCONSTRUCT. Each issue carries a theme analysed in detail by different specialists from the service. Specific advices are grouped in articles, workshops and courses. The technological advice service also publishes brochures and papers, such as 'Practical use of concrete in the agriculture' and 'Potato storage: Construction and storage practices'.



6.3.4 Laboratory of Spray Technology

The Laboratory of Spray Technology of CLO-DVL has been BELTEST- accredited since spring 2002 (certificate 259-T ISO 17 025). The accreditation comprises four tests involving spray equipment viz. 1) the flow of individual nozzles at a given working pressure, 2) the liquid distribution of an individual nozzle at a given pressure and spray height, 3) the liquid distribution of a set of nozzles installed on an idealised spray boom at a given pressure, spray height and offset angle and 4) the liquid distribution of a field sprayer at a given spray pressure, spray height and offset angle. Besides the four accredited measuring set-ups the laboratory also possesses a wide range of additional measuring and testing equipment to characterise spraying machines and/or their parts. The laboratory carries out these accredited tests as a support for intern research projects concerning spray technology but offers also its services to external clients such as manufacturers of spraying equipment and accessories, scientific institutes, government services, etc.

For promotion CLO-DVL organised an international visitor's day to the Laboratory of Spray Technology on 9 May 2005. The goal of this day was to present and make public the extensive range of expertise, measuring and test set-ups of the laboratory to potential customers and partners of Belgian and foreign research institutes, government agencies and private companies involved in spraying technology and plant protection products. This activity attracted a good and varied response (25 attendants) including a number of foreign participants and a rather large group of foreign interest from the Netherlands, France, Germany and Poland. As a result of this visitor's day two projects have already been received and a common research and advisory project was written for submission.

6.3.5 Quality control in connection with the maintenance of milk installations

The project group 'Control' coordinates all activities in Belgium concerning the maintenance tests of milk installations (milking installations, refrigerated farm milk tanks) and organises the training and support of milk cooling equipment technicians, milking machine technicians and milk yield specialists. The operation of 'Control' takes into account the requirements set out by IKM (Integral Quality Monitoring of Milk) and the international standards for milk installations viz. ISO 3918, ISO 5707 and ISO 6690.

All measuring and advisory reports of milk installations and refrigerated farm bulk tanks made in 2004 were inventoried during the past year. Subsequently all technicians were evaluated by means of a fully automated evaluation report made in Excel. Therefore, 5% of all reports from each technician, with a minimum of five, were selected at random and checked for accuracy and correctness. Since the start of this project a clear increase in this score has been observed. The measuring equipment of each technician was also checked: pulsator tester, vacuum meter, air flow meter and thermometer.

The above results demonstrate the importance of a periodic maintenance of both milk installation and refrigerated farm bulk tanks. Maintenance must be carried out correctly. Hence, providing a qualitative training and regular specialisation courses for technicians are essential. The annual evaluation of service technicians not only serves the goal of supervising their work but must also solves the technical questions and problems that they may encounter with the maintenance of milk installations or milk cooling equipment. Since the start of the project in 1996 a remarkable improvement in the maintenance of milk equipment has been observed.



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7. PUBLICATIONS

7.1 Articles published in journals and included in the Science Citation Index

CLO-DVP

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CLO-DVL

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CLO-DVZ

Calewaert J.-B., Lescrauwaet A.-K., Mees J., Seys J., Hostens K., Redant F., Moulaert I., Raemaekers M., Demaré W., Belpaeme, K., Maelfait H., Kyramarios M., Tak P., Maes F., Douvere F., Overloop S., Peeters B. (2005). Hoofdstuk 8 Kust en Zee: Te weinig vis, te veel vervuiling. In: Van Steertegem M. (Ed.) (2005) Milieurapport Vlaanderen Thema's MIRA-T 2005. LanooCampus, Leuven, 145-159. www.milieurapport.be

Roose P., Cooreman K., Vyncke W. (2005) PCB's in cod (Gadus morhua), flounder (Platichtys flesus), blue mussel (Mytilus edulis and brown shrimp (Crangon crangon) from the Belgian Continental Shelf: relation to biological parameters and trend analysis. In: Roose P. (2005). Volatile organic compounds and related microcontaminants in the Scheldt estuary and the southern North Sea: method development and monitoring. PhD thesis Universiteit Amsterdam, 203-216.

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8. THESES AND TRAINING REPORTS

CLO-DVP

Berghman Evelien. Effect van de hoge licht intensiteit en kruisingen op de stabiliteit van de juncties in transgene *Arabidopsis* lijnen. Graduaat Farmaceutische en Biologische Technieken, KaHo St.Lieven, Gent. Begeleider: Nina Papazova

Coppens Jessica. Event-specifieke kwantificering van transgene maïs- en koolzaadlijnen door middel van real-time PCR. Graduaat laboratorium- en voedingstechnologie (LVT), optie Farmaceutische en Biologische Technieken (FBT), KaHo Sint-Lieven, Gent. Begeleider: Isabel Taverniers

Delforche Maarten. Kwalitatieve en kwantitatieve detectie van genetisch gemanipuleerde maïs- en suikerbietlijnen. Graduaat laboratorium- en voedingstechnologie (LVT), optie Farmaceutische en Biologische Technieken (FBT), Hogeschool Gent, Departement Gezondheidszorg Vesalius. Begeleider: Isabel Taverniers

Glorieus Elien. **Studie van het effect van stresscondities op de stabiliteit van plant/T-DNA juncties in transgene lijnen van** *Arabidopsis.* Bio-Ingenieur in de cel- en genbiotechnologie, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent. Begeleider: Erik Van Bockstaele en Nina Papazova

Janssens Jeroen. **Genetische analyse van divergente selectie** voor kroonroestresistentie in raaigrassen op basis van fenotype en genotype, Industrieel Ingenieur in chemie optie biochemie, Hogeschool Gent, Departement Industriële wetenschappen, BME-CTL. Begeleider: Hilde Muylle

Leus Leen. Resistance breeding for powdery mildew (*Podosphaera pannosa*) and black spot (*Diplocarpon rosae*) in roses. Doctoraat 13/12/05 UGent. Promotor: Erik Van Bockstaele

Robyn Inge. **Isolatie en regeneratie van protoplasten bij Araceae**. Bachelor Agro- en Biotechnologie, KATHO Roeselare. Begeleider: Tom Eeckhaut

Taverniers Isabel. Development and implementation of strategies for GMO quantification in an evolving European context. Doctoraat 03/03/05, UGent. Promotor: Erik Van Bockstaele en Marc De Loose

Van Daele Inge. Identification of genes related to selfincompatibility in ryegrass (*Lolium perenne* L.). Doctoraat 29/11/05, UGent. Promotor: Erik Van Bockstaele en Isabel

Roldán-Ruiz

Vandenbruwaene Joke. **Ploïdieveredeling bij rozen als** ondersteunende techniek voor interspecifieke kruisingen, Industrieel Ingenieur in chemie optie biochemie, Hogeschool Gent, Departement Industriële wetenschappen, BME-CTL. Begeleider: Tom Eeckhaut

Vanstechelman Yves. **Opsporen van genetische merkers voor FHB resistentie in tarwecultivars bestemd voor de Belgische landbouw**, Bio-Ingenieur in de cel- en genbiotechnologie, Faculteit Bio-ingenieurswetenschappen, UGent. Begeleider: Hilde Muylle en Rebecca Zwart

Verleysen Hans. **strategies for woody ornamentals in plants**. Docotraat 02/06/05, UGent. Promotor: Erik Van Bockstaele

CLO-DFE

Beck H. **CMC- en Groencompost in steksubstraat** *Hedera helix* **'Esther'.** KAHO Sint-Lieven, departement Sint-Niklaas, Graduaat Agro- en Biotechnologie. Begeleider: ir. Koen Willekens

Cornelis A. Voorspellen van maïsopkomst op basis van de koudetest. Hogeschool Gent, Graduaat Agro- en Biotechnologie. Begeleider: Dr. ir. Johan Van Waes

Roelandts P. Onderzoek naar de waarde van CMCcompost in teeltsubstraten. Hogeschool Gent, Departement Biotechnologische Wetenschappen, Landschapsbeheer & Landbouw, Graduaat Agro- en Biotechnologie. Begeleider: ir. Koen Willekens

CLO-DGB

Tolani A.R.. **Phylogenetic identification and genetic variability** of *Pratylenchus* species, especially *Pratylenchus coffeae* from Africa. Universiteit Gent.Begeleiders: Lieven Waeyenberge & Maurice Moens

Vanbesien Pieter. **De beuk**. Graduaat/Bachelor Agro- en Biotechnologie, optie Groenvoorziening, KATHO, Roeselare. Begeleider: Johan Witters

Van Gansbeke Annick. Karakterisering en PCR-DGGEmonitoring van *Pseudomonas* geassocieerd met nerfrot bij botersla. Graduaat/Bachelor Chemie, KAHO Sint-Lieven Begeleiders: Bart Cottyn & Martine Maes

Van Puyvelde Eric. **Roofmijten en spintmijten bij beuk**. Graduaat Agro- en Biotechnologie, optie Groenmanagement, KAHO Sint-Lieven, Sint-Niklaas.Begeleider: Johan Witters



Van Vooren Tineke. **Studie van overleving, kruisingstype** en genetische diversiteit van *Phytophthora ramorum* bij Rhododendron. Graduaat/Bachelor Chemie, keuze Biotechnologie, Hogeschool Gent. Begeleiders: Isabelle De Dobbelaere & Kurt Heungens

Van Wuytswinkel Jan. *Pratylenchus penetrans* en *P. crenatus*: **kweek, identificatie en onderzoek naar onderlinge competitie**. Graduaat Agro-en biotechnologie, optie Landbouw. KAHO Sint-Lieven, Sint-Niklaas. Begeleiders: Nancy de Sutter & Nicole Viaene

Wastyn Robbe. **De natuurlijke vijanden van de** witloofmineervlieg. Graduaat Landbouw en Biotechnologie, Hogeschool Gent. Begeleider: Hans Casteels

CLO-DVV

De donder Sven. **Onderzoek naar evaluatie van oneven keten vetzuren in functie van de adaptatie van pens bacteriën**. Departement Biotechnologische wetenschappen, Landschapsbeheer en Landbouw, Hogeschool Gent. Eindwerk, academiejaar 2004-2005. Promotor: Daniël De Brabander

Ir. Valckeners Damien. Influence de la désynchronisation des apports azotés et énergétiques dans le rumen sur la digestion et le métabolisme azoté du taurillon Blanc Bleu Belge culard. Doctoraat op 10/05/05 aan de Faculté des Sciences Agronomiques, Université de Gembloux. Jurylid: Leo Fiems

Van Assche Wim. Stage van 27/9 tot 21/10/2005. Graduaat Landbouw - Hogeschool Gent. Stagerapport Departement Dierenvoeding en Veehouderij. Begeleider: Marc De Paepe

Willen Mario. Invloed van lactatiestadium op het vetzuurpatroon van melk. Departement Biotechnologische wetenschappen, Landschapsbeheer en Landbouw, Hogeschool Gent. Eindwerk, academiejaar 2004-2005. Promotor: Daniël De Brabander

CLO-DVK

Coorevits A. Biodiversiteit van aërobe sporenvormers uit rauwe melk afkomstig van biologische en conventionele melkveebedrijven. Licentiaat Biotechnologie,UGent, 2004-2005. Begeleider: Heyndrickx M.

Coorevits P. Moleculaire typering van besmettingsflora van MA verpakte versneden kookwaren. Industrieel ingenieur, Hogeschool Gent, 2004-2005. Begeleider: Heyndrickx M.

Dewaele B. Groei van Salmonella Enteritidis op het dooiermembraan in eieren bij verschillende temperaturen. Gegradueerde/Bachelor in Agro- en Biotechnologie, optie Celen Genbiotechnologie, KAHO Zuid-West Vlaanderen Roeselare, 2004-2005. Begeleider: Grijspeerdt K.

Knockaert D. Invloed van condens op de eischaal en hittestress bij hennen op de penetratie en contaminatie van eieren met *Salmonella* Enteritidis. Gegradueerde/Bachelor in Chemie, optie Biochemie, KAHO Sint-Lieven Gent, 2004-2005. Begeleider: De Reu K.

Lever S., Provinciaal Instituut voor Tuinbouwonderwijs, Mechelen, stage 21-25/03/2005. Begeleider: Mortier L.

Maes M. Invloed van condensvorming op de eischaal op de bacteriële penetratie doorheen de eischaal en de bacteriële contaminatie van de ei-inhoud met *Salmonella* Enteritidis. Gegradueerde/Bachelor in Voedings- en Dieetkunde, KHBO Brugge–Oostende, 2004-2005. Begeleider: De Reu K.

Nols S. **Opsporen van tetracyclinen in melk en kippenvlees met** LC-MS/MS. Gegradueerde/Bachelor in Chemie, optie Chemie, Hogeschool Gent, 2004-2005. Begeleider: De Ruyck H.

Sichien L. Kwantificering van *Campylobacter* spp. op kippenkarkassen via fluorescentie *in situ* hybridisatie (FISH). Gegradueerde/Bachelor in Chemie, optie Biochemie, KaHo Sint-Lieven Gent, 2004-2005. Begeleider: Messens W.

Van Pamel E. Differentiatie van *Listeria monocytogenes* stammen op basis van hun virulentie voor de mens. Bioingenieur in de Cel- en Genbiotechnologie, UGent, 2004-2005. Begeleider: Van Coillie E.

CLO-DVL

Creve J. Invloed van hokgrootte en verrijking op de mate van fluctuerende asymmetrie bij vleeskonijnen gehuisvest in groep. Scriptie voorgelegd tot het behalen van de graad Licentiaat in de Biologie, optie Dierkunde (Universiteit Gent). Begeleiders: F. Tuyttens & E. Van Poucke.

Derijcke L. **Temporele patronen van fluctuerende asymmetrie bij vleeskippen tijdens de ontogenie.** Scriptie voorgelegd tot het behalen van de graad Licentiaat in de Biologie, optie Dierkunde (Universiteit Gent). Begeleiders: F. Tuyttens & E. Van Poucke.

Joly F. Etude sur la conception assistée par ordinateur avec Inventor 8, Stageverslag van kandidaat Landbouwingenieur (ENESAD, Dijon). Begeleider: J. Vangeyte.



Lambers J. Verslag van de testen voor een sorteeropstelling. Stageverslag van kandidaat Landbouwingenieur. Begeleider: J. Vangeyte.

Vanleene S. **Mechanische schadepreventie op aardappelrooiers door vermogens- en snelheidssturing.** Eindwerk Industrieel Ingenieur Landbouw en Biotechnologie, optie Landbouw (BIOT Hogeschool Gent). Begeleiders: T. Van Canneyt, S. Windey & B. Sonck.

Van Overbeke S., Van Zandweghe S. **Invloeden van een vachtborstel bij melkvee.** Eindwerk Bachelor Agro- en Biotechnologie, 3^{de} jaar Landbouw (Katholieke Hogeschool Zuid-West-Vlaanderen). Begeleiders: F. Tuyttens & A. Van Nuffel.

Vanparys D. **Kanaalventilatie in vleesvarkensstallen.** Eindwerk Industrieel Ingenieur Landbouw en Biotechnologie, optie Landbouw (BIOT Hogeschool Gent). Begeleiders: H. Cnockaert & B. Sonck.

Willemsen J. **De invloed van rooitechnische vaiabelen op de aardappelkwaliteit.** Eindwerk Industrieel Ingenieur in Landbouw en Biotechnologie, optie Landbouw (KHK Geel). Begeleiders: T. Van Canneyt & S. Windey.

CLO-DVZ

Debaenst I. **Bepaling detectielimiet bij analyse op metabisulfiet in garnalen via destillatie.** Scholengroep Sint-Rembert, Vrij Land- en tuinbouwinstituut, Torhout. Begeleiders: Karen Bekaert en Sabrine Derveaux, DVZ

De Clercq I. Authenticiteitsbepaling van vis, schaal- en weekdieren met behulp van PCR-SSCP. Universiteit Gent, Faculteit Bio-ingenieurswetenschappen, 2^{de} proef Cel- en gentechnologie. Begeleiders: Stefan Hoffman en Daphné Deloof, DVZ

Manaffar R. DNA Molecular Marker Development for differentiation between Artemia parthenogenetic and Urmia Artemia. INCO Programma Universiteit Gent, Faculteit Bioingenieurswetenschappen, ARC (Artemia Reference Centre). Begeleiders: Stefan Hoffman en Daphné Deloof, DVZ

Rodriguez S. DNA Molecular Marker Development for Authentication of Chilean Commercial Bivalve Species. INCO Programma Universiteit Gent, Faculteit Bioingenieurswetenschappen, ARC (Artemia Reference Centre). Begeleiders: Stefan Hoffman en Daphné Deloof, DVZ

Rodriguez S. DNA Molecular Marker Development for

differentiation of South American Artemia persimilis populations. INCO Programma Universiteit Gent, Faculteit Bioingenieurswetenschappen, ARC (Artemia Reference Centre). Begeleiders: Stefan Hoffman en Daphné Deloof, DVZ

Standaert F. Optimalisatie en validatie van de procedure voor de bepaling van het gehalte aan Totaal Vluchtige Basen in visserijproducten. Hogeschool Gent, Departement Biotechnologische Wetenschappen, Landschapsbeheer en Landbouw, Campus Melle. Begeleiders: Karen Bekaert en Sabrine Derveaux, DVZ

Van Dewalle N. **Onderzoeken van de lineariteit bij bepaling van totale vluchtige basen in vis.** Scholengroep Sint-Rembert, Vrij Land- en tuinbouwinstituut, Torhout. Begeleiders: Karen Bekaert en Sabrine Derveaux, DVZ

Zubiate Sologaistoa L. **Trainee in chemical and biological monitoring. Praktische opleiding in Mariene Wetenschappen** in het kader van de samenwerkingsovereenkomst tussen Universidad de Vigo en CLO-DVZ. Begeleiders: Koen Parmentier en Kris Hostens, DVZ



9. COMMUNICATION

9.1 Press contacts

- 22nd of March: Press conference: "GGO's in Europa". Het Pand, Gent; organisation CLO-DVP
- 21st of April: The name-giving ceremony of a new azalea "Prinses Claire". Gentse Floraliën; organisation CLO-DVP
- 12th of July: RTL-TVI: shooting for the programme "Coûte que coûte". "Antibiotica-residu analysen in honing" under the authority of Test-Aankoop, CLO-DVK
- 8th of September: Agricultural Press on the occasion of a work visit by the Minister-President Yves Leterme, CLO-DVK
- 18th of September: CANVAS: "Vlaamse vissers: het water aan de lippen", CLO-DVZ
- 4th of December: CANVAS: shooting for the programme "Overleven". "Een gen in de soep: GGO's", CLO-DVP
- 19th of September: ÉÉN: programme "Witte Raven".
 "Geografisch vormgever/model wordt visser", CLO-DVZ
- 23th of December: FOCUS WTV: "De prijs van de Vlaamse garnalen", CLO-DVZ

9.2 Activities and workshops organised by or in cooperation with CLO

- 7th to 9th of January: Agriflanders, Gent. Participation
 CLO-DVP, CLO-DFE, CLO-DVV
- 2th of February: Information meeting for the sector.
 "Procedure critical use of methyl bromide", CLO-DGB, organisation REO-veiling, Roeselare
- 3th of February: 2^{ed} workshop TAD FarmCOMPOST. "Return from soil and compost analyses", CLO-DFE
- 14th to 18th of February: Workshop on Work packages 3 & 4 of the EU-project EASE (European Advisory System Evaluation) in order to identify an appropriate allocation of resources to data collection to support fishery management issues, CLO-DVZ, Oostende; organisation and contributions CLO-DVZ
- 26th of February: Workshop Strawberries and Small Fruits. "Compost, good for more than soil structure". In association with CLO-DGB and CLO-DFE, organisation Koninklijk Verbond van de Aardbeikwekers (National Royal Organization of Strawberry Growers), Roosdaal-Pamel
- 1st to 4th of March: Plenary meeting of the "ICES" Planning Group on commercial catch, discard and biological sampling (PGCDBS)", Oostende, organisation and contributions CLO-DVZ
- 9th of March: collaboration to workshop "Omega-3vetzuren en CLA in de landbouw: perspectieven voor boer en consument", CTL-Gent, organisation MVG, ABKL, VLT
- 17th of March: Workshop "In het oog van de storm: de Vlaamse visserij op de drempel van de 21^{ste} eeuw".
 Scarpoord, Knokke; organisation CLO-DVZ in association with VLIZ and SDVO.
- 18th of March: Workshop cut flowers. "Nematodes: what are they and what do they do?", CLO-DGB, organisation Proefcentrum voor Sierteelt, Destelbergen
- 21th of March: Meeting with the practice centres.



"Nematodes. The good, the bad and the ugly", "Scientific research in the lab of entomology", "Fungi in the ornamentals / research activities CLO-DGB", "Company hygiene rediscovered", CLO-DGB

- 22th and 24th of March: Workshop Provinciaal Proefcentrum voor de Groenteteelt, Kruishoutem.
 "Recognising pests and natural enemies – part 1: Acarology", CLO-DGB
- 23th of March: Workshop about the improvement and maintenance of permanent grassland. "Oversowing with white clover", Bocholt, organized by the Agricultural Advisory Centre Limburg, in association with CLO-DFE
- 24th of March: "Vlaanderendag Open huis bij de Vlaamse Overheid". Open house at CLO-DVZ, Oostende. 1600 visitors in the presence of Minister-President Yves Leterme. Organisation: MVG and CLO-DVZ, in association with Dienst voor Zeevisserij
- 26th of March: collaboration to WPSA-workshop, CLO-DVV
- 15th to 24th of April: Floraliën, Gent. Participation CLO-DVP
- 4th of May: Co-organisation WRSA-workshop. Faculty of Veterinary Medicine of the UGent, Merelbeke, in association with CLO-DVV
- 14th of June: Workshop "Potential of clover and Lucerne on a progressive cattle farm", Merelbeke, CLO-DFE, in association with CLO-DVV
- 17th of June: Workshop "Facets of the agricultural research at the CLO: pillar for the future ILVO".
 "Biological control of root-knot nematodes with *Pochonia chlamydosporia*: a step towards reduced use of methyl bromide", CLO-DVP
- 14th of July: Workshop "A future for legume fodder crops", meeting in the framework of a demonstration about protein crops on the farm, Merelbeke, CLO-DFE
- 14th of September: Visit Joe Borg, EU-Commissioner

for Fisheries and Maritime Affairs and Minister-President Yves Leterme to CLO-DVZ.

- 19th to 22th of September: 35th Conference of the West European Fish Technologists Association (WEFTA), Antwerp; organisation CLO-DVZ in association with RIVO
- 23th of September: "Evolutie van analytische technieken in het voedingslaboratorium". Symposium as a result of 10 years BELTEST accreditation by CLO-DVK.
- 24th to 25th of September: "Internationale Werktuigendagen", Oudenaarde. Participation CLO-DVP, CLO-DFE, CLO-DVV, CLO-DVL
- 6th of October: Workshop organic vegetable culture in the greenhouse. "Biofumigation", CLO-DGB, organisation Biokas (the Netherlands) and the Interregproject "Cross-border organic farmers", in association with het Provinciaal Proefcentrum voor de Groenteteelt, Kruishoutem
- 27th of Oktober: Collaboration to WPSA-workshop on CLO-DVV
- 17th of November: 3th workshop TAD FarmCOMPOST. "Assessment soil quality and management", CLO-DFE
- 6th to 11th of December: Agribex, Brussel. Participation CLO-DVP, CLO-DFE, CLO-DVV
- 6th of December: Study evening early potatoes at Tielt.
 "Bad stands of potatoes in rotations with vegetables or field crops frequently caused by nematodes",
 Tielt, organisation Interprovinciaal proefcentrum voor de aardappelteelt vzw, with CLO-DGB
- 9th of December: Presentation of the "Belgian descriptive and recommended variety list for forage and green manure crops 2006", CLO-DFE
- 28th of December: Workshop 'Innovatiecentrum Duurzame & Ecologische Visserij (IDEV), organisation and contributions CLO-DVZ

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