



Activity Report 2003 – 2004

Flemish Agricultural and Fishery Research

Agricultural
Research
Centre



Ministry
of the Flemish
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INTRODUCTION

This report – the first since the Agricultural Research Centre (CLO) is part of the Ministry of the Flemish Community - presents an overview of the research activities and results of CLO over the past two years. In this period CLO was the scene of a series of screenings and audits. The outcome of these forms the cornerstone of the CLO future whereby the knowledge-building and policy-supporting research activities as well as its services to the community will be included in the Institute for Agriculture and Fishery Research – ILVO – in three units: plant, animal and technology and nutrition.

The mission of ILVO will look as follows:

- Contributing towards carrying out and coordinating policy-supporting scientific research in view of a sustainable agriculture, horticulture and fishery in an economic, ecological and social perspective.
- Building-up knowledge required for updating and improving production methods, for monitoring the quality, for safeguarding the end products and for improving the policy instruments as a basis for the development of the sector and the agricultural rural policy.
- Disseminating the research results to policy-makers, the agricultural sector and to society.

Research is an evolutionary and self-adapting activity directed towards the future and confronted with policy goals and social values. This is also the framework in which the consultations with the administration and the cooperation with the universities, colleges, research institutes, demonstration centres and the whole sector are situated.

Despite the time and energy invested during this transition period, the dynamics of research were never under threat. On the contrary, the success of attracting funds from the market resulted in a pool of enthusiastic collaborators. We hope that in future CLO/ILVO will be able to continue to act as selector for filling in vacancies in research projects.

This hitherto biennial activity report of CLO is already based on the future structures and focuses on a number of results linked to our strategic goals. It documents the CLO contribution to support the government policy for establishing sustainable agriculture and fisheries. Besides the latter, a series of remarkable achievements of the past two years are extra highlighted. The listed publications are another indicator pointing to our output.

The researchers and collaborators are the chief assets of CLO. We may be proud of their high quality and professionalism. The results of their efforts are briefly described in this report. On behalf of CLO I am very pleased to express my gratitude to all who have, directly or indirectly, made this possible and have supported us and gave us their confidence.

I am very much indebted especially to Ir. Herwig Keymeulen who kept the CLO-ship afloat during the past sixteen years despite the reorganisation and the regionalisation.

Prof. dr. ir. Erik Van Bockstaele
acting general director



Ir. Herwig Keymeulen, general director of the Agricultural Research Centre for the past 16 years, has completed his active career on 31 December 2004.



His successor **Prof. dr. ir. Erik Van Bockstaele**, acting general director of the Agricultural Research Centre from 1 January 2005.

1. ONE AGRICULTURAL RESEARCH CENTRE/SEVEN DEPARTMENTS/THREE KNOWLEDGE UNITS

CLO, the Agricultural Research Centre, consists of seven departments, which are described in brief below. They are located in Merelbeke and Melle, except the Department of Sea Fisheries which is located in Ostend.

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

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The key tasks of the department focus on research to generate new knowledge and to support policy in the field of plant genetics and breeding. A multidisciplinary approach has a central place in the various programmes of the department. In the context of the implementation of European regulations on GMOs, techniques are developed and evaluated for detection and quantification. DNA marker technology is also used for the development of new selection and breeding techniques and the study of biodiversity in various crops. In the framework of international agreements, maintaining the genetic heritage is an important aspect. 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 the exploitation of interesting genetic characteristics for the development of sustainable agriculture and horticulture on the other hand. Crossings, which focus on developing pre-breeding material, are linked to this. Improving the resistance to diseases and plagues is a priority in this respect. A better use of nitrogen by new varieties of grasses is also an important point for attention. In



ornamentals, interspecific hybridisation techniques, which increase the genetic variation by means of pollination between species, are frequently used. Research into alternative applications and uses of crops focuses on the variation and heredity of the inulin chain length in chicory, on the backgrounds of the production of bioactive prenylflavonoids in hops, and on the possibilities of improving the protein level in grasslands.

In the context of developing sustainable agricultural systems, the department is examining the possibilities of biological seed propagation and integrated weed control in seed production.

In addition to the above-mentioned emphases in research, the department carries out activities with regard to authenticity control, GMO detection, post control and seed certification to provide services for government, the agricultural and horticultural sector, as well as third parties. It is also responsible for producing high quality starting material for the agricultural and horticultural sector.

1.2 Department Crop Husbandry and Ecophysiology

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The Department of Crop Husbandry and Ecophysiology (CLO-DFE) conducts scientific research on the behaviour of grass, fodder crops and ornamental plants under different growth conditions. The aim is an improvement of yield and quality as part of a sustainable agriculture and horticulture with respect for man, animal and environment. The research at the department is part of the policy support and gives service to the sector.

In relation to the production of arable and forage crops, much attention is given to the rotation schemes, tillage practices, carbon sequestration in grassland and urban areas, leguminous crops on the farm level, solutions for less mineral (N and P) surpluses and losses, the reduction of nitrate residues in the soil after the growth and grazing season, the chemical composition and feeding value in the relation to growth stage and fertilisation level. According to the Recommendation of the EU Commission research is going on concerning guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming. Test for the DUS and the VCU of new varieties of agricultural crops, according the EU Directive 2002/53, remains an important research theme. Besides the official trials, a descriptive and



recommendation list of varieties is edited. For EU (DG SANCO) “community comparative trials” for winter wheat were organised in 2003 and 2004. The accredited laboratory for the control of certified seed lots and tubers was incorporated in the activities of the department. The fundamental and applied ecophysiological research focusses on the photosyntheses of plants grown in sub-optimal conditions (lower N availability) and stress situations (cold stress). Algorithms for morphologic characterisation of crops via image analyses are under development. The department has a technological advisory service (TAD-IWT for the production and use of microbial controlled compost (CMC compost) in agriculture and horticulture. A well equipped chemical laboratory can analyse (inter) departmental samples of water, soil, substrates and forage crops, but may also support outside partners. CLO-DFE has a good expertise on the federal, national and European level and maintains a good relationship with Belgian and foreign research institutes, universities and scientific institutes, especially in Central and Eastern-Europe.

1.3 Department of Crop Protection

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Scientists of the Crop Protection Department are active in five different disciplines: viz. acarology, bacteriology, entomology, mycology and nematology. In each of these disciplines they contribute to durable plant production by both their policy supporting and knowledge acquiring research. They organise their research along three major lines: (i) improvement of plant pathogen identification and detection; (ii) improvement of the knowledge on plant-pathogen relationships and the factors that influence these relationships; (iii) development of integrated control methods. The first line applies modern biotechnological techniques to characterise species and, if necessary, subpopulations. The second line uses field observations and precise, if needed on the genetics, laboratory observations to increase the knowledge of the ecological (biotic and non-biotic) conditions determining the plant-pathogen relationship. The development of management strategies aims at the reduction of input of pesticides, whilst solutions are tried to be found via resistance and/or biological antagonists.

Via the Diagnostic Centre for Plants, the expertise acquired in the research is put at the service of both public authorities and private persons. In this Centre diseased plants are diagnosed and pathogens are searched for in substrata, plants or plant products.



1.4 Department Animal Nutrition and Husbandry

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CLO-DVV carries out applied scientific research in animal production aiming at a more efficient and therefore environmental friendly animal nutrition and a sustainable, consumer oriented and socially accepted 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.

The society is concerned about the environment and asks for more diversity on the countryside. Therefore, efforts are done 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 by dietary as well as housing techniques. Examples are ad libitum



feeding of pregnant sows with fibre rich feeds, group housing of rabbits and sufficient physical structure in the diet for cattle.

Due to the growing awareness and demand for a healthy human nutrition, research is increasingly focused on the healthy value of animal products. Dietary possibilities are studied to enrich milk, meat and eggs with nutrients important for the health of the consumer. This is called functional animal nutrition. Furthermore, CLO-DVV takes care of quality in animal production by participating in excretion studies of residues of animal medicines and contaminants in animal products.

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

1.5 Department Animal Product Quality and Transformation Technology

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DVK-CLO performs research aimed at the safety, authenticity and functional quality of food of animal origin in order to sustain a durable policy, a better protection of the consumer and an improvement of the market position of the Flemish producer. Research is carried out on the behaviour of zoonotic microorganisms in the food production chain. The development, evaluation and application of molecular identification, detection, typing and gene expression techniques play an important role in this research. In order to break contamination cycles, the use of adapted feed strategies is evaluated for the reduction of the shedding of pathogens. The problem of the carry-over of harmful aerobic sporeformers in raw milk and of egg shell contaminations is studied. Through risk assessment the effect of diverse remediation measures is quantitatively evaluated. Detection methods for contaminants and residues of veterinary medicines in diverse matrices are being developed. Microbiological tests and receptor- and immunoassays are being developed and evaluated. For the confirmation of a positive



screening result chromatographic-mass spectrometric methods are being developed and validated. This is necessary in order to trace and remediate the contamination source. Aspects as faecal recycling, cross-contamination on the farm and migration get special attention. In the frame of fraud control research is carried out on techniques to determine species and treatment authenticity. Chemical-physical research is carried out to give foodstuffs a higher added value by means of a changed composition or technology. Problem solving research is done in relation to farm dairy products. A technological pilot infrastructure is available for the Flemish farm dairy processors and the food industry. DVK is the national reference laboratory for milk and milk products and has accredited laboratories for service, advice and counter expertise. DVK carries out the scientific guidance of external laboratories, such as the Flemish Interprofessional Organism responsible for the composition and quality determination of the raw milk.

1.6 Department of Mechanisation, Labour, Buildings, Animal Welfare and Environmental Protection

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

The research into farm buildings focuses on the construction and equipment of animal houses with special attention to the reduction in the emissions towards the environment (ammonia, dust, etc.) and to the well-being of man and animal.

Studies concerning labour organisation and management on the farm and integration of technical means (like mechanisation, automation, robotics) can lead to a substantial improvement in the welfare of the agricultural and horticultural worker. To improve the welfare of farm animals research is carried out into the development of methodologies for measuring and monitoring animal welfare (both on farm level and on experimental level). Using these methodologies several existing and new and more animal-friendly production systems are evaluated (for cattle, pigs and poultry).

The environmental research at the department is mainly directed on the reduction of emissions originating from animal husbandry: from animal



houses as well as manure stores and the field.

Concerning the latter various spreading techniques for animal slurries and mineral fertilisers are tested and measuring protocols for assessing these techniques are optimised.

Research into agricultural mechanisation focuses on the development of measuring techniques, procedures and equipment to determine the technical characteristics of agricultural and horticultural machinery.

New technologies are applied for optimising the performance of these machines and for improving the quality of the products and the protection of the environment.

In 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 especially to maximise the quality of the harvested, stored and processed products.

Besides the applied scientific research the department possesses a knowledge and service centre consisting of technological advice centres viz. AgriConstruct and AgroMech, a Spray Technology Laboratory, the Inspection Service for Spraying Machines and the Project 'Control' (quality management: maintenance of milking equipment).

1.7 Sea Fisheries Department

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The main task of the Sea Fisheries Department is to supply fisheries policies and fishery-related environmental policies with sound scientific advice, on request of international scientific committees, management bodies, national and international authorities and the fishing industry. Our scientific knowledge creates the necessary basis for a rational and sustainable exploitation of the marine environment from a biological, technical and socio-economic viewpoint.

Internationally, there is a growing need for policy advice (in casu towards fisheries) based on integrated and multidisciplinary scientific data. Starting point is an 'ecosystem approach' where the marine environment is regarded as a habitat for different sea fishery products, with the quality of these products determined by the quality of the marine environment itself.

To contribute to this ecological approach, the Department aims at a thorough integration of her studies in the field of: (1) fisheries biology (population dynamics, exploitation, stock assessment, discards, sexual maturity); (2) aquaculture and restocking (fish and shellfish culture, regeneration of natural stocks); (3) technical fisheries research (sustainable fishery techniques, new fishing equipment, methods, materials and testing methods);

(4) quality of the marine environment (biological, physical and chemical effects of anthropogenic activities, baseline studies on biodiversity of benthic organisms and demersal fishes); (5) quality of fishery products (genetically based authenticity, freshness and traceability of different fishery products).

The objectives of the Department will help to develop optimized sampling protocols and analytical methods in support of management measures and models for fisheries, aquaculture, the marine habitat and the quality of products from fisheries and aquaculture. Eventually, the biological and technical research can be extended with a socio-economic part (e.g. the proposal to culture mussels in open sea). The mutual integration will lead to a better insight in the structural and functional processes in the marine environment, resulting in a more sustainable exploitation of that environment and the protection, conservation and recovery of the commercial fish stocks.



Display of the OMEGA mesh gauge



DVZ-stand at the Ostend Fair

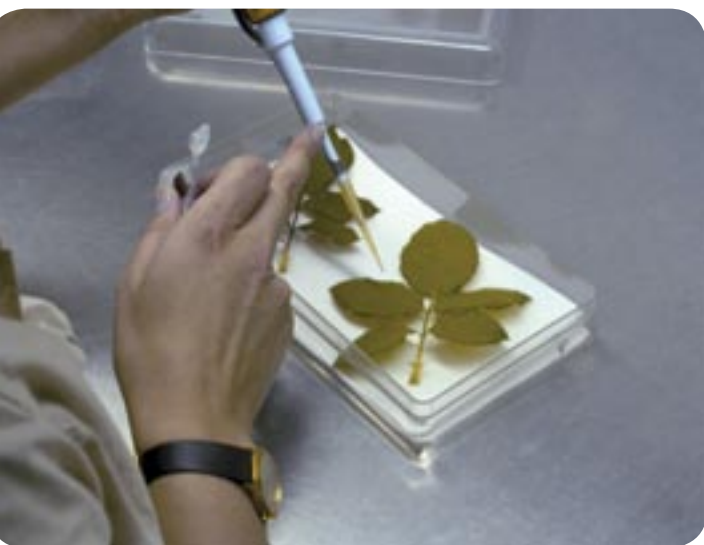
2. KNOWLEDGE UNIT PLANT

2.1 Genetic biodiversity: source for breeding

Exploitation of the existing natural genetic variation for most agricultural and horticultural crops is limited at present. Nevertheless wild species form an important gene pool that can be used to add new and desirable traits to crops. An example of this is the rose. The genetic basis on which modern rose cultivars are established is very small. Only between 8 and 20 species out of about 200 have contributed to the origin of our present-day rose cultivars. Many of the as-yet-unexploited wild species contain genes and traits of interest, such as fragrance, esthetical value, attractive hips, strong bushiness (as natural fences), winter hardiness or disease resistance. The utilisation of this wild germplasm is an important strategy for the conservation of species. CLO-DVP works in Flemish and European (GENEROSE) research projects involved in screening the genetic diversity of wild roses and investigating the possible use of these species in pre-breeding programs.

Wild populations were sampled in recent inventories of autochthonous rose species in Flanders and Europe in view of conservation strategies. Using AFLP-markers and microsatellite DNA data, the genetic diversity within and between species is being studied. A more in depth study on the complex genetic base of autochthonous populations is being made in collaboration with other international research groups. The first results show clearly that the genetic biodiversity between wild rose species is great, and this variation is also expressed phenotypically. A determination of this so-called functional diversity (determination of useful and horticultural relevant traits, such as flowering, hip formation, growth characteristics and disease resistance), is therefore a prerequisite for the exploitation of the present biodiversity in practical rose breeding.

At this moment disease resistance is the most important research topic. Screening techniques





which make it possible to test resistance against two of the major rose pathogens, black spot and powdery mildew, have already been developed. In addition, crosses are being made between diploid cultivars and species in order to create segregating populations useful in resistance research. Knowledge of the segregation is essential for specific breeding goals in the future. The possible use of molecular markers, which can gain significant time in the breeding process, will also be evaluated.

Interspecific hybridisation between species and cultivars is frequently limited by crossing barriers, which are mainly caused by differences in ploidy level. Modern rose cultivars are mostly tetraploid, while wild species and older cultivars are diploid. Species from the section *Caninae* are pentaploid but produce haploid pollen via a special meiosis. In combination with the possible formation of unreduced gametes, this aberrant meiosis offers possibilities for ploidy breeding. Crosses between diploid and tetraploid roses frequently result in sterile triploids. This sterility



is however not always absolute. Observations show that fertile pollen, both haploid and diploid, are produced. The use of this diploid pollen can offer an alternative crossing route via chemical-induced chromosome doubling (i.e. with oryzaline). Another strategy used to overcome ploidy differences is dihaploidisation. In this strategy the culture rose is pollinated with dead pollen that have been irradiated. Embryos are formed without fecundation taking place. After regeneration of plants from these embryos crosses can be made at the diploid level. Today, CLO-DVP implements all these techniques in its crossing programmes. Within a few years, this should lead to the development of new pre-breeding material with important genetic traits that can be used by breeders for developing resistant rose cultivars.

2.2 DNA-markers help to create sustainable plant varieties

In the context of sustainable agriculture, the main objective of forage production systems is to maximise the productivity and the quality of the forage, while minimising negative environmental effects. Feed produced on-farm is essential for sustainable ruminant livestock production. Thus, in order to fulfil this objective the development of improved production systems for forage grasses is required. In contrast to cereals, forage grasses allow for economical, environment-friendly feed production under low levels of pesticide and fertiliser application. Until recently, the main breeding objectives for forage grasses were to increase yield and persistence. However, in the future fodder grass breeding programmes will have to pay more attention to the specific necessities of livestock and the environmental impact of farming activities. Breeding methods are less advanced for perennial ryegrass (*L. perenne*) compared to other crops, despite the worldwide

agricultural and economic importance of perennial ryegrass (*L. perenne*) as a forage crop.

In the past decade the amount of information regarding the biological function of hundreds of genes has increased dramatically. In most cases, this information was derived from studies on the model plant *Arabidopsis thaliana*. Also genes putatively involved in important traits for forage grasses have been identified and characterised in *Arabidopsis*. CLO-DVP participates in one European project (GRASP), which aims to implement this knowledge in perennial ryegrass breeding programmes.

Plant breeding relies on the slight differences between alleles (or forms) of a given gene. The characterisation of these slight differences in genes involved in relevant traits is of particular importance for the development of competitive breeding programmes. These slight allelic differences are 'allele-specific' DNA-markers called SNP (or Single Nucleotide Polymorphism) markers. One of the main objectives of the GRASP project is to identify and validate SNP markers in genes of relevance for sustainable ryegrass breeding. These genes of interest are related to forage quality and environmental sustainability, and include N-use efficiency, water soluble carbohydrate content, leaf elongation, rooting, stress tolerance, disease resistance and flowering. This approach will allow us to study the function of relevant genes and constitutes a realistic, applicable strategy for the improvement of forage grasses.

In collaboration with the different GRASP partners, 100 genes for agronomically relevant traits have been identified and isolated in perennial ryegrass to date. Currently, the different allelic forms of these genes are being studied in a set of 20 perennial ryegrass plants. The main objective is to develop SNP-markers within these genes. Furthermore, populations of perennial ryegrass that have been subjected to divergent phenotypic selection for different traits will be used to investigate the involvement of these genes in the determination of the traits studied. A significant

difference in allelic frequencies is expected for those genes involved in the traits under selection. Thus, we will be able to identify the allelic forms of genes associated with an improvement of the traits under investigation. The technologies for the screening of SNP-markers are relatively cheap and automatic, and thus will be directly applicable in perennial ryegrass breeding programmes.

2.3 Sustainable management of diseases and pests

2.3.1 Soil sampling for a better management of nematodes in field-grown vegetables

Pratylenchus penetrans is a migratory endoparasitic nematode with a large host range. The nematode is widely distributed in Flanders and is especially important in tree and rose nurseries and field grown vegetables. *Meloidogyne chitwoodi* and *M. fallax* are sedentary endoparasitic nematodes with quarantine status. These latter reduce the quality of carrots and black salsify in the North of the Provinces Antwerpen and Limburg.

A precise estimation of nematode densities is the corner stone of their integrated control. Nematode densities are accurately estimated by soil samplings that consider the distribution of the nematode. This depends on the host plant, the time of the year and the soil type. In order to develop a suited sampling strategy for *P. penetrans* we monitored the nematode population dynamics under several crops over a full year, i.e. also in the post harvest period. In all of the crops the population density increased during the cropping season and reached a maximum at a few weeks before the harvest. During the remaining time before the harvest and during the fallow period that followed, the population decreased continuously. Under maize, black salsify, carrot and potato the nematode population density decreased with 62%, 44%, 66% and 67%, respectively. The increase and



decrease could be modelled with a double logistic curve. As the plant development also follows a logistic curve, the presumption that nematode densities are proportional with the size of the feeding source, is confirmed.

We also examined the vertical distribution of *P. penetrans* over a soil profile up to 70 cm depth and monitored its densities in the organic and mineral fractions of the soil. In the majority of the crops the

organic fraction contained more than 50% of the total amount of nematodes. The results confirm that both fractions should compose a soil sample if one wants to estimate the nematode density accurately. Except under maize, *P. penetrans* was not detected at depths greater than 50 cm. The distribution of the nematode was independent of the sampling time. From these results it can be concluded that the optimum sampling depth for maize, black salsify, carrot and potato is 45, 25, 25 en 35 cm, respectively.

To optimise soil sampling for *M. chitwoodi* we examined its distribution in other fields. Under sugar beet, the greatest number of nematodes was detected at the level between 30 to 50 cm. Obviously, the standard procedure limiting sampling at the first 20 cm underestimates the infestation.

Meloidogyne chitwoodi dies in great numbers during winter but can survive these adverse conditions in the resting egg stage. This form of eggs is produced when adult females feed on roots of senescing plants. Adding root diffusates whilst incubating this kind of eggs, stimulates juveniles to hatch and allows for a better prediction of the population.

2.3.2 Quarantine diseases in potato

The potato production in Belgium has been challenged repeatedly by plant health problems in the past few years. The incidences of brown rot (*Ralstonia solanacearum*) in ware potatoes and of ring rot (*Clavibacter michiganensis* subsp. *sepedoncus*) in seed potatoes have caused great concern on the sanitary condition of our potato production, which by return would affect the trade position of the whole sector. A programme of systematic monitoring of domestic and imported potato stocks is implemented yearly. Relevant ecological key issues for dissemination and



Potato tuber infested with Meloidogyne chitwoodi, the maize root not nematode

persistence are surveyed complementary. 1000 sample points are established in various rivers and canals associated with drain points of potato industry sewage and household sewage treatment stations. The various potato processes from washing to transformation are surveyed in 150 potato processing premises.

Survival and persistence are also the key elements in the research on both bacteria which objective is not only to provide insight in the ecology but also to generate reliable instruments for containment of infection and for safe handling and disposal of contaminated potato stocks. The fate of *Ralstonia solanacearum* in soil is particularly monitored in the potential viable but non-culturable condition, by which the pathogen remains viable in the soil but cannot be isolated on culture media and consequently escapes from being detected. Field persistence for more than one year after infection of brown rot was demonstrated in weed rhizospheres, specifically in *Solanum nigrum*, black nightshade. However, in case of failure of isolation persistence in a non-active form was not expressed in bioassays. Other studies identify the parameters by which contact contamination is transformed into infection in the progeny tubers. Differences in transmission were scored for contamination on the skin at the heel end or at the eyes. Elaborated protocols for disinfection of tubers or decontamination of materials and surfaces involve the use of peroxygen based compounds which are most reconcilable with security for the producer and safety for the environment.

Also nematode problems are of concern in the potato growing industry. A survey performed a few years ago, revealed that the potato cyst nematode (*Globodera spp.*) is wide-spread in some potato growing areas. Data showed that 95% of the infested fields contained *G. rostochiensis* and 5% were infested with *G. pallida*. Lately, many of the soil samples taken by growers in problem fields, contain *G. pallida*. In many cases, potato cultivars with resistance to *G. rostochiensis* are grown in these fields, resulting in an increase in *G. pallida*. A monitoring system for the species and pathotypes in potato fields is therefore warranted. PCR-techniques

are available for this and a test for choice of cultivars has been optimized with which the pathotype can be determined indirectly.

A small survey for antagonists of potato cysts revealed that 80% of the cysts are infected with fungi or bacteria. Instead of counting the number of cysts as a degree of field infestation, it is therefore more important to determine the live content of the cysts. Other quarantine organisms that are spreading in potato cultivation areas, but also in field-grown vegetables and other field crops, are the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*. The optimal detection method of these nematodes in potato tubers was examined. The distribution of the nematode in the tuber was determined and several extraction techniques were compared. A fast detection technique was developed and allows for screening of large quantities of potato tubers.



Bioassay to determine the susceptibility of *Rhododendron* species and cultivars for *Phytophthora ramorum*

2.3.3 *Phytophthora ramorum* on *Rhododendron* in Flanders

Phytophthora ramorum is a newly identified *Phytophthora* species, responsible for extensive mortality of local oak species at the US west coast (Sudden Oak Death or SOD). In Europe, the fungus is mostly found on *Rhododendron* and *Viburnum* in horticultural settings but a few findings on oak, beech and chestwood in England and the Netherlands show that European trees can also be affected. The danger for an epidemic in commercial horticulture and the risk for spread of the pathogen to public park and forest settings have led to EU phytosanitary measures. These include inspections at all premises where *Rhododendron*, *Viburnum*, and *Camellia* are grown and led to serious consequences for the nurseries where *P. ramorum* is found. The problems with *P. ramorum* are complex because they involve commercial aspects (direct and indirect effects on the horticultural sector) and aspects of public concern (risk for infection of local trees). Research at CLO-DGB concerning this pathogen is aimed towards optimal control in nurseries and towards scientific analysis of the current phytosanitary measures. Part of this research is conducted in collaboration with FAVV, which is the Belgian agency responsible for the execution of the EU phytosanitary measures. In regard to the first aspect, the optimal control of the pathogen in nurseries, the relative susceptibility of 63 *Rhododendron* species and cultivars was examined. Also, with the aim of optimal and sustainable use of fungicides, the effectiveness of potentially useful fungicides was tested. Other aspects of the biology of *P. ramorum* which are studied are survival at different temperature and humidity regimes and identification of the most important conditions and plant parts involved in infection. In regard to the second aspect, the scientific analysis of the current phytosanitary measures, the spread of *P. ramorum* at the level of nurseries is investigated. This involves extensive sampling at a site where *P. ramorum* has been identified. The location of all findings is registered and used to evaluate the relative spread of the pathogen inside and outside the current quarantine zones.

2.4 Crop production with reduced input and under sub-optimal growing conditions.

2.4.1 Cold tolerance of agricultural crops: Is the growing season starting earlier?

In the frame of a sustainable agriculture, a crop should cover the soil the whole year. By this, there is a continuous uptake of nutrients and soil erosion is reduced. Grass can be seen as a model crop, as it protects the soil during the whole year. For annual crops (i.e. maize and chicory) this is not the case. In springtime the crop is sown, only when a period with improved climatic conditions is reached. Early sowing is full of risks due to the cold sensitivity of the crop. However, an early sowing is desirable, since this implies early nutrient uptake and reduction of soil



Cold tolerance with maize

erosion (environmental aspect) but also encourages an early growth and a longer growing period with a higher yield (crop related aspect). Furthermore, a crop which is sown earlier on the field, will cover the soil faster, and by competition it will reduce weed growth. Growth of young crop plants is being hampered by the cold growing conditions. Especially in combination with high light levels, plants are being stressed more.

Growth of plants is being controlled by the available temperature, needed for the chemical processes, and

the amount of available photosynthetic active light to drive the photosynthesis process and production of sugars, enabling plant growth. Due to a too low a temperature, chemical processes are slower and building components for growth are being made and/or released at a slower rate. Furthermore, a combination of high light levels and low temperature (i.e. during springtime mornings (high light levels and frost)) can lead to photoinhibition of photosynthesis. Photoinhibition is at first a series of regulating processes to start inefficiencies at the level of the photosynthesis system to protect this system.

The photosynthesis process consists of 2 sub-processes: light reactions and the dark reactions. The light reactions take care of the conversion of light energy to chemical energy (ATP and NADPH) and are light dependent. The dark reactions are temperature dependent. CO₂ and energy are being used to produce sugars. When the temperature is too low, there is not enough warmth to maintain the process speed of the dark reactions. Due to the reduced use of high energetic molecules (ATP and NADPH) in the dark reaction, a feedback will occur at the level of the light reactions. This means that at high light level in combination with a low temperature the excess of absorbed light needs to be dissipated. As light is being caught by the "light harvesting complex", a chlorophyll molecule is being excited and brought in "singlet state". This molecule falls back to the ground state after passing the energy in one of four possible ways: (1) the energy is being passed to reaction centres for photochemistry (that means the dark reactions, but at low temperatures this is only a small sink); (2) the energy is reemitted as light with a longer wave length (a small energy loss occurs), this is chlorophyll a fluorescence; (3) energy can be dissipated through thermal processes (heat production); (4) the "singlet state" chlorophyll can be converted to a "triplet state" which in turn can pass its energy to O₂, forming a very reactive oxygen radical. This can result into damage to the chloroplast. When light/temperature stress holds on damages in the leaves can occur due to the formation of radicals (very reactive chemical components) which are destructing leaf structures. The way the different pathways are being used will

determine the tolerance of the leaf (plant) against photoinhibition and consequently the youth growth under cold conditions.

At the Department of Crop Husbandry and Ecophysiology (CLO-DFE) the cold resistance of different varieties of different crops is being tested. It is important to find the processes which are most influenced by this stress. The aim is to develop a fast non-destructive method/experiment to test the resistance of plants/varieties against this stress. This objective test can then be used, as well in variety trials as in breeding programmes.

2.4.2 Variety behaviour in normal and vulnerable regions of Flanders

In the framework of the current fertilization problematic (MAP-regulation) there are in Flanders regions with sharpened fertilization norms (i.e. in regions with water collection and zones for extension). The strong extension of vulnerable regions in Flanders (almost 50 % of the surface) means for most cultures an important reduction of N-fertilization. In these vulnerable zones, the culture of especially forage crops (grasses, silage maize) shall be executed under sub-optimal conditions for fertilization. Questions about the profitability of the culture will be formulated by the farmers.

An important factor for the profitability is the variety choice. The varieties, which are now on the market, are bred under optimal conditions (high input) of fertilization. From the point of view of farmers with agricultural land in zones with sharpened fertilization norms, there is the question about the variety behaviour from the best varieties of the actual recommended list: will these varieties also be the best under lower input? To give an answer to this question, research was executed at CLO-DFE during several years with silage and corn maize. Recently perennial ryegrass and timothy varieties were also tested at different N-input.

For the trials with silage and corn maize a selection of varieties was made, based on the Belgian descriptive



and recommended variety list. Different agricultural and morphological characteristics were important: earliness (early & late type), yield (low and high), early vigour and quality. The trials were set up in sandy soils in the extension zone of a region with water collection. There were trials at 2 farms. On the first farm the trial was set up on a field with no input of N during the last 4 years (low input). At the second farm maize was cultivated in a rotation with grasses. Every year 25 ton/ha of manure was given, supplemented with anorganic fertilization; the total amount of fertilization was based on a soil analysis.

Between both trials for silage maize, no different ranking order for early vigour and flowering date was found. For the low input the plants were much shorter, compared to the normal (high) input. The total dry matter yield (average 2001 till 2003) was 1/3 lower at the low input field. For this parameter the ranking order of the varieties was different, especially the high productive varieties are scoring much lower under low input. A higher fertilization resulted in general in a later ripeness but the ranking order of the varieties was nearly the same. Differences in quality (digestibility, starch) were very small between both

trials. The differences in N-content were 0,75 % (low) and 1,2 % (high); for this characteristic the ranking order was also the same. N-export (dry matter yield x % N) was on average 100 kg N/ha at the low and 210 kg/ha at the high input, with an analogous classification of the varieties as for the total dry matter yield.

For corn maize the differences between the varieties were higher, compared to silage maize. The ranking order for earliness was the same at both trials. Concerning the grain yield some varieties attain at the low input only ½ of the yield at high input; others have only a reduced yield of 30%. Between both trials there was a different ranking order for the varieties.

The study for the N-efficiency for grasses started in 2003 at 2 locations (Merelbeke – sandy loam; Geel – sandy). Four semi-early and 4 late varieties of perennial ryegrass and 3 varieties of timothy were incorporated in the trials. The varieties were tested at 2 fertilization levels: 225 and 350 kg N/ha/year. The mowing dates were in function of the grass growth; they were different for both N-levels. The following parameters will be evaluated: % of dry matter, yield of dry matter, N-content, N-export and digestibility. The N-level in the soil will be analysed in spring and in autumn.

As conclusion we can state that a good knowledge of maize varieties, especially for the culture of grains, will be very important in regions with sharpened fertilization norms. Under extreme fertilization conditions, varieties can show a different behaviour. Therefore it is important to test varieties of the catalogue in regions with severe norms of fertilization. Differences in variety behaviour of grasses under both N-levels will only be clear after several years.

2.5 Sustainable crop systems: Forage legumes, a biological source of nitrogen

Forage production in Flanders is almost completely based on grass and maize. Maize silage is the energy source whereas grass and concentrates (imported soya) deliver the protein to balance the ration. A strong limitation in the use of N and P (Flemish manure action plan), the increase in price of the high-protein concentrates and subsidies are responsible for a renewed interest in forage legumes. The Flemish farmer is especially interested in the use of white clover for grazing purposes in permanent grassland and to a lesser degree in red clover (lucerne) as a temporary crop, eminently suitable for silage cropping.

CLO-DFE is concentrating on research concerning the potential of production (dry matter, energy and protein) of these crops in monoculture or in association with grasses, feeding value and adequate management. CLO-DVP is breeding improved varieties of white and red clover. The selection criteria are different for both crops. A major challenge in white clover breeding is the improvement of the persistency: new varieties must be more persistent and reliable in permanent grassland under grazing and a moderate N-fertilisation management. A good winter hardiness and an early spring growth are crucial in this matter. The most important selection criteria in red clover are disease resistance, yield, good re-growth after cutting, persistency and a high seed yield. The CLO-DVP varieties are suitable for the European market.

Experiments with white clover in permanent grassland are mainly executed under grazing conditions. It is more complicated and time-consuming in comparison with a cutting regime but the results are more relevant for the farmer. Research focused on the effect of a decrease in N-fertilisation in combination with the use of grass clover mixtures on dry matter yield, feeding value and botanical composition of the sward started in 1996 and will

First cut grass-clover mixture



be finished in 2005. The first results show a small difference in net dry matter yield (approx. 0.9 ton ha^{-1}) and a comparable feeding value between an intensive managed grass sward with 330 kg N ha^{-1} and a grass clover sward with 170 kg N ha^{-1} . There is a lower sward density on the grass clover treatment caused by a very high level of white clover. Three systems of clover sowing in existing grassland were compared by measuring the white clover frequency during 3 consecutive years in 3 locations. Slot seeding was clearly most successful. Direct drilling and especially broadcast oversowing after harrowing achieved very variable results. Recently, research

intends to investigate the influence of white clover in the sward on the nitrate content in the soil profile (0-90cm) at the end of the growing season as a parameter of potential nitrate leaching to the ground water. The relationship between clover frequency and management on the one hand and nitrate content of the soil on the other is investigated on pastures with a high clover content. In another experiment the behaviour of white clover is studied in monocultures or mixtures of perennial ryegrass and timothy to ensure a good long term clover development. Researchers from CLO-DFE/DVP are collaborating with colleagues of several West-European countries

in the COST 852 project. In 2002, a common experiment was set up to investigate productivity and persistency of mixtures with several clover varieties in comparison with a single clover variety.

In a cutting system of forage crops, red clover (lucerne) can be considered as a crop with a high potential in the crop rotation. Topics of this research are: the potential of sowing in a cover crop, the sowing period (autumn, spring), the association with perennial and Italian ryegrass or timothy for 2-3 years and the effect of a supplementary N-fertilisation on the clover content in the forage and the potential yield.



3. KNOWLEDGE UNIT ANIMAL

3.1 Animal welfare

3.1.1 Ad libitum feeding with fibre-rich diets of pregnant sows housed in group

Already today group housing of pregnant sows is obliged when starting renovation or new construction of sow stables. From 2013 onwards all pregnant sows have to be housed in group. Besides the problems concerning the behaviour of sows in group, their nutrition also deserves attention. For the moment, the majority of pregnant sows are fed restrictedly. Applying restricted feeding in group housing conditions implies relatively high investment costs

(adapted infrastructure, animal identification...). Moreover, restricted feed provision still has negative implications for animal welfare. Ad libitum feeding would be a less expensive and more animal friendly solution. With conventional gestation diets this leads to too fat sows with less favourable production results. Most feed companies offer an adapted ad libitum diet, however with variable success. Initially, the use of large quantities of sugar beet pulp seemed to be the most obvious solution. Yet the often too high price of this product urges the sector to search for alternatives. From preliminary research results it seems that chicory pulp, maize gluten feed, alfalfa and oat hulls have potential to restrict the voluntary feed/



energy intake of pregnant sows in ad libitum feeding conditions to a more or less acceptable level. Wheat bran, rapeseed meal and sunflower meal, on the other hand, are not suitable alternatives for sugar beet pulp.

3.1.2 Monitoring animal welfare on farm level

European consumers and citizens attach increasingly more importance to animal welfare. Consumers expect that animal products be produced with (greater) respect for animal welfare. To meet this concern a reliable system for monitoring animal welfare on-farm needs to be developed. A European standard for assessing animal welfare and providing information on it to the consumer offers optimal guarantees for the European market position, for the transparency of the system towards the European consumer and for improving the welfare of farm animals in Europe.

Various research teams, however, use different definitions on animal welfare and consequently adopt different welfare indicators and methods to integrate these indicators into an overall welfare assessment. Hence, a consensus is urgently required on (1) which

indicators need to be measured, (2) the procedures to measure these indicators on farm level, and (3) the relative weight that must be attached to the selected indicators in order to reach an integrated judgment on animal welfare.

Since a procedure to monitor animal welfare must be sufficiently flexible to measure accurately, reliably and objectively animal welfare in Belgium as well as in other EU member states a close cooperation is realised on this theme with other European research institutes. A typical example thereof is the EU Cost Action 846. Through this programme two experts of each EU member state that signed this Cost Action can attend the meetings and workshops where a standard method to assess animal welfare on European animal farms is developed. Besides an overview of the methods that are used by the different research teams to assess animal welfare on-farm a list of potential animal welfare indicators for pigs, cattle and poultry is drawn. The reliability, validity and practical applicability of each indicator has been evaluated. However, it became clear that these criteria for most of the indicators required further investigation. As a result of this Cost Action, a EU Integrated Project was successfully applied for within Priority 5 (Food Quality and Safety) of the Sixth Framework Programme. The Integrated Project entitled: 'Integration of animal welfare in the food quality chain: from public concern to improved welfare and transparent quality' was launched in 2004. The aims of this ambitious project, involving some 50 research teams, are: (1) to develop practical strategies and methods to improve animal welfare, (2) to develop a European on-farm welfare assessment standard, (3) to develop a European animal welfare information standard and (4) to integrate and interrelate the most appropriate specialist expertise in the multi-disciplinary research field of animal welfare in Europe. Also on national level there exists a cooperation with various universities to assess or to optimise particular animal welfare indicators. Thus, methods were developed to score body condition of cattle and to automatically quantify by means of image-processing techniques the behaviour of laying hens in enriched cages. Innovative animal welfare indicators were also developed and validated. In this



connection a research project is going on since a few years to establish whether the degree of fluctuating asymmetry is a reliable, user-friendly and integrated indicator for the welfare of broilers and rabbits. Fluctuating asymmetry refers to small, random differences between the left and right-hand side of bilateral symmetrical traits. Under optimal conditions the left and right-hand side of these morphological traits should develop according to an identical pattern. The degree to which an animal is incapable to reach such a stable development of the phenotype can be a relatively easy and objective measure for the quality of both the individual and the housing conditions during development. It was indeed demonstrated that broilers from organic husbandry systems are less asymmetric than chickens from conventional intensive production systems and that a more animal-friendly environment (low stocking density and environmental enrichment) reduces the degree of fluctuating asymmetry in fattening rabbits. Surprisingly though, the results of our latest experiment do not appear to confirm that various stressors increase the degree of fluctuating asymmetry in broilers. Further research, especially into the ontogeny of fluctuating asymmetry, is needed.

3.1.3 Group housing of broiler rabbits

Housing in small cages restricts the natural behaviour pattern and is by consequence considered as animal unfriendly. In earlier experiments, the possibilities of group housing of broiler rabbits in small pens were demonstrated. Nevertheless, there remains a lot of uncertainty to achieve a sustainable compromise between welfare and profit such as the minimal area required by an animal to feel comfortable as well as the optimal group size. Therefore, the pens were rebuilt and equipped with an automated and self-positioning UV camera set-up on the ceiling. In a first series of experiments, the effect of housing density is studied. In each of the pens, with size varying from 1.1 m² to 3.1 m², 22 young rabbits are housed. The construction of the pens is completely symmetric to judge the minimal space needed by an animal in function of his age. Moreover, half of the pens are enriched with a multifunctional wood construction



that allows minimal hiding and gnawing. Apart from the welfare related parameters, the production indices and eventual skin lesions are judged.

3.2 Efficient and functional animal feeding

3.2.1 Energy supply for double-muscléd cows during the indoor period

Double-muscléd cows are sometimes fed poor-quality feeds during the indoor period. As such, energy requirements are not always fulfilled. It is assumed that cows can mobilize their body reserves, which can be built up again during the subsequent grazing period. Research during several years with 126 Belgian Blue cows showed that some energy restriction is acceptable. However, a restriction of more than 20% coincided with a higher calf mortality. This should be dissuaded, as the calf crop is the most important source of income in suckler cow husbandry. Primiparous cows warrant extra attention when parturition occurs at the start of the indoor period and when they are suckled for a long period. From a theoretical approach, energy should be restricted by



no more than 15%. In order to reduce calf losses, it is recommended to watch the body condition of the cow and to adapt the diet, if necessary.

3.2.2 Valorising wheat for dairy cattle

The use of home-grown wheat in dairy cattle nutrition gained interest over the past few years. Wheat can partially substitute commercial concentrates and hence reduce feeding costs. The grain is mostly fed dry and rolled or ground. Besides, ensiled immature wheat and soda treated wheat have been claimed to improve the feeding value and therefore the performance of dairy cattle. However from a comparative trial with these three wheat forms, it appears that the net energy value is not significantly different. In comparison with rolled wheat, soda wheat contains 22% more and ensiled wheat 24% less true protein digested in the small intestine. At an intake level of about 5 kg wheat, soda treatment

increases the production of milk, milk fat and milk protein, but reduces weight gain. Ensiled wheat lowers milk production, but increases milk fat content.

3.2.3 Effect of specific additives to improve the digestion in poultry

Diets for both broiler chickens and meat turkeys are characterized by a high nutrient density, which needs an important energy contribution from fat. The fat quality in terms of bio-available energy value can vary widely, mainly due to differences in chemical composition with important consequences on emulsification-dependent digestibility and absorption. The dietary supplementation with emulsifiers can clearly improve the digestibility of fat. The composition as well as the concentration of non-starch carbohydrates (NSP) vary from one feedstuff to another. The anti-nutritional effect of insoluble NSP (cell wall components) is related to their capability



to include nutrients, while the soluble NSP enhance the viscosity in the gastro-intestinal tract. The dietary supplementation with specific enzymes can enhance the MEN-content of wheat with 2 to 12%. This beneficial effect appears more pronounced in young broilers than in adult poultry.

3.2.4 Alternatives for antibiotic feed additives in poultry

The zootechnical performance and feed efficiency in poultry are highly related to both the quantitative and qualitative microflora in the gastro-intestinal tract and the nearby environment as well. Poultry has a rather limited naturally native resistance and immunity against colonization and infection by potentially pathogenic micro-organisms. Antimicrobial feed additives have made a tremendous contribution to the profitability in the intensive husbandry thereby providing human population

with healthy and nutritious poultry products. As a consequence of the increasing concern about the potential for antibiotic resistant strains of bacteria, the European Commission decided in 1999 by invoking the precautionary principle to ban in 2006 the 4 remaining commonly used feed antibiotics. There are a number of non-therapeutic alternatives, including enzymes, (in)organic acids, probiotics, prebiotics, herbs and etheric oils, immunostimulants and other management practices. It must be emphasised that these measures will help to compensate only partially, but not replace, antibiotic growth promoters, and will have an indirect and more variable efficacy. There is, however, no clear view on the ultimate cost of the ban on zootechnical performance, morbidity and mortality, slaughter-yield and return-on-investment.

3.2.5 O-carotenoids determine the egg yolk colour

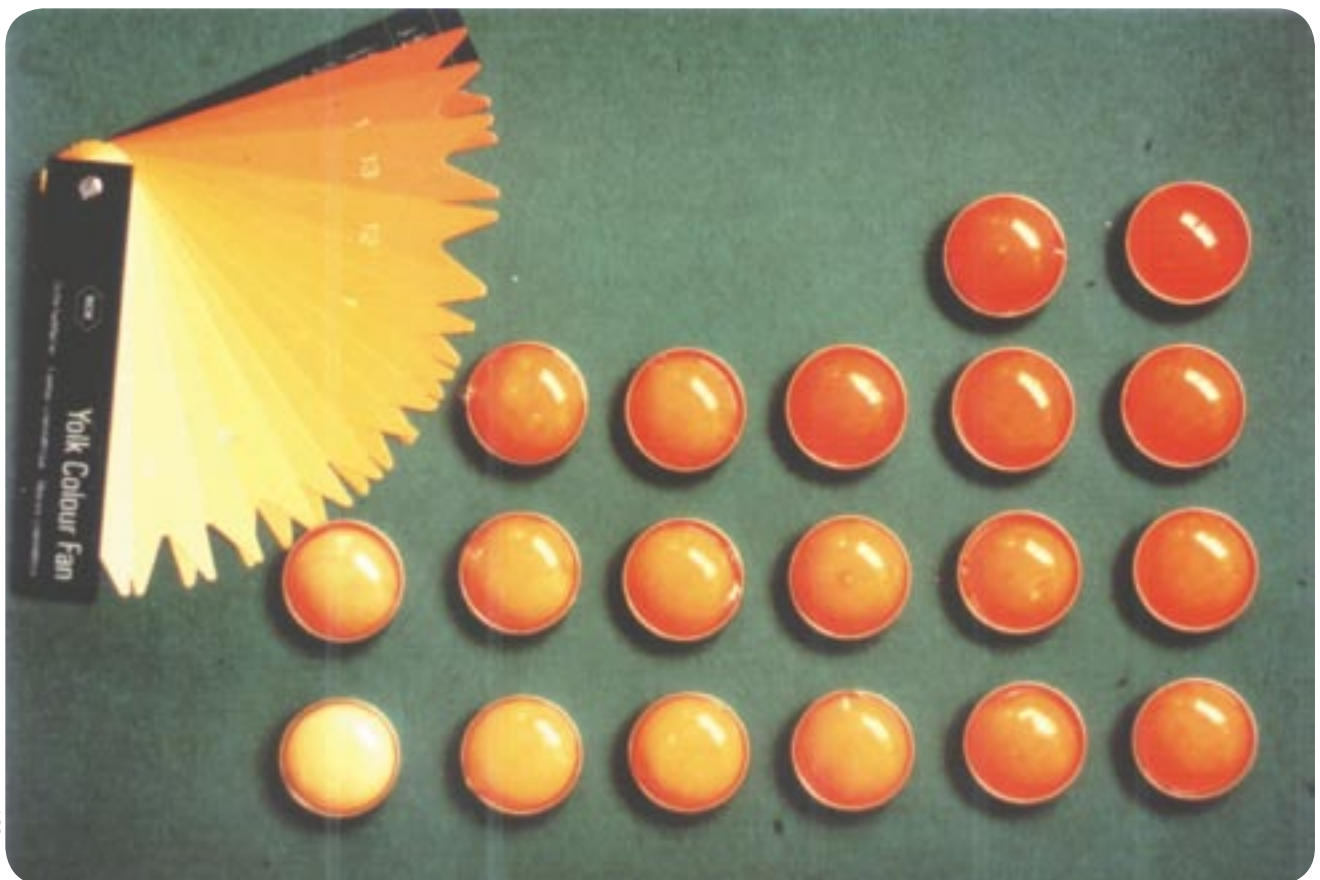
The egg quality is a multi-factorial characteristic depending on both external and internal factors. The internal factors are related to e.g. the egg yolk with its nutritional value (protein, fat and fatty acid profile,...). The physical appearance and colour are important factors because of their impact on consumer preference. The target egg yolk colour with variations from yellow over orange to red is strongly dependent on geographic location. The Belgian consumer prefers a colour varying from deeply orange to red.

The egg yolk colour is mainly determined by both the

concentration and physico-chemical and biological properties of the O-carotenoids in the diet. One distinguishes molecules with either a yellow or a red colour hue. The yellow O-carotenoids are mainly found in yellow corn, alfalfa and marigold extractions, while the red O-carotenoids are found in paprika extractions. Besides, also nature-identical preparations are available.

3.2.6 Rabbits as model animal to judge dietary omega-3 fatty acids

The favourable effects of unsaturated fatty acids and particularly the omega-3 fatty acids in human nutrition are increasingly recognized. They claim an increased protection against cancer and an



Egg yolk colour

enhancement of the immunity status. In animal nutrition too, interest is growing to utilise them as promoter of health and performances, in the meanwhile enriching the animal end-products with these fatty acids. Due to their quick reproduction rhythm and small size, rabbits are an excellent animal model. In an experiment females were fed during 6 cycles with a concentrate based on extruded linseed, known for its high content of omega-3 fatty acids. This not only resulted in a strong change of the fatty acid pattern of milk, but also increased longevity of the females and reduced mortality of the young after weaning.

3.3 Sustainable animal husbandry

3.3.1 Improved knowledge of the amino acid requirements for slaughter pigs as a means to reduce the N-excretion

One way to reduce nitrogen excretion via pig manure is the source oriented approach, which consists in lowering the protein content of the diet and fine tuning the dietary protein supply in order to match the requirements of the pig. This implies that the amino acid requirements for optimal animal performances are determined in typical Belgian housing and husbandry conditions, by using the typical Belgian meat pig being a Piétrain with hybrid cross. The experimental diets are formulated based on the ideal protein concept, in which dietary amino acids are always present in optimal reciprocal proportions. In the trials the effect of different levels of ideal protein on daily gain and feed conversion ratio (kg feed necessary for 1 kg gain) are studied. The trials are executed for barrows and gilts separately, because the growth curves are sex-dependent, in particular with regard to protein and fat accretion. The requirements are derived for the different weight categories from piglet to slaughter pig, from 8 to 25 kg, from 25 to 40 kg, from 40 to 70 kg and from 70 to 110 kg.

3.3.2 Lower ammonia emission in poultry production by daily adjustment of feed composition

In view of the 'Protocol to the Convention on Long Range Transboundary Air Pollution on Persistent Organic Pollutants', Flanders has engaged itself to reduce the ammonia emission in 2010 with 43 % against the emission of 1990. To achieve this, the emission of ammonia by animal production has to be lowered considerably. Next to an adjusted broiler house concept, there is the possibility of optimising the protein/amino acid feeding of the broilers. Instead of applying the commonly used two or three feeding phases, it seems interesting to make even a daily adjustment of the feed composition to feed the birds more according to their needs.



3.3.3 Better knowledge of the P-sources and the application of phytase in broilers

About two thirds of the phosphorus (P) in vegetable sources is present as phytic acid, a form with very limited availability for broilers. This implicates that the standard diet has to be supplemented with inorganic P-sources and phytase in order to meet the P-requirement. Phytases are exogenous enzymes with the ability to convert the P in vegetable sources into an available form for the animals. The application of phytases resulted in a reduction of the total dietary P, which in its turn reduced P excretion. On the other hand, P requirements are a critical issue mainly because of the leg problems of the present quickly growing broilers. Moreover, there are data showing that the true P-availability in phytase-supplemented diets might be lower than the theoretical value, resulting in a P deficiency. Research has been done in order to determine the biological efficacy of inorganic P-sources and microbial phytase in order to formulate diets with a lower total P concentration without adverse effect on the zootechnical performance and animal welfare of the broilers.

3.4 Sustainable sea fishery

3.4.1 Macrobenthos as a monitoring tool to study the effects of dredge dumping offshore the Belgian Coast

The biological monitoring research of the Sea Fisheries Department focuses on the impact of anthropogenic disturbances of the benthic ecosystem of the Belgian Continental Shelf (BCS). For example, sand extraction on the sand banks or dumping of dredged material coming from the main shipping channels and harbors can have an impact on the composition of the bottom fauna. In this framework ongoing sampling campaigns have been conducted twice a year for several decades. The sampling zones cover five dumping zones, two spare dumping zones and three reference zones. Several other zones of the BCS are sampled in the framework of other programs (e.g. sand extraction, OSPAR, windmill farms). The

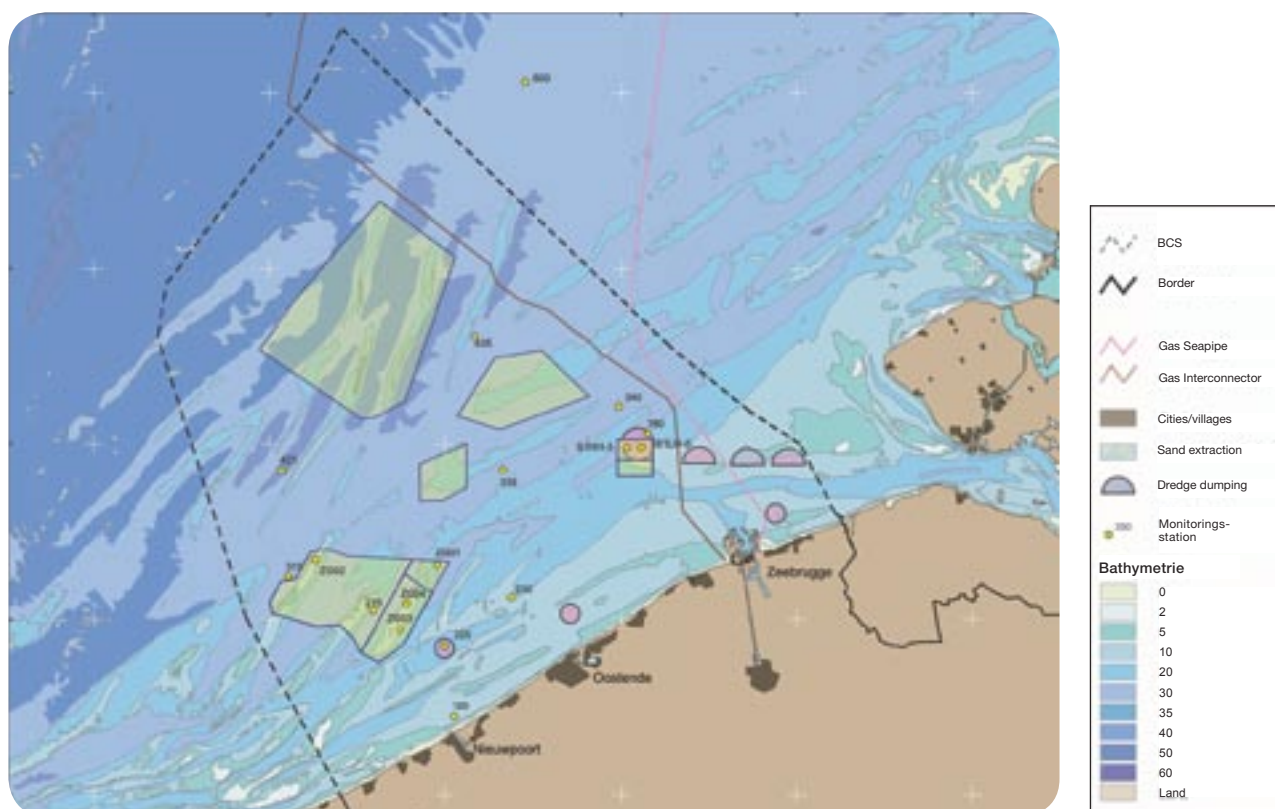
main objective is to investigate the possible effects of different anthropogenic activities on benthic organisms (macro- and epibenthos) and demersal fish, mainly in relation to changes in sediment composition and the possible pollution by heavy metals and organic substances.

Benthos is the name used for animals living in, on or just above the sea floor. These organisms can further be divided according to their size or the place they occupy relative to the sea floor. Macrobenthic organisms are living in the sediment and as such are not very mobile. Their main size varies between 1 and 100 mm. These organisms comprise an important link in the trophic organization of the marine benthic ecosystem, as a food source for the epibenthos and the demersal fish fauna. The most important representatives of the macrobenthos are polychaetes, amphipods, cumaceans, bivalves, ophiuroids and echinoids.

Because of their relative immobility and their dependency on the bottom, macrobenthic organisms are well suited to monitor the benthic ecosystem. The presence and absence of different short-living species (e.g. polychaetes and amphipods) is related to seasonal and yearly fluctuations in the environment and to the availability of food. On the other hand, long-living species (e.g. larger bivalves) are typically found in relative stable communities. After disturbance such species do not reappear rapidly.

To study the macrobenthos bottom samples of 10 liters are taken with a 'Van Veen grab'. These samples are preserved in a diluted formaldehyde solution and rinsed on a sieve with a mesh size of 1 mm. Then all organisms are determined using a stereo microscope, counted and weighed. Different ecological parameters are calculated, such as species richness, diversity, dominance, density and biomass. Next to the macrobenthos also the grain size of the sediment is analyzed.

The tolerance of benthic organisms towards dumping of dredged material is dependent on the rate of the successive discharges and secondly, on the



Overview of sampling stations, dumping and sand extraction zones

amount, type and quality of the dumped material. As long as the sediment deposition is limited to 5 cm, no negative impact has been found on most macrobenthic species. Additionally, many benthic species are more tolerant for fine sand than for mud. On the other hand, the re-colonization of macrobenthic organisms (through planktonic or pelagic larvae from undisturbed populations) is dependent on the type of the dredged material, the water depth, the mobility of the deposited sediment and the current velocities at the dumping sites in

relation to the dilution of the mud fraction.

The sediment at dumping zones Oostende (LO) and Zeebrugge Oost (LZO) is characterized by a changing grain size with a dominance of mud. As such, no stable fauna community can develop at these sites. The variability in the sediment can be attributed to either the dumping of dredged material or to the local currents. Also, the community analysis reveals strong yearly fluctuations in species composition and diversity of the macrobenthos at these dumping sites. This can be explained by the fact that higher

Macrobenthos

1. *Magelona johnstoni* (polychaete)
2. *Poecilochaetus serpens* (polychaete)
3. *Bathyporeia guilliamsoniana* (amphipod)
4. *Diastylis bradyl* (cumacean)
5. *Mysella bidentata* (bivalv)
6. *Echinocyamus pusillus* (echinoid)



mud concentrations can limit the development of many sedentary benthic organisms. Still, higher density peaks are occasionally noted at both dumping zones, which are mainly attributed to the quick re-colonization of opportunistic, short-living species like the polychaete *Polydora* spp. and the amphipod *Corophium* spp. that profit of the influx of detritus associated with the mud.

Based on the long-term monitoring studies it can be concluded that stations in the vicinity of the dumping zones preserve their typical community, which means that dumping of dredged material can be seen as a local disturbance. Only for some specific species,

like opportunistic or newly introduced species a clear variation is observed. However, on a long-term basis the dumping zones show a comparable trend in number of species, diversity and density with the reference areas or other zones of the BCS as can be found in the literature. To a large extent this can be attributed to the highly dynamic character of the BCS, characterized by strong tidal currents and a large meteorological variability.

Generally, it can be stated that it is difficult to discern the effects of dumping of dredged material on the environment and on the present macrobenthic fauna from the natural variation in the benthic ecosystem.



4. KNOWLEDGE UNIT TECHNOLOGY AND NUTRITION

4.1 Food safety

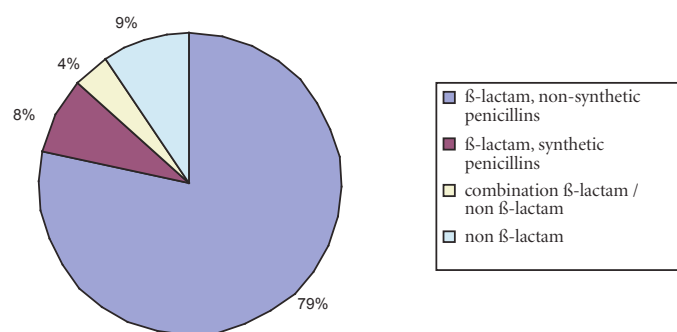
4.1.1 Group specification and identification of inhibitory substances in positive Flemish farm milk samples

In order to enlarge the knowledge about the antibiotic compounds present in positive milk samples in the framework of the official quality determination of raw milk, additional residue analyses were performed at CLO-DVK. A group specification was performed on all Flemish milk samples, penalised for inhibitory substances in the period of May-June 2003. This group specification was based on the microbiological inhibitor test Delvotest MCS after pre-treatment of the milk with penase/penicillinase and group specific Charm II receptor tests. Identification and semiquantification of the residues, using liquid chromatography coupled to mass spectrometry (LC-MS/MS), was performed on a part of the samples containing β -lactam antibiotics.

In total 181 farm milk samples were analysed. In line with the monitoring results, the main group of antibiotic residues causing penalisation are β -lactam antibiotics (164/181 or 90.6 %). Further research revealed that in 142 cases the residues belong to the group of the non-synthetic penicillins, in 15 samples the residues belong to the group of the synthetic penicillins or the cephalosporins and in 7 cases it concerns a combination of β -lactam/non- β -lactam antibiotics. Finally there were 17 farm milk samples with residues not belonging to the β -lactam group but mainly to the group of sulphonamides.

Of the samples containing β -lactam residues 60 samples were analysed with LC-MS/MS in order to identify and quantify the residue. In nearly all cases the presence of penicillin G or cloxacillin was confirmed. In 11 samples residues were found with a concentration exceeding at least 10 times the

DISTRIBUTION INHIBITORY SUBSTANCES



Results group specification of inhibitory substances in 181 positive Flemish raw farm milk samples

respective MRL (Maximum Residue Level).

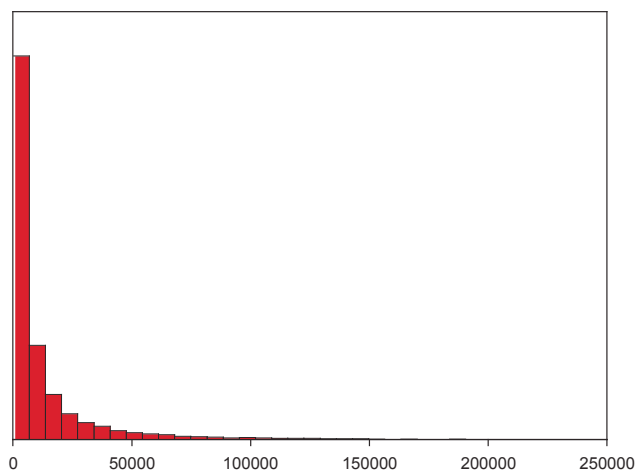
Research as such gives interesting information that should be linked to inquiries on the farm to check what type of treatment was carried out or which compounds were causing the presence of residues in the milk. In that way, actions can be taken to contribute to a decrease of the amount of positive milk deliveries.

4.1.2 Risk assessment of *Salmonella* Enteritidis in hen eggs in Belgium

Salmonellosis is one of the most frequently occurring foodborne diseases. Approximately 64% of the cases in Belgium are due to *Salmonella* Enteritidis infections (SE). SE is mainly associated with the consumption of hen eggs and egg products. The Belgian egg food chain was simulated with a

quantitative risk model in an attempt to identify the bottlenecks and assess the potential impact of preventative measures.

The World Health Organisation has formulated a general template for a risk model for SE in eggs and egg products. The risk model in this study is based on this template, adapted as much as possible to the specific Belgian situation. An important aspect of any risk assessment is the so-called exposure assessment, where the level of exposure of the population to the bacterium is calculated. In this case, the exposure assessment can be subdivided in three modules: the production module, the distribution and storage module and the preparation and consumption module. The production module leads to the prediction of the fraction of contaminated eggs and the associated SE concentration. Aspects like flock prevalence, within-flock prevalence and percentage contaminated eggs produced by infected hens are taken into account. The evolution of the internal egg temperature as function of time is described in the distribution and storage module. Important variables are the surrounding temperature and the residence time in the packing stations, during the transport and in the sales outlets. The output of this module is the



Yearly prognosis of salmonellosis cases according to the model

distribution of the number of SE in the contaminated eggs. The preparation and consumption module describes the trajectory of every contaminated egg from the sales outlet to the preparation phase. There are a number of different trajectories possible. A contaminated egg might go to retail or institutional users. It might or might not be pooled with other



eggs. A contaminated egg might be served as an egg-based meal or as an ingredient. Therefore, the effect of cooking depends on which path it follows. The number of servings to which an egg contributes also depends on its pathway, which is associated with a certain probability. The output of the preparation module is a variability distribution for the number of SE per serving for each of the possible pathways. This distribution is then combined with a dose response model leading to a distribution of salmonellosis cases. Bacterial growth and survival is modelled similarly for all the possible pathways. Growth is dependent on time and temperature. Only growth in the yolk is considered, which is possible only after the yolk membrane is sufficiently deteriorated.



Typical results of a quantitative risk assessment are given in the form of histograms. A large number of assumptions had to be made while implementing the model, and the available data are not always of the best quality. Inevitably, the results are very variable and should consequently not be judged on the basis of absolute numbers. The biggest benefit of a risk assessment is its value in assisting identifying the critical points in the chain. For this specific example it appears that the temperature throughout the chain is the most important factor in reducing the annual number of salmonellosis cases. The introduction of cooling in the whole chain could be the most effective strategy to counteract egg related SE infections, apart from making the flocks totally SE-free. At this moment, the model is limited to shell eggs. Egg products will be introduced in a later stage.

4.1.3 Expression of virulence genes in pathogenic bacteria

Not all the genes present on the genome in bacteria will be expressed at the same time. The genetic information present in DNA of the bacteria is only transcribed to messenger RNA (mRNA) under specific conditions. Each gene encodes a unique protein that performs a specialized function in the cell. The first step in gene expression is called transcription. In a second step, the translation, mRNA is translated to a specific protein. At DVK, by means



of real-time reversed transcriptase PCR, briefly real-time RT-PCR, the expression of different virulence genes of *Salmonella* and *Listeria monocytogenes* were investigated under circumstances present in animal, human and food. By identifying differences in expression of certain virulence genes of the bacteria, strains were further differentiated to determine the most virulent strains.

Reverse transcriptase (RT) is used to copy RNA to cDNA which operates as template in the real-time PCR reaction. In real-time PCR, the PCR product is detected as it accumulates and this allows monitoring of the PCR reaction during the exponential phase and quantification of the initial starting amount. The detection is based on the sequence-specific binding of SYBR Green I Dye in the minor groove of the double-stranded DNA. For the quantification of the gene expression relative *quantification* is used and expressed against one or several control genes. These are genes that are essential for the bacteria and are constitutively transcribed.

First of all for every gene of interest different growth phases and growth conditions were tested. In a second phase different strains of one species were tested under a well defined growth state and condition and differences in expression were correlated with the phenotypical property of the bacterium.

For *Salmonella* the expression of the *mntH* gene was determined under different growth conditions. MntH (H^+ -coupled Mn^{2+} transporter) is important for survival of the bacterium in the phagosome of the host macrophage. In the exponential phase of growth the *mntH* expression was determined in a nutrient rich and a nutrient poor medium with various concentrations of iron. The nutrient poor medium mimics the conditions present in the phagosome of the macrophage. The expression was determined before and after induction with hydrogen peroxide that simulates the defence mechanism of the host. In the nutrient poor medium, with low iron concentration, the *mntH* expression is strongly induced after hydrogen peroxide treatment.

For *L. monocytogenes* isolates of different origin, isolated from patients (clinical isolates) or isolated from food or the environment (not-clinical associated isolates) the expressions of *ami* and *inlB* were

determined. Both genes are important virulence genes of *L. monocytogenes* for adhesion and invasion of hepatocytic cells. A statistical significant difference was observed in *inlB* expression, with clinical strains showing a lower *inlB* expression level than non-clinical associated strains, whereas no significant difference was noticed for the *ami* expression. The results of this study indicate that a differential *inlB* expression level possibly plays a role in the virulence capacity of *L. monocytogenes* strains.

4.1.4 Residues of anticoccidials in eggs due to carry-over after preparation of feed containing anticoccidial additives

In Belgium and in other countries of the European Union, a number of anticoccidials are allowed to be used as feed additive at a certain concentration during a certain time interval for broilers and pullets, but not for laying hens. Due to carry-over in the production lines of the feed mill, low concentrations of additives can be present in feed intended for laying hens while this feed should be free of additives. This can lead to the presence of unwanted residues in eggs. Although





the pharmaceutical industry tackled this problem by introducing granular formulations that give less rise to contamination in the installations of the feed mills, positive egg samples are still found regularly. Within the European Union different action limits are applied because no maximum residue limits (MRL) are available for the anticoccidials. This is due to the fact that these products are registered as additives and not as medicines. The European Union is aware of this issue and works at the moment on a report that should be finalized by 2008 to be able to take a definitive decision about this group of substances. In Belgium, the scientific committee of the Federal Agency for the Safety of the Food Chain gave an advice concerning action limits for the presence of

residues of certain additives and veterinary medicines in foodstuffs when no MRL is available. For certain substances, among which anticoccidials, a value of 10 $\mu\text{g}/\text{kg}$ was set as reference value for taking action in case there is no MRL. For the substances in question, this threshold value in foodstuffs of animal origin does not lead to an exceeding of the acceptable daily intake.

In collaboration with the Centre d'Economie Rurale, Laboratoire d'Hormonologie, a project was set up in which an integrated approach was developed for the determination of residues of these substances in eggs. Besides immunological methods that can be used to analyse samples in a relative cheap and quick

way, also mass spectrometric confirmation methods were developed. Mass spectrometric methods are much more expensive but are able to give evidence of identity and quantity of the residue present. These methods were fully validated according to the most recent European legislation. Then the methods were used to analyse eggs coming from laying hens fed with feed containing in each case one anticoccidial, and this at 2 different concentrations : firstly the concentration allowed in broilers and secondly the concentration that could be present in the case of 5 % carry-over at the feeding mill. The results show, that even for the lowest concentrations, it takes 8 days after cessation of the administration of feed containing anticoccidials until the residue concentrations in eggs are below the proposed action limit of 10 µg/kg. To obtain total residue-free eggs this time can run up to 17 days depending on the compound studied.

Also the influence of those substances on the zootechnical parameters, yolk colour and shell quality were studied. For the yolk colour, the shell quality, the feed intake and feed conversion no significant differences with the blank group were observed. For the average egg weight, laying percentage and daily egg mass, a downward tendency was observed for eggs coming from laying hens receiving feed containing a combination of nicarbazin and narasin.

4.2 The Quality Index Method (QIM) for the assessment of fish freshness

The fish sector does not escape from the increasing demand for freshness, quality and traceability of their product. To facilitate the communication between buyers and sellers of fish, to satisfy the exigencies of the inspection authorities and the requirements of the consumer, the development of an objective, standardized method for the freshness determination of fish is needed. The recently developed Quality Index Method (QIM) could bring a solution to this issue.

There are different physical, chemical and sensorial

methods to measure the deterioration of fish.

The oldest, and still most applied chemical method, is the determination of the total volatile bases (TVB), mainly composed of TMA (trimethylamine), DMA (dimethylamine) and ammonia. These are all deterioration products formed in fish, produced by bacterial activity. Regarding the physical methods, the so-called “fishtester”, measuring the electrical resistance in fish, has to be cited. As the fish putrefaction progresses, this resistance diminishes because the permeability of the fish cell wall increases. Nevertheless, these methods remain unsuitable to estimate fish freshness. For example, no difference can be made between fish, kept on ice for 1 or 3 days. In addition, a lot of the used methods are destructive. Therefore, sensory evaluation currently stays the most important method for freshness evaluation in the fish sector.

Sensory evaluation is based on characteristic sensory changes which occur in appearance, odour, taste and texture of fish when they deteriorate. The most frequently used sensory methods are based on structured scaling as the European Union scheme. Within the EU scheme, three levels can be distinguished: E (Extra), A and B, where E represents the best quality and where the level below B is unsuited for human consumption. The EU scheme is commonly used for the sensory assessment of raw fish; however its validity has been questioned. This scheme does not take into account differences between species because only general parameters are used.

On the contrary, the QIM method evaluates the different attributes separately, in a well-determined order. When evaluating each characteristic, a score is given from 0 (good quality) to 3 (bad quality). The sum of all the scores results in the final “QIM score”. No excessive emphasis is laid on a single attribute so a sample cannot be rejected on the basis of a single criterion. Minor differences in results from any one criterion do not unduly influence the total QIM score.

The QIM-score increases linearly with keeping time

Attribute	Description	QIM Score
SKIN APPEARANCE	fresh, bright, red-orange colors, metallic-bronze sideline, cream-white belly	0
	less bright, less red-orange, some greyish	1
	dull, greyish, putrefaction colors	2
SKIN MUCUS	clear, not clotted	0
	milky, slightly clotted	1
	yellow-brown, clotted	2
EYES FORM	convex	0
	flat, slightly sunken	1
	sunken	2
EYES PUPILS	clear cornea, clear and black pupil, clear yellow border around pupil	0
	dull pupil, yellow border less defined, cornea slightly cloudy	1
	milky pupil, milky cornea	2
	grey pupil, milky cornea	3
GILLS ODOR	fresh, seaweed, grass, metallic	0
	neutral, slight musty, metallic	1
	musty	2
	sour, rotten, faecal	3
GILLS COLOR	bright, red	0
	less bright, red	1
	discolored, yellow spots	2
	yellow-brown discoloration	3
GILLS MUCUS	no mucus or clear mucus, lamellae slight sticky	0
	milky mucus, lamellae sticky	1
	yellow-brown mucus, lamellae sticky	2
TEXTURE	in rigor	0
	firm, elastic	1
	less firm, less elastic	2
	soft	3
FINS	bright brown-green-bleu colors, drawing well differentiated	0
	less bright colors, drawing less differentiated	1
	grey-brown colors, drawing not differentiated	2
TOTAL		22

on ice, a relation that results from a major number of experimental data obtained with a trained sensory panel. In that way, the QIM approach limits the subjectivity of the sensory analysis. Since the potential keeping time on ice is known for each species, the so called “shelf life” (number of days that the fish can still be kept on ice) can be calculated from the QIM score. Both data (shelf life and time kept on ice) can only be achieved with the QIM method and can produce particular interesting information for the commerce in fish products. As the deterioration process of every fish specie proceeds in a different way, a QIM scheme is developed for each fish species.

The introduction of selling on distance via internet (e-commerce) has strongly encouraged the interest for objective assessment of fish freshness. The internet buyer has a continually higher need for a uniformed, objective determination of fish freshness and quality. This idea motivated the three Flemish fish Auctions in 2000 (Zeebrugge, Oostende, Nieuwpoort), in cooperation with the Sea Fisheries Department, to set up a collective project about freshness assessment, the so-called FFQA project (Flemish Fish Quality Assessment). The aim of this project was to familiarize the workers with the QIM system. Thanks to the success of this initiative, the project was extended in 2002 with the Fish Quality Assessment (FQA) project. During this 3-year project, 13 new QIM schemes are being developed (whiting, lemon sole, dab, tub gurnard, lesser spotted dogfish, ray, pout, haddock, bass, brown shrimp, red mullet, scallop and Norway lobster) at the Sea Fisheries Department. A major number of keeping tests and validation tests are set up to develop the different QIM schemes. The project runs in cooperation with the “rederscentrale” (association of ship-owners) as promoter and the three Flemish fish auctions. At the same time, eight

inspectors of the fish auctions and the Flemish Public Employment Service (VDAB) are trained to assess the freshness of these species. The aim of this training is to familiarize the trainees with the QIM system and to maximize the uniformity of the assessment within the group and within the Flemish fish auctions. The FQA project is realized with financial support of the European Union and the Flemish Community.

4.3 Authenticity

4.3.1 Research on rest proteolytic activity in milk powders of different heat classes

Rest proteolytic activity is the remaining enzyme activity in milk and milk products after heat treatment. Since milk powders often are used as intermediary products undergoing further processing as ingredient in milk derived food products, rest proteolytic activity could play an important role in the storage properties of the respective products, especially in the case their thermal processing was rather low. On basis of whey protein denaturation, milk powders are classified in low heat, medium heat and high heat powders. Milk powders classified as high heat powders seem to contain no proteolytic activity. Of all studied medium heat powders, one showed proteolytic activity as well as the low heat powders which all contained considerable quantities of rest proteolytic activity. As to the low heat powders, 85% of the proteolytic activity could be inhibited by aprotinin. This is an indication that this proteolytic activity could be attributed to active plasmin, an endogenous protease in milk. The resulting 15% has to be attributed to the presence of bacterial proteases. These are less heat sensible metalloproteases that can be inhibited by EDTA. The rest proteolytic activity in the medium heat powder is lower than in the low heat powders. Moreover, not more than 50% of this proteolytic activity can be inhibited by aprotinin. The larger contribution of bacterial proteases in medium heat powder could be explained by the fact that these bacterial enzymes are better heat resistant than plasmin.

It is clear that especially low heat powders still contain proteolytic activity and that this fact should be taken into account when further processing such powders. Also quality of the raw material before the drying process is of major importance since prolonged storage of the raw milk or storage under unfavourable conditions before processing could cause the presence of a considerable amount of bacterial proteases better surviving heating and spray drying than endogenous proteases (plasmin).

4.3.2 Research on the species origin of milk used in dairy products

Research was carried out on methods allowing the detection of species origin of consumption milk and cheese. Most attention has been paid to methods for the detection of bovine milk in goat's milk, goat's cheese and ewe's cheese. Such frauds could also be traced by chromatographic or electrophoretic techniques, but these methods are laborious and time-consuming and for that reason less interesting as routine methods.

For such research a large number of immunological tests and antibodies are available on the market. After optimization of the different immunological (ELISA) tests, practicability was verified on raw milk, pasteurized milk, UHT and sterilized milk as well as on Feta cheese. The most sensitive tests allow the detection of 0,1% of cow milk in raw goat's milk, 0,2% in pasteurized goat's milk and 0,5% in UHT goat's milk and Feta cheese. The research has also demonstrated that immunological detection of antibodies directed to whey proteins were of no use in UHT and sterilized milk. On the other hand, this is possible with tests using antibodies directed against caseins. This can be explained by the higher sensitivity of whey proteins for heat denaturation.

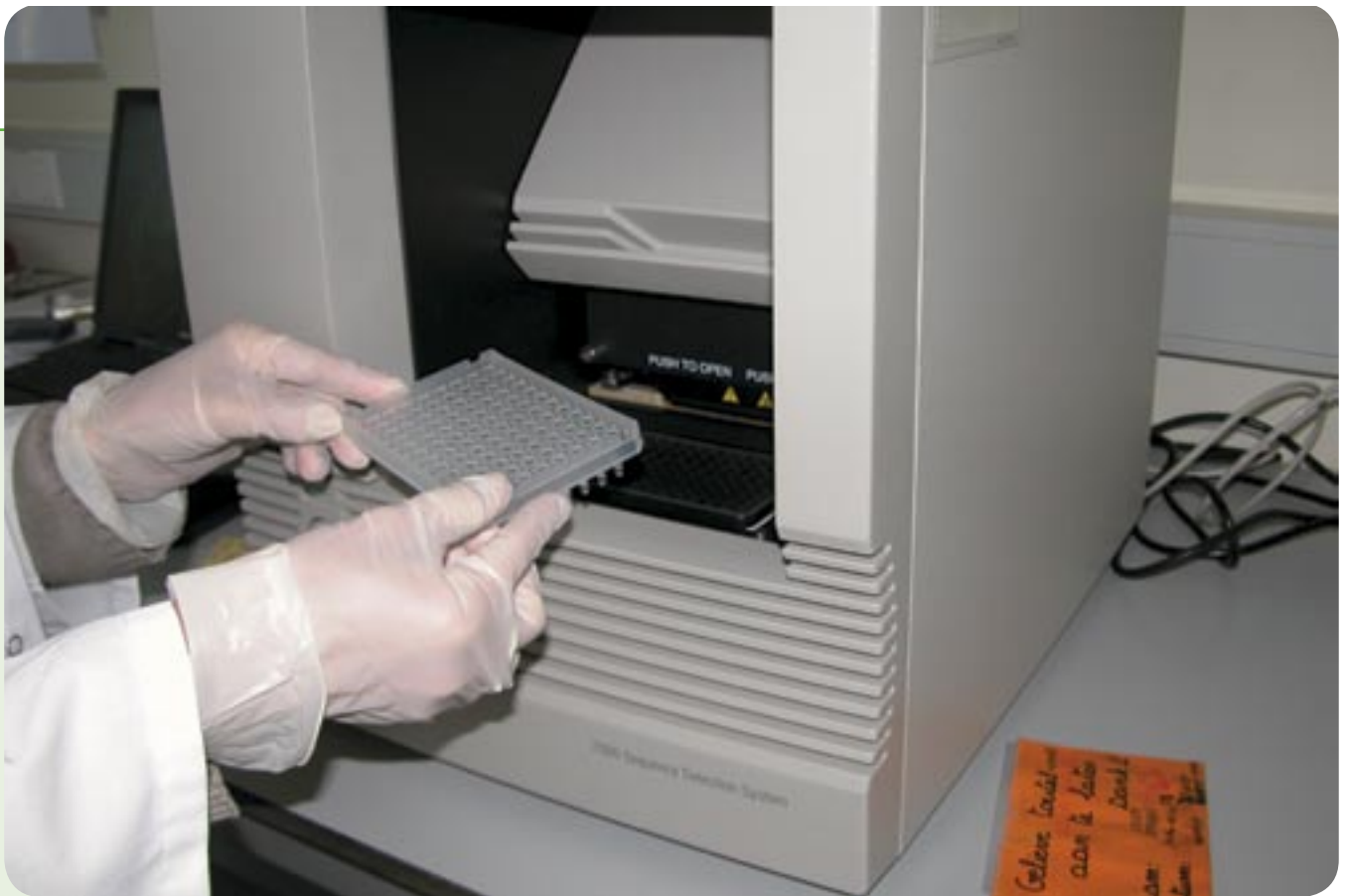
Also the practicability of test strips was investigated. They allow very fast detection, but are of no use for quantitative purposes. Their sensitivity is lower and they cannot be used for UHT and sterilized milk.

4.3.3 Research in function of support of the establishment and implementation of GMO regulation

During the last six years, a research group within CLO-DVP has built up expertise in the domain of characterisation and detection of GMOs (genetically modified organisms). CLO-DVP has put effort into evaluating molecular techniques and, if necessary, in optimising such techniques, for the evaluation of the safety of new GMOs and for the detection of transgenes. The accomplishments of this research and the main resulting applications are applicable in the light of a number of evolutions in the European regulatory framework on GMOs.

The motivation for GMO research was, and still is, to support the establishment and practical implementation of regulations concerning the control of GM products. A new, strict and comprehensive legal framework has been in force in the EU since







April 2004 with two new regulations: 1829/2003 on GM food and feed and 1830/2003 on traceability and labelling. A threshold value of 0.9 % means that all products with a higher relative GMO content require mandatory labelling as “contains/is derived from genetically modified soybean/maize/etc.”. A first focus in the research was on GMO quantification and the interpretation of legal threshold values for compulsory labelling. Before an analytical technique can be put forward in a detection and control procedure, a number of parameters must be known and fixed. The CLO-DVP research has contributed substantially towards a description and the generation of the unique information needed

for detection of a GMO. This unique information has been generated independently of the involved economic actors by means of an in-house developed anchor PCR method. This method concerns the junction region between the inserted DNA and the plant DNA. Recently, legislation included the obligation to submit such information together with the dossier for authorisation of a GMO. This information in fact forms the direct link between the description of a GMO and controlling the presence of the GMO in derived products.

A second important purpose of performing analyses to provide a control system for GMOs is to ensure

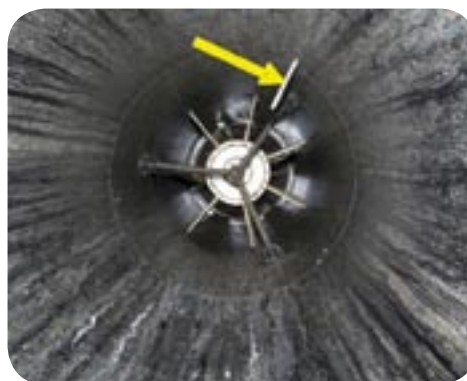
the compatibility between the raw experimental data and the results that are reported as a requirement of the legislation. Since the labelling thresholds have been put in force, questions have arisen concerning the interpretation of relative percentages of GMO. We have demonstrated that for the same sample, GMO percentages expressed as seeds, based on weights of material or on haploid genome copy numbers, are not necessarily the same. At the European level, a choice has been made for GMO analysis to be performed with DNA-based techniques. As a consequence, a Commission recommendation states that the analytical result should be expressed based on haploid genome copy numbers. This new and straightforward definition of measurement and expression units for GMOs solves a lot of problems and simplifies the harmonisation of detection strategies at the international level.

A third requirement for performing quantitative analyses is the availability of standards and control samples. These can be product-based or pure analytic materials. The production and distribution of standard materials calls for traceability, the assurance of uniform and high quality production processes, universal availability, and an acceptable cost price. Matrix-based standards that have been used traditionally possess a number of problems. Therefore, CLO-DVP has carried out research on the use of alternative, pure DNA-based standards. This work involves broader collaboration at national and European levels (BNGL and ENGL). In our approach, DNA fragments of interest are built into plasmid vectors. Plasmid DNA samples can easily be produced, and by mixing this DNA in certain ratios with conventional, non-transgenic plant DNA, a suitable calibrator for quantitative GMO analysis is obtained. The main advantage of this approach for competent authorities and governments is that the plasmid standards can be made independently of the involved companies. The development and use of pure plasmid DNA standards as calibrators in real-time PCR also supports the new recommendation on the interpretation of percent thresholds, and enables horizontal implementation of the legislation (for seeds, food and feed).

4.4 Environmental protection

4.4.1 Measuring ammonia emission from livestock buildings

In a European context the ammonia emission in Flanders has to be reduced with 40% by 2010. A law of October 2003 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



Sampling tube in ventilation shaft

poultry categories. From now on the authorities want to determine the emission factor ($\text{kg NH}_3/\text{animal place/year}$) for existing and newly developed animal houses. As a consequence ammonia emission measurements will be necessary. Today, ammonia emission measurements from commercial pig and poultry houses are expensive due to the specialised equipment that is needed (e.g. photo-acoustic gas monitor), the labour and the long-term measuring periods (up to 200 days). A compromise procedure for field measurements consists of reducing the number of measuring days.

The main objective of this project is to develop a procedure for calculating the annual ammonia emission based on a short measuring period (approximately 16 days) in mechanically ventilated



Photo-acoustic multi-gas monitor with 8-channel multi-sampler

livestock buildings.

With the shortened measuring method the ammonia concentration in a specific animal house is at each moment simulated based on an ammonia model. This ammonia model expresses the ammonia emission or concentration in function of simply measurable climate variables that are continuously available throughout the year (inside temperature, outside temperature, humidity and ventilation rate) and management variables (weight, number of animals).

On days that the ammonia concentration is not measured, the latter is calculated using the ammonia model. The parameters and the structure of this ammonia model are determined based on the measurements on a limited number of measuring days when the ammonia concentration is measured anyway (in combination with the earlier mentioned

climate and management variables). These parameters and the model structure are only valid for that particular animal house and during the period around the measuring days. This means that the model can vary with time for the same animal house. Measurements on particular days, spread over the entire year are therefore always necessary for every animal house. In this project the model structure and the choice of the optimum number of measuring days were determined for the various animal categories. For fattening pigs, for example, the number of measuring days can be reduced to 24 on a yearly basis.

During this project CLO-DVL has built a mobile measuring apparatus and developed two test devices. These consist of a photo-acoustic multi-gas monitor combined with an 8-channel multi-sampler and the accompanying sample tubes. This equipment makes it possible to measure the ammonia levels at eight different locations. Indoor air samples were taken in the exhaust chimney and transported through tubes to the measuring device. Climate parameters were registered with the equipment available in the animal house. A calibrated ventilation rate sensor measured ventilation rates in the ventilation shafts. The ammonia emission can be calculated from the measured ammonia concentrations and the registered ventilation rates.

4.4.2 Development of a test procedure and mobile test device for the determination of the spatial distribution of fertiliser granules by various types of fertiliser spreaders

Throughout Europe environmental awareness in general and a growing concern particularly for the quality of freshwaters and groundwater and for the safety of our food can be observed.

Attention is mainly focused on agriculture and especially on areas with intensive farming that come under increasing pressure. The applied quantity of agricultural nutrients should be strongly reduced. Organic fertilisers are applied as much as possible while the amount of supplementary mineral fertilisers is continuously further reduced.

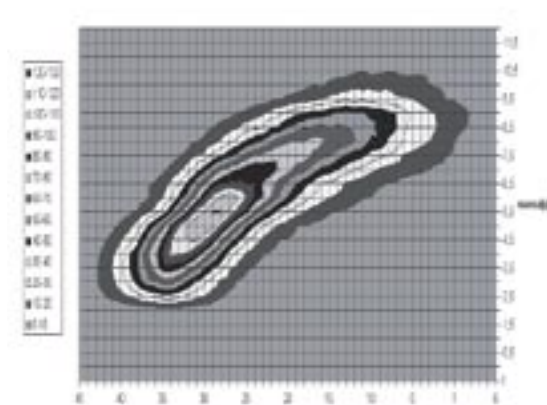
An optimised application of this restricted amount

of mineral fertiliser is necessary and this can only be achieved by a precise and homogeneous spread pattern. A good spread pattern is obtained with high-quality fertiliser and high-performance fertiliser spreaders. The performance of the fertiliser spreader depends not only on its design but also on a correct adjustment. However, the user is often hardly familiar with the adjustment and maintenance of spreaders. Simple, straightforward procedures and test devices for the measurement of distributions of fertiliser

grains by means of spreaders are lacking. This research aims at developing a test device that serves as a tool to evaluate the spread pattern on farm level. The method is based on measuring speed and direction of the fertiliser grains. The results of these measurements are entered into a ballistic model that calculates the trajectories and the landing points of the grains. Speed and direction are determined using photographic techniques. Initially, the trajectories of the grains were recorded with a high-speed camera.



Visualisation of the grains



The spread pattern



Test device with measuring unit

The camera pictured the grains 1,000 times per second. The advantage of this technique is that a high-resolution image can be obtained. On the other hand the high cost associated with this technique is an important drawback. In a later stage a low-cost technique using a commercial digital camera was developed. With this device high-frame rates, such as with the high-speed camera, cannot be obtained. By using a high-frequency stroboscope at 1,000 Hz combined with a long shutter time the high-frame rate could be simulated. During the exposure time the grains are illuminated at least three times. Three successive positions of the grains are then visualised on the photograph. Commercial stroboscopes capable of producing these high frequencies are however very expensive. Furthermore, several such stroboscopes are needed to create sufficient lightening. Therefore, a special led stroboscope with ample light intensity was developed. Only under optimal light conditions can the grains be pictured correctly and the determination of the exposure settings for these optimal conditions represents an important part of the research. To calculate direction and speed of the grains, the grains need to be recognised as objects on the pictures. Dedicated software was developed to determine the coordinates of the grains. Once the coordinates are known speed and direction are calculated and entered into the ballistic model. The model then calculates the landing points. The determination of the spread pattern from the landing points is ongoing.



4.5. Agromechanisation

4.5.1. Potato quality: Reducing mechanical damage

In today's strongly mechanised agriculture potatoes are subjected to many mechanical treatments before they can be served on the consumer's plate. Each step in the processing chain - from harvesting to packing - can bring about impacts between potatoes and between potatoes and machine parts. If impact forces become too high the risk exists that tissues and cell walls under the potato skin will be crushed. This

may give rise to bruises, cracks, cell necrosis and the formation of melanin. A quality potato is expected not to show these signs: it improves its appearance, the potato is less susceptible to fungi and bacterial infections and can be stored better. Moreover, end products such as crisps and fries will not show deviations in colour and taste, the waste flow will be minimised and the financial return for the grower and processor will be increased.

In order to minimise mechanical damage to potatoes, an electronic potato is introduced. This wireless sensor is carried through the processing machines and

installations and signals the locations where the risk for damage is the highest. The transmitted signal also includes detailed data of the intensity and the number of impacts to which the potatoes are subjected.

During a research covering a number of years (and supported by IWT-Flanders) the maximum allowable measured value of the sensor before potatoes are liable to damage was investigated. Since the degree of damage is also subject of a number of other factors



such as temperature, fertilisation and dry matter content of the potatoes, the maximum allowable measured level of the sensor varies with the situation. Several statistical models have been developed describing the degree of mechanical damage as a function of the influencing variables. This enables CLO-DVL through its Technological Advisory Service 'Agromech' to give advice throughout the year concerning the correct adjustment of potato harvesters, bin filling equipment, trailers, sorting and packing lines. Farmers and contractors can call upon the expertise of CLO-DVL to adjust the machines correctly and to formulate advice for a soft handling of the potatoes. If the wireless sensor registers excessive values, then machines need readjusting, after which new measurements can be carried out until the desired handling intensity is reached. In the framework of this research a comparative study on anti-shock systems has also been carried out for tippers and trailers. It concerns a study which undeniably demonstrates the value of anti-shock systems and which arouses great interest through various channels in the potato trade.

4.5.2 Agrotextiles

Agrotextiles are technical textile fabrics of the industry that are applied in agriculture as wind barrier, shading fabric, groundcover sheet, insect netting and energy saving screen. Furthermore, agrotextiles are also used for very specific applications.

The research into agrotextiles was in first instance directed at their use as wind barriers in fruit, vegetable and ornamental growing. The research on the influence of wind barriers was performed under controllable circumstances in the wind tunnel of Ghent University with a constant wind speed and wind direction, and under natural circumstances with fluctuating wind speeds and variable wind directions. Wind barriers separate the incident wind in two parts: one part passes through the barrier and another part moves up. The former depends on the open area of the barrier. For the wind direction perpendicular to the barrier on a horizontal flat surface, comparable with freshly mowed grass, all barriers with an open area between 0 and 60% give a wind speed reduction

of at least 30% for leeward distances between 5 and 20 times the barrier height at heights smaller than or equal to the barrier height (Fig.1). Turbulent eddies make impervious or quasi-impervious wind barriers less effective than wind barriers with an open area of 20%. Wind barriers with an open area of up to 50% are very effective in reducing wind speed when the wind direction stands perpendicular to the barrier. Depending on the kind of barrier and the turbulence, the original wind speed is recovered for 80% at a leeward distance of 15 to 20 times the barrier height measured halfway the barrier height. The greater resistance of the thicker natural wind barrier will decrease the wind speed more than the thin synthetic wind barrier with the same open area. Therefore, the open area of the thinner synthetic barrier should be smaller to obtain the same result.

The research also revealed that the incidence angle between wind direction and wind barrier largely determines the efficiency of the wind speed reduction. Wind barriers are more efficient when they stand perpendicular to the wind direction. The leeward distance to the barrier where the maximum wind speed reduction occurs, decreases and, consequently, approaches the barrier with decreasing incidence angle between barrier and wind direction. Not only the sheltering distance decreases but also the speed reduction, and even an increase in wind speed may

occur from a certain leeward distance on, depending on the incidence angle between barrier and wind direction. In this case, barriers with a greater open area seem to be more effective because of an increase in the sheltering distance and a slower rise of the wind speed (Fig. 2). The research also showed that successive wind barriers should not stand too close to each other to be most effective, and their mutual distance should be definitely greater than the distance where the maximum wind speed reduction appears. Differences in wind turbulence due to rougher approach flow conditions or obstacles in the approach flow region, may cause a different sheltering. Wind barriers are less effective with an irregular rough approach flow zone or with isolated roughnesses in the approach flow zone which diminishes the leeward distance of wind speed recovery.

Agrotextiles are usually exposed to the natural weather conditions what results in a decrease of the mechanical properties like tensile strength. The experiment in which various kinds of agrotextiles were exposed to the natural weather conditions for five years has shown that the decrease in mechanical properties is rather limited when UV-stabilizers were added to the raw material. Extrapolation of the experimental data indicates that the lifetime of agrotextiles is guaranteed for a long period (more than 20 - 30 years).



Synthetic wind breaking cloth to protect fruit growing.

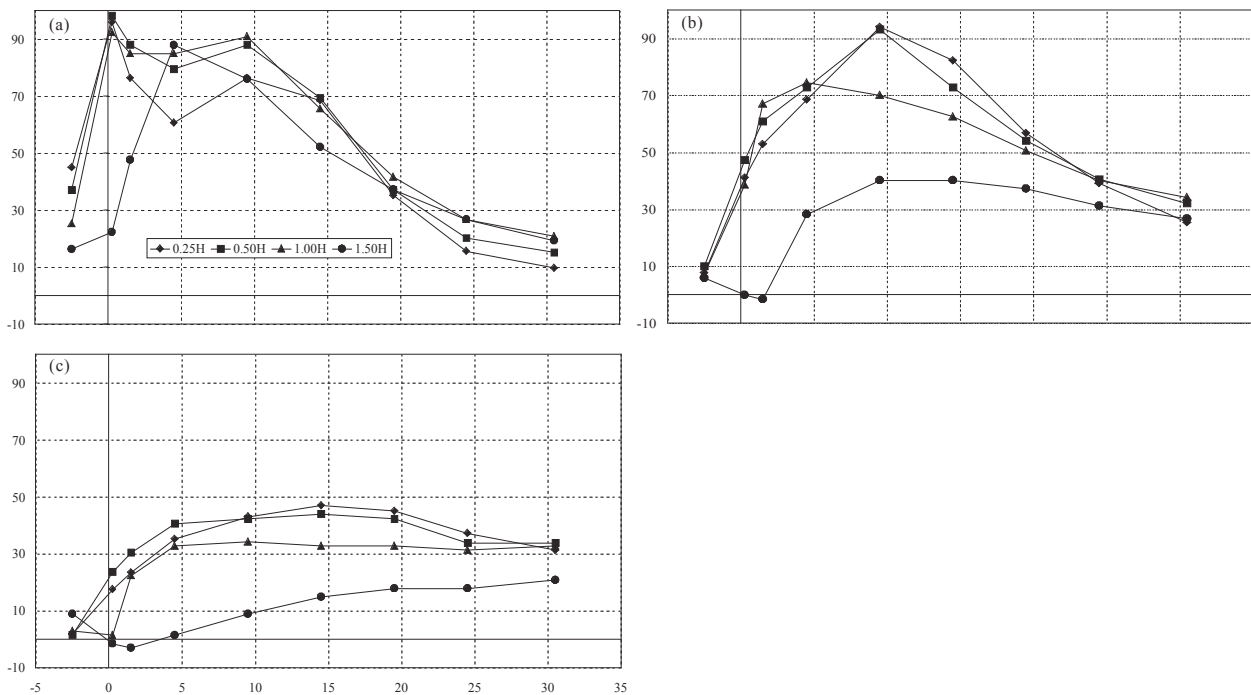


Fig. 1. Wind speed reduction leeward of a wind barrier with an open area of (a) 0%; (b) 35.7% and (c) 62.6%, measured at four heights.

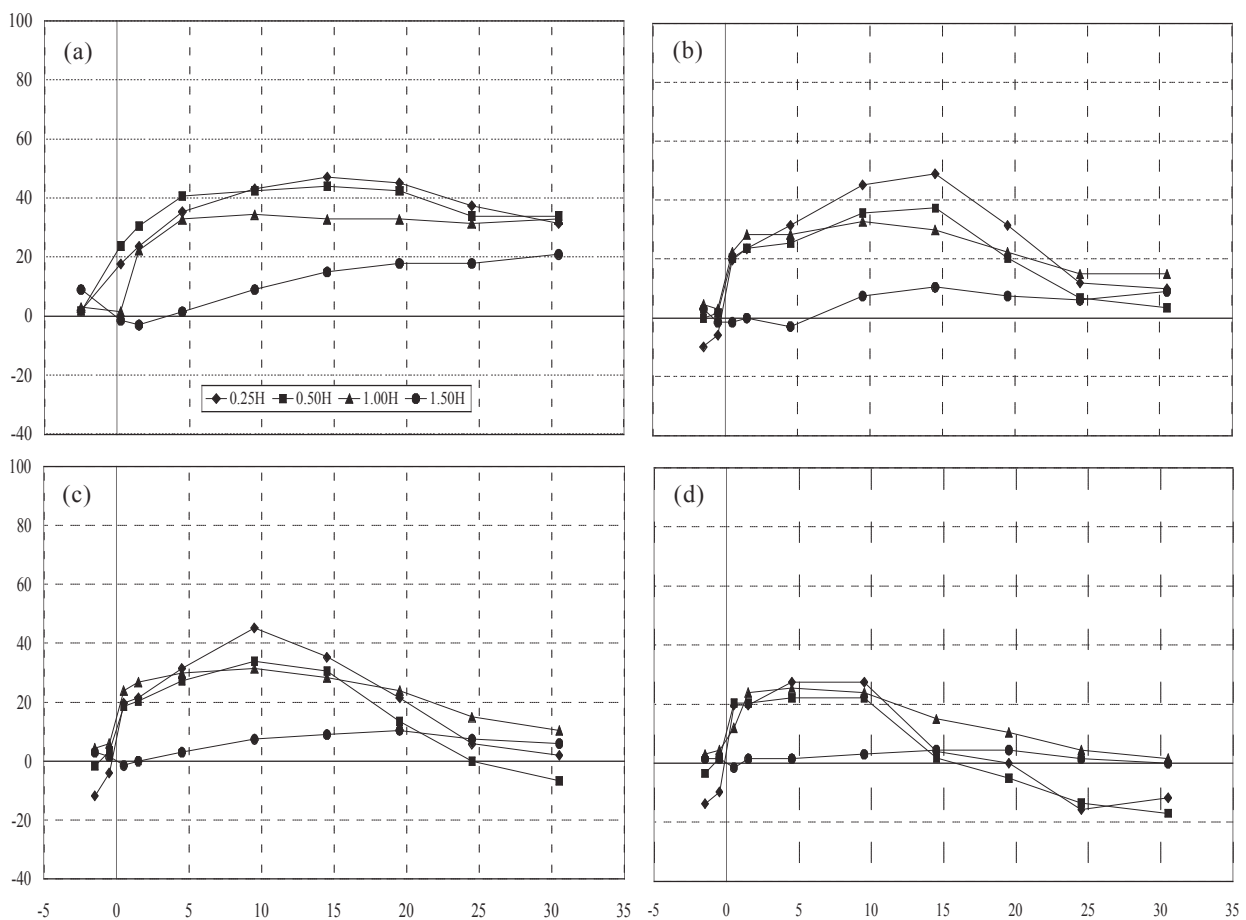


Fig. 2. Wind speed reduction leeward of a wind barrier with an open area of 62.6%, at incidence angles between wind direction and barrier of (a) 90°, (b) 60°, (c) 45° and (d) 30°, measured at four heights.

5. EXTRA HIGHLIGHTED

5.1 PLANT

5.1.1 The use of biological organic waste in the production of high quality compost for plant growing

Organic rests from agriculture and horticulture don't reach the big composting installations. They are carried back unaltered on the field or leave the farm to dumping-grounds. However, each imaginable organic rest is a potential basis for plant nutrition and

soil fertility. Some types of rests are: the remainder when making vegetables (e.g. leek) marketable, crop residues (e.g. tomato), bad green-fodder (e.g. hay), rest of silage (e.g. maize), sorted roots incl. earth (e.g. Belgian endive), etc.

At CLO-DFE the expertise is developed to transform such rest-products into an eminent product by an approved composting method, CMC or 'Controlled Microbial Composting'. It concerns aerobic transformation of fresh organic matter to humus and



Turning over compost



Cultivation of strawberries without and with compost

beneficial micro-organisms. Composting organic rests from the farm in combination with mown-grass, manure, green and woody materials guarantees an optimal composition of the initial mixture. The ratio between the materials determines the process but also the quality of the end-product. A microbial starter is used. The composting process is directed by providing the organisms with sufficient water and oxygen during the whole composting period. An intensive decaying phase with a sufficient rise of the temperature (65°C) is followed by a building phase by which the degradation products are converted and stabilized. Nutrients are incorporated maximally in micro-organisms' biomass and organic matter is stabilized. The stabilization process is favored by the presence of clay minerals, which are initially added via clay or soil with a certain content of clay.

On-farm composting on the basis of own and imported organic rest-products is an actual phenomenon. There is sufficient know-how and the advisory service FarmCOMPOST, located on CLO-DFE, delivers the necessary support. It concerns a SYMBIOS project of sustainable agriculture that is financially supported by IWT. Also the required technology exists, e.g. compost turner, compost cover,

measuring-instruments, etc.) to execute composting on farm-scale.

A well-elaborated legal arrangement concerning the allowance and the linked exploitation conditions is lacking. Therefore the government is contacted.

The intrinsic quality of the compost product urges a renewed view on soil fertility. Compost application facilitates a fast building-up of soil organic matter without negative environmental effects. Compost revives the soil. Soil life activity in combination with plant activity delivers a sound and productive agroecosystem that can limit external input of chemicals (fertilisers and pesticides) to a large extent. Mature compost possesses a certain nitrogen potential. By its positive contribution to the soil condition and the completion of the soil food web the return of other fertilisation is heightened and nutrients in crop residues are recycled in a more effective way. Research in the application of CMC-compost is stimulated and started up at the Agricultural Research Centre (CLO).

It is stated that the activity and diversity of organisms introduced in soil or substrate with compost can provide protection against soil-born diseases, based on competitive and antagonistic activity towards

the soil pathogens. Besides this, CLO-DGB/DFE research has now demonstrated that high quality compost can have a far reaching effect on the growth condition for the plant, which can result in resistance towards air-born diseases. Up till now, our model plant is strawberry. It has been grown in CMC-compost substrate mixes. In these substrates the strawberry plants develop a broad-spectrum resistance. Challenged with Botrytis, powdery mildew, spider mites and aphids that spontaneously occur in a strawberry culture, none of these pathogens cause an epidemic, whereas the traditional culture grown in peat substrate with mineral fertiliser has been seriously affected and has to be treated with pesticides. We have to conclude that a 'different' plant is produced in this compost substrate and that this stage of high disease resistance is surely linked to the different management of nutrients and water in the compost substrates when compared to the traditional substrate. It is accepted that the soil microbiology has a substantial role in regulating the nutrient concentrations available to the plant.

Further CLO-DGB research focuses on the impact of soil microbiology and plant nutrition on disease resistance. To which extent compost determines bacterial and fungal occupation of the plant rhizosphere is studied.

The understanding of these interactions would enable the development of procedures for assessment of the quality of composts and soils regarding disease control and plant nutrition.

The input of organic matter via compost improves the soil structure and water holding capacity. The stimulated activity of soil organisms benefits the structure too. Each type of soil organism contributes in its own way. Fungi and bacteria build up soil aggregates, digging organisms make porosity. Susceptibility to erosion is affected by soil porosity and thus by the stability of soil aggregates and the water holding capacity of the soil. By application of high quality compost erosion is counteracted at the source.



The ICES mesh gauge



The OMEGA mesh gauge

5.2 ANIMAL

5.2.1 The OMEGA mesh gauge

Many technical measures for the protection and the recovery of commercial fish stocks are based on the implementation of minimum mesh sizes. The aim is to ensure a minimum selectivity of the fishing gear. Since a few decennia the present methods for the determination of the mesh size are more and more contested.

Fisheries inspectors use the flat wedge gauge which is pushed into the mesh until it is stopped by the resistance of the netting material. Measurements made with the wedge gauge are not objective because the result depends on the hand force exerted by the inspector. The outcome of the measurement often differs from country to country and within a country from inspector to inspector. As a consequence fishermen often use nets with slightly different mesh sizes depending on the fishing area, and netting manufacturers have to build up stocks of these slightly different netting materials. The lack of objectivity of legally performed mesh measurements is the most used argument to contest the correctness of the measurement.

Scientists use the ICES gauge which measures the mesh opening under a fixed longitudinal force of 4kgf. A proposal to lay down the ICES gauge as standard mesh gauge in an ISO standard was rejected because

the measuring force of this gauge is controlled by a spring, the characteristics of which may change over time.

Minimum mesh sizes are defined on the basis of selectivity experiments in which the ICES gauge is used. However, mesh sizes measured with the ICES gauge are much smaller than those obtained with the wedge gauge used by fisheries inspectors. As a consequence the anticipated selectivity is not achieved in commercial fisheries. These problems have led to an increasing demand for standardisation of mesh measurements by scientists, fisheries inspectors and the industry, netting manufacturers as well as fishermen.

With a view on the development of an objective mesh gauge a combined R&D and demonstration project was set up under the auspices of the 5th Framework Programme of the European Commission. The project, known as the OMEGA project, started in October 2002 and ends in February 2005. The consortium is composed of 6 fisheries research institutes, 6 fisheries inspection services and a joint venture of 2 instrument makers. The Belgian participation consists of the Sea Fisheries Department of the Agricultural Research Centre and MARELEC, a company specialised in marine measuring systems.

The selection of the instrument makers and of the conceptual design of the new mesh gauge took place in a former project, also coordinated by the Sea



The wedge gauge

Fisheries Department. The OMEGA gauge has a fixed jaw with a built-in load cell and a movable jaw driven by an electric motor. The mesh to be measured is stretched by the movable jaw until the pre-set measuring force is reached. The measured length is derived from the number of revolutions of the motor. The measuring force, the mesh opening and the serial number of the measurement are displayed on a LCD screen. On acceptance of the measurement by the operator, these data are stored in the memory of a built-in computer which also controls the operation of the mesh gauge. At the end of a sequence of measurements the average mesh opening can be read. The memory can hold 1000 measurements. The instrument is battery operated. The batteries

are rechargeable and have a capacity sufficient for 1000 measurements. Functions such as choosing the measurement force, consulting the measurement data, control of the remaining battery capacity and memory space, are accessible through a menu. An infrared connection allows downloading of the stored data to a laptop or PC.

The range of the OMEGA gauge is 10-300mm, the precision is 1mm. For meshes smaller than 40mm or larger than 200mm the jaws must be changed. The force measurement has a range of 0-180N. Fixed measuring forces are 40 and 100N respectively for meshes smaller than, or equal to or larger than 55mm. These measuring forces are recommended

by a Study Group of the International Council for the Exploration of the Sea (ICES). The instrument is constructed of materials resistant to corrosion by sea water, and is also waterproof (IP56) and shockproof. The accuracy of the force and length measurement can easily be checked with a set of calibration weights and a test plate with calibrated slots.

At the start of the project a test instrument was built and tried out. Special attention was paid to the ergonomics of the instrument. A series of modifications and improvements led to a prototype of which 30 instruments were built. This prototype was extensively tested and compared with existing mesh gauges during a series of laboratory tests. At sea the OMEGA gauge was compared with the wedge gauge during routine fisheries inspections and with the ICES gauge during scientific fisheries cruises. The data collected by the project partners were supplemented with data from about twenty other European and non-European fisheries inspection services, research institutes, netting manufacturers and fishermen's organisations. Juridical advice was gained for drafting a measuring protocol that can sustain the rigours of European Court procedures.

The test programme carried out showed that the OMEGA gauge meets the preconceived technical and ergonomic requirements. The results of the mesh measurements are free of human influence and not biased by material characteristics, such as friction between the netting material and the jaws. From a legal point of view the new instrument is acceptable to European Courts. The protocol offers the opportunity to formulate an improved legislation. The aim is to replace the present wedge gauge by the OMEGA gauge for inspection purposes and to adapt existing ISO and CEN standards to the new situation. It is as good as certain that ICES will adopt the new mesh gauge as the standard mesh gauge for scientific research.

5.2.2 Minimal physical structure level in beef cattle diets: a necessity for animal welfare and efficiency

Ruminants need a minimal amount of physical structure in the diet for an optimal rumen function. Belgian blue bulls are generally fed diets high in energy containing marginal



amounts of roughage. Because roughage (fibrous feed) provides the necessary structure, its portion in the diet cannot be reduced endlessly. Fibrous feedstuffs excite the rumen wall causing continuous contractions. As a consequence, rumen gasses can escape and the rumen content is mixed thoroughly allowing the rumen flora to function properly. At the same time, nutrient absorption through the rumen wall and passage of smaller particles to the intestine is increased. Moreover, fibrous feedstuffs stimulate ruminating and hence production of saliva, which in turn is a buffer that helps to prevent rumen acidosis. A lack of physical structure in the diet can reduce feed intake, decrease rumen digestion and nitrogen efficiency and lower growth. Moreover, preventing acidosis is important for animal welfare, because it may induce inflammation of the rumen wall, hoof lesions and liver abscesses and in the most severe case can cause death.

To derive physical structure requirements for Belgian Blue bulls, experiments were carried out during 6 years using in total 312 bulls. Thereby, the influence of the structural value of the diet on feed intake, growth and feed conversion during the growth and finishing period ($\pm 340 - 660$ kg) was studied. At slaughter, carcass and meat quality were determined as well as parameters to assess possible damage of the rumen wall or claw disorders.

The structural value of a feedstuff indicates to what extent the feedstuff contributes to a stable rumen fermentation and helps to prevent rumen acidification. From previous research with dairy cattle follows that the structural value mainly depends on crude fibre content and the particle length for

roughages and on the carbohydrate nature and the particle size for concentrates.

Based on the results of all trials a standard for physical structure requirement for beef cattle of 0.60 per kg dry matter is advised for total mixed rations with pelleted concentrates. This standard is also safe for non-pelleted concentrates (ground or unground). For not mixed rations however, one should aim at a somewhat higher value.

5.3 TECHNOLOGY AND NUTRITION

Two new accreditations:

5.3.1 Accreditation for the organisation of ring trials by DVK

The “Department of Animal Product Quality and Transformation Technology” CLO-DVK and the “Department of Quality of Agriculture Productions” C.R.A. Gembloux (DQPA) organize coordinated, applying the calendar which is composed every year in mutual consultation, ring trials for the scientific guidance of the Belgian Milk Control Laboratories, charged with the official determination of the quality and composition of the milk delivered to buyers and the Belgian dairy industry. For the organization of the ring trials DVK obtained an accreditation following the ILAC-G13:2000 norm during the last Beltest audit of 17 and 18 June 2004.

The ring trials are organized for the following parameters: total flora, coliforms, freezing point, inhibitory substances, disinfectants, filtration test, somatic cell counting and the fat- and protein content. For each of the desired parameters the Milk Control Laboratories and the other participating laboratories (dairy industry) receive series of samples, which they have to analyze following their routine methods. The laboratories of DVK-Melle, DQPA-Gembloux and FAVV-AFSCA carry out the reference analyses on these samples.

Using certain criteria, the results of the Milk Control Laboratories and the dairy industry are compared

with the reference results. If too big differences are obtained the concerning laboratory has to take action immediately. Herewith they can count on advice of DVK-Melle and DQPA-Gembloux. The ring trials are organized monthly. Besides the monthly reports of the results, twice a year a reunion is organized with the responsible of the Milk Control Laboratories and FAVV-AFSCA to present and to discuss a report with the results of the previous period. Finally all the results of the Milk Control Laboratories are processed in an annual report and presented at the “Dairy work group”.

These ring trials are very important for maintaining the accreditation system of the Milk Control Laboratories and have an important role in the auto control system of the Belgian dairy industry. In the past the reference analyses for the ring trials were already carried out under accredited circumstances, but now the organization (production of the samples, transport, treatment,...) of them is also accredited and several supplementary controls are carried out. This means mainly homogeneity and stability controls, but also the quality assurance of reagents, apparatus used, equipment, conditions during transport, processing of data, etc. DVK is one of the first organizations in Belgium that obtains an accreditation for the organization of ring trials. Supplementary the organization of a new ring trial was started in October: determination of the fat content in cream.

5.3.2 Accreditation for CLO-DVP

GMO analyses are carried out for the implementation of the EU legislation. As such, it is important to guarantee the accuracy of results. For this reason, in collaboration with CLO-DVK, CLO-DVP has invested in the establishment of a laboratory system that guarantees high quality. The system includes a standardised procedure for sample receipt, procedures for sample preparation, the DNA analysis as such, reporting of the results, and informing the body responsible for the sample. In addition, this system includes a comprehensive procedure for the monitoring and control of the quality of the

infrastructure used, as well as training of highly qualified personnel.

A consequent and horizontal implementation of the new legislation, asks for a clear plan for GMO analysis in the laboratory. At CLO-DVP a structure was built up, allowing for rapid screening, unique identification of GM events, as well as accurate quantification of GM events. This strategy is based on the principle of modularity of the procedure and uses one technique for different purposes, i.e. real-time PCR. Based

on the specific question asked and the type and composition of the sample (matrix), a decision support system, with the structure of a tree scheme, will determine the different steps in the procedure to follow.

Based on a technical audit carried out by BELTEST, since June 2004, CLO is accredited for qualitative and quantitative GMO analyses on raw and processed products according to the standard ISO 17025,.



6. PUBLIC SERVICE

6.1 PLANT

6.1.1 Delivery of prebasic and basic seeds

Before a cultivar arrives at the consumer it must first be successful in official tests and then multiplied. In the scope of granting services, CLO-DVP ensures that sufficient starting material of the created cultivars is available by producing prebasic and basic seed.

The integral quality control on the production system guarantees purity and that the DVP-cultivars are true to type. This starting material is provided to the seed business for commercial seed production.

The total delivery of prebasic and basic seed in 2004 was about 7% more than in 2003. Traditionally, two-thirds of the seed provided were Italian and Westerwoldicum ryegrass. This was because these DVP-selections are widely spread and have a relative high consumption every year. The most important cultivars in this group are Lemnos, Meroa and Gemini. All of these varieties are tetraploid, with a wide listing and use in Europe. They guarantee high yields in both grass production and in quantities of seeds.

The delivery of basic seed of white mustard has decreased despite, in general, the expansion of green cover in Flanders, and in specific, the increase in the use of cruciferous green manure crops. This is mainly due to the large numbers of high yielding European productions, in 2003 and a strong offer of cultivars from Eastern Europe. On the other hand, the delivery of fodder radish basic seed remained almost at the same level.

About 27% of the basic seed was further multiplied to R1-seed in Belgium. In 2004, approximately 1000 ha of Perennial and 2100 ha of Italian ryegrass were controlled by the certifying authorities in Belgium, of which 60 and 80%, respectively were DVP-cultivars. In addition to these inland productions, the majority of the multiplications of DVP-cultivars were performed in Germany.





6.1.2 Diagnostic Centre for Plants

In the Diagnostic Centre for Plants, plant pathogens from four disciplines are isolated and identified: bacteriology, mycology, entomology and nematology. The diagnostic activities are put at the service of civil services, plant growers, advisers, private persons and research centres. In the different disciplines expertise is built and made available in an independent and objective way. Beside diagnosis, wherever possible, advice is also given for the control and the prevention of plant growth problems. To satisfy the quality requirements by the government and the European authorities, a number of procedures according to the norm NBN EN ISO/IEC 17025 are drawn up and implemented.



Diabrotica virgifera (male)



The spider mite Eotetranychus fagi (the male on the left, the female on the right) (microscopic view)

Some facts from the diagnosis centre:

- In 2003, 15.050 analyses were performed, whereas there were only 11.352 analyses in 2002. This increase in number of analyses continues in 2004.

-For decades the seed potato production in Belgium was free from bacterial ring rot. However, in 2002 *Clavibacter michiganense* subsp. *sepedonicus* was detected for the first time in seed. Altogether 7 lots of the processing variety Santana were found infected. Analysis of these lots showed that out of 20400 tubers tested, only 3 showed symptoms and infection levels varied from 0,08% to 1,32%. Finally, 3000 tonnes of seed potatoes on 111 ha across 8 farms and 9800 tonnes of ware potatoes on 369 ha across 8 farms were affected. A more intensive sampling and testing programme is implemented to overcome the problems associated with latency of the disease and the relatively low infection rates in a potato lot.

-The quarantine fungus *Phytophthora ramorum* was detected several times in 2003. Until now the pathogen was only detected in Flanders on *Rhododendron* sp. and *Viburnum* sp. Because the European host range of *P. ramorum* is still expanding, the necessary attention is also given to the monitoring of these other host plants.

-The quarantine nematode *Hirschmaniella* sp. was determined on imported water plants; 18% of the samples were found contaminated.

-The western corn rootworm *Diabrotica virgifera* was found for the first time in Belgium in September 2003. This beetle, initially an important insect pest of maize in North-America, was introduced accidentally in Bosnia at the beginning of the 1990s and spread in Western-Europe. The Federal Agency for the Safety of the Food Chain started a survey in July 2003 in collaboration with the Department of Crop Protection to monitor this beetle in our country. To do so, pheromone traps (sticky plates with bait) were placed in maize fields around military airports, civil airports, as well as in the neighbourhood of seaports, at the CEI and near parking places along

international motorways coming from North-Italy, South-Switzerland, Austria and France. Pheromone traps with suspected material were investigated at the Diagnostic Centre for Plants. This quarantine insect was first detected in our country near Zaventem at the beginning of September 2003. Later on in September the beetle was also found near Kampenhout. Three focus zones (Zaventem, Machelen-Steenokkerzeel en Kampenhout) were defined around the sites where the beetles were trapped, and surrounded by one safety zone. In order to eradicate the pest insect and to avoid further spread, the European Commission enforced a number of emergency measures for focus zones and safety zones. These measures are applied in the year of detection and extend to one year after detection. Monitoring of the organism was intensified in 2004. On 7 September 2004 the western corn rootworm was detected again in Kampenhout. The infested field was situated in the existing safety zone. Half September the beetle was found again on two additional fields in the safety zone. A new focus zone was defined around the infested fields, the existing focus zones were adapted and the safety zone was extended. On 5 October the beetle was found once again in a field in the safety zone (Wezenbeek-Oppem). The small numbers of beetles detected in 2004 show that the emergency measures taken in 2003 had a useful effect.

-The spider mite *Eotetranychus fagi* was detected for the first time in several places in Flanders on *Fagus sylvatica* in the autumn of 2002, and later in 2003 and 2004. Because of the sucking activity of this mite the leaves become brown and fall early. Because of the popularity of beech as hedge plant, further spread of this pest is expected.

-Before starting the export of Belgian tomatoes to Japan our country has to prove that the Mediterranean fruit fly *Ceratitis capitata* is absent in the production area. Because of the extensive import of fruit in our country this fruit fly can be imported and survives (in glasshouses). Larvae of this fly parasitize more than 260 fruits, vegetables, flowers and nuts. Last year's monitoring of this fly was carried out with pheromone traps on a number of places with high risk (Brucargo, CEI, port of Antwerp, production area).

In the Diagnostic Centre for Plants more than 3000 pheromone traps were checked yearly on the presence of this quarantine fly. Thanks to this monitoring action the export of our quality tomatoes to Japan is a fact.

6.1.3 Scientific and logistic support of pre- and post-control and seed quality control of agricultural crops

In 2003 pre and post control trials were set up, under order of ABKL – NCPP, for grasses (864 samples from 15 species) and flax (570 samples). For 2004, 674 samples of grasses (from 15 species), 528 samples from flax and 369 samples from potatoes were tested.

On order of EU-DG SANCO (Health and Consumer Protection) comparative trials for winter wheat were set up for the growing period 2002–2003 and 2003–2004.

In 2003 and 2004 respectively 133 and 125 samples were evaluated for purity (off-types) and identity, based on the UPOV-description. The results were discussed during a meeting in June, in combination with a field visit with presentations of the certification services from all EU-countries.

In view of integration of the official seed laboratory (ex Gentbrugge) the training of technicians at CLO-DFE was started. From the 1st of April 2004 the seed laboratory was operational in the building of CLO-DFE. For the support of the activities of this accredited laboratory, technicians of variety research and post control were incorporated at peak moments.

6.1.4 DUS and VCU research for the national and European variety catalogue

Under order of the Administration for Quality of Agricultural Production (ABKL – NCPP – Flemish Region) and “Direction de la Qualité des Produits” (Walloon Region) DUS and VCU trials were set up, evaluated and reported in view of the elaboration of the national variety catalogue of agricultural crops. The national variety catalogues are the basis for the drawn up of the common European variety catalogue of agricultural crops. The protocols for the trials are put forward by the Technical Interregional Working Group.

For the VCU trials in 2003 and 2004 respectively 377 and 357 varieties, from 18 crops, were tested at 6 to 7



locations in different agricultural regions. Furthermore, 7 new varieties of industrial chicory were tested for DUS research and evaluated to a reference collection (38 varieties) according to the UPOV guideline TG/172/3.

In 2003 and 2004 respectively 4 and 7 begonia and tuber hybrida cultivars were tested for plant breeders rights on order of the Community Plant Variety Office (Angers). These cultivars were compared to 5 reference varieties.

6.1.5 Reference laboratory for research on Plant and Soil

In the agreement document, between the competent minister and CLO, the development of a reference laboratory as a mandated service for research of soil and plant is agreed. The aim is to develop fast, accurate, and animal friendly analysis techniques which reflect the knowledge and the understanding of the chemical composition of water, soil (as growth medium) and plants. Therefore the newest developments in chemical and physico-chemical analysis techniques are followed up and the best possibilities are examined to implement them in the laboratory.

Once these analysis techniques are converted into useful methods, the accreditation is the next step. A chemical laboratory, at service for the authorities and its scientific research has the duty to test itself on accuracy and reproducibility. The accreditation and the maintenance of it is hereby the best guarantee. The accreditation will be organised according to the ISO-17025 norm: "General requests for the competence of testing and calibration laboratories".

Procedures dealing with the quality handbook are drafted. In this quality handbook, the practical implementation of the system in the quality division of the department is described. Besides some test procedures with additional validation are drafted.

At the beginning only the analyses for which the authorities have any interest will be accredited, being some routine determinations in forage crops like dry matter content, crude protein and nitrogen content.

6.2 ANIMAL

6.2.1 Reference laboratory for animal feeds

In the frame of contract research with feed manufacturers, the government and other research institutes, CLO-DVV carries out feeding experiments with cattle, pigs, poultry and rabbits. Besides the study of the animal performances, there is specific expertise to execute digestion and balance trials, in sacco rumen incubations and in vitro determinations. The feeds, excreta and end products (milk, meat, eggs) from these experiments are analysed by the quality section. The latter consists of four labs: chemistry-physics, chromatography, digestion physiology and meat research. Recently, BELTEST was asked for a pre-audit in order to obtain the EN ISO/IEC 17025 norm in the course of 2005 for the main chemical analyses (e.g. Weende and Van Soest parameters), fatty acid composition and cellulase digestibility in feeds and fermentation characteristics in silages.

6.3 TECHNOLOGY AND NUTRITION

6.3.1 The ISO 17025 accredited laboratories of the Quality Division of DVK

To prove the quality of their products to their buyers, the industry and the producers are more and more in need of objective analyses performed by competent laboratories. The government, as supervisor, has also to make appeal to such laboratories. The Belgian accreditation organization BELTEST offers laboratories the possibility to express their competence and provides them a national and international reference. Since 26 September 1995 the laboratories of the Quality Division of the DVK-CLO department have the analytical competence according to BELTEST-criteria (NBN EN ISO 17025 guideline). Towards the end of 2004 the Quality Division covered 50 accredited analyses distributed over the laboratory Physics-Chemistry and organization of comparative studies, the laboratory Chromatography, the laboratory Microbiology and Genetically Modified Organisms (GMO's) and the laboratory for detection



of Antibiotics.

In 2003 the extension of the scope of the Microbiological laboratory with the detection of GMO's in soy, maize and derived products was started and this in cooperation with the Department of Plant Genetics and Breeding (DVP-CLO) also belonging to the Agricultural Research Centre. In June 2004 the accreditation of these analyses was obtained. Twelve of the fourteen microbiological analyses previously only accredited for the matrices milk, milk products, meat and meat products are since September 2004 performed on all foodstuffs under accreditation.

The comparative studies organised in the framework of the scientific guidance to the Belgian Milk Control laboratories and the Belgian dairy industry are since September 2004 also organized according to BELTEST-criteria. Those activities were added to the scope of the laboratory Physics-Chemistry. The Quality Division takes now into account not only the NBN EN ISO 17025 but also the ILAC-G13:2000 guideline.

In the Chromatographic laboratory the determination of ionophoric coccidiostats was added to the scope in 2004, using the procedure of flexible scope.

Finally, in 2003, the laboratory for detection of

Antibiotics received an accreditation for the detection of β -lactams, tetracyclines, and sulphonamides with the Parallax Test and the detection of β -lactams with the Charm MRL Beta-lactam-Test in raw milk, milk, milk powder (and cream). A flexible scope for the detection of chloramphenicol in foodstuffs was also obtained. In 2004 the scope was again extended with the detection of inhibitory substances in raw milk, milk, milk powder and cream with the CMT Copan Milk Test and with the detection of tetracyclines in honey with the Tetrasensor Honey test. A flexible scope for the screening of inhibitory substances in foodstuffs with a microbiological inhibitor test and the screening of antibiotics and chemotherapeutics with receptor-, immuno- and Charm II (receptor) tests with radioactive labelling was obtained.

The detailed scope with the analysis methods can be found on the website www.clo.fgov.be/dvk/consulting_quality_lab.htm or via the BELTEST website <http://Beltest.fgov.be>

6.3.2 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 the 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 220 individual advices each year. The main reasons for the 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 is 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, 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'.

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.

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: fertilizer spreaders (spreading pattern), potato harvesters (prevention of discolouration), chicory harvesters, sprayers (incl. lance sprayers), etc. This kind of service necessitates a visit to the farm. After completing the necessary observations a report is made up that is



provided to the people concerned.

Not all recommendations are of this size. The largest number of 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.

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).

Quality Management: maintenance of milking equipment.

The project group 'Control' coordinates the activities for testing milking equipment (milk installation, refrigerated farm milk tank) and organises the training and the support of the technicians of milking machines, refrigerated farm milk tanks and milk extraction equipment in Belgium.

In the functioning of Control the demands made by IKM (integrated quality control milk) and the international standards for milk installations (ISO 3918, ISO 5707, ISO 6690) are taken into account.

Last year an inventory was made of all the measuring and advisory reports of 2003. Each technician was subsequently evaluated by means of a completely automatic evaluation report, developed in Excel. This was done on random samples (5%) of all reports made by the technician (with a minimum of five reports). The selected reports were checked for accurateness and correctness. Since the start of the project there has been a clear increase in the quality of the reports.

Also the measuring equipment (pulsation tester, vacuum gauge, air flow meter and thermometer) of the maintenance technicians was checked for proper operation.

The results clearly illustrate the importance of a regular (annual) maintenance of both milk installation and refrigerated farm milk tank. Maintenance activities must be carried out professionally. Hence, a qualitative training of the technicians is necessary. The annual evaluation of



the technicians does not aim solely at the work of the technicians but is also intended to solve technical questions or problems related to the maintenance of the milking installations or refrigerated farm milk tanks. Since the start of this project (1996) a remarkable improvement in the maintenance of the installations has been observed.

The courses for milking machine technicians continued in October and nine participants attended the courses.



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