



2003-2004

ACTIVITY REPORT





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ACTIVITY REPORT

2003 - 2004

WALLOON AGRICULTURAL RESEARCH CENTER



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Directeur général a.i. : P. Meeùs

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C O M P O S I T I O N

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(from 16 January 2004)

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(until 15 January 2004)

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College of Heads of Department

President:

P. Meeùs, Acting Director General, Walloon Agricultural Research Centre, Gembloux

Members:

B. Watillon, Head of Biotechnology Department M. Frankinet, Head of Crop Production Department M. Cavelier, Head of Biological Control and Genetic Resources Department Y. Schenkel, Head of Agricultural Engineering Department N. Bartiaux-Thill, Head of Animal Production and Nutrition Department P. Dardenne, Head of Quality of Agricultural Products Department

Managing Board of the Legal Entity

(until 15 January 2004)

President:

P. Meeùs, Acting Director, Gembloux Agricultural Research Centre

Members:

R. Piscaglia, Acting Assistant Managing Director, Gembloux Agricultural Research Centre G. Detiège, General Secretary, Phytophar G. Houins, Director of the Control Policy Department, Federal Agency for the Safety of the Food Chain (AFSCA) Ph. Van Eyck, rue de l'Abbaye, 50, 5030 Gembloux B. Watillon, Head of the Biotechnology Department, Gembloux Agricultural Research Centre P. Dardenne, Head of Quality of Agricultural Products Department

Observers from the Walloon Regional Government:

J.M. Godefroid, representing the Minister, José Happart E. Reuter, representing the Minister, Serge Kubla J. Renault, Acting Director General, Department of Agriculture, Ministry of the Walloon Regional Government

PREFACE

Two significant events marked the 2003-2004 period for the Research Centre, its operation and its future aims. Firstly, the regionalisation begun during the previous reporting period took concrete form and secondly, the Centre was reorganised as a public-interest organisation, called the Walloon Agricultural Research Centre. While its new status gives the CRA-W greater autonomy, it also brings fresh responsibilities.

The reforms are not yet complete. For one thing, the Centre's scientific activities will be carried out under the supervision of the Agricultural Research Orientation and Evaluation Committee. Moreover, the status enjoyed by researchers for over forty years is to be replaced by the new code applicable to the Walloon civil service.

Fortunately, the changes that are in progress have not damped our researchers' enthusiasm, as is evident from this 2003-2004 research report. Each Department has striven to meet the requirements of the Walloon Region and its aim of extending the benefits of research and research undertakings not only to farmers but to society as a whole. While continuing to work on the research programmes established within the Federal framework, the CRA-W has endeavoured to meet a threefold challenge: to anticipate changes, to develop its expertise and to disseminate its knowledge.

The success of this ambitious programme is due to the multidisciplinary and complementary skills of the CRA-W's Departments and Units, which cover the different areas relevant to agriculture. Now more than ever it is imperative to pool activities under common headings, such as food quality and safety, traceability along the food chain, environmental management, sustainable production systems, rural development and sustainable use of natural resources.

The future will bring fresh challenges for the CRA-W, particularly in terms of society's demands. To meet those challenges, on going investment will be required in fundamental and applied research and the Centre's service activities.

Directeur général a.i. : **P. Meeùs**

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A C R O N Y M S А A B R E V I A T I O N S N D

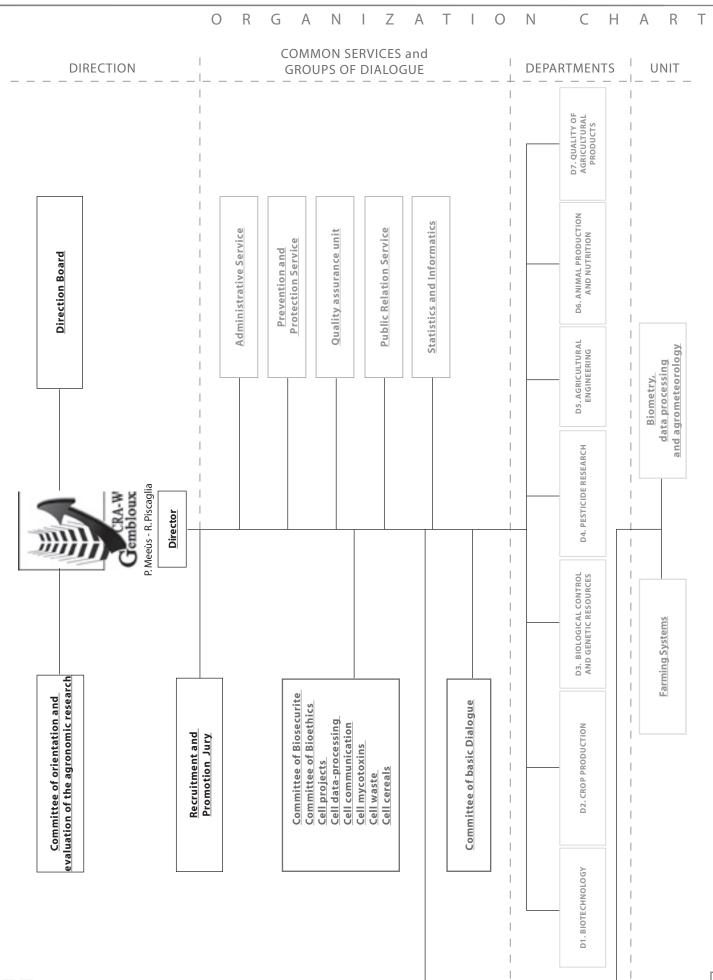
ACLSV	Apple chlorotic leafspot virus
AEI	Agro-environmental indicators
AFPP	Association Française de Protection des Plantes
AOC	Appellation d'origine contrôlée (registered designation of origin)
ApMV	Apple mosaic virus
ASGV	Apple stem grooving virus
ASPV	Apple stem pitting virus
AWEX	Agence wallonne à l'exportation (Walloon Export Agency)
BaMMV	Barley mild mosaic virus
BASE	Biotechnology, Agronomy, Society and Environment
BaYMV	Barley yellow mosaic virus
BDPA	Biometry, Data Processing and Agrometeorology
BEPN	Bureau économique de la Province de Namur
BNYVV	Beet necrotic yellow vein virus
BYDV	Barley yellow dwarf virus
CBD	Convention on Biological Diversity
CEE	Commission of the European Community
CEHW	Centre d'Essais Horticoles de Wallonie
CGMS	Crop Growth Monitoring System
CLO-DvP	Departement Plantengenetica enveredeling
CNES	Centre National d'Etudes Spatiales
COLEACP	Comité de liaison Europe-Afrique- Caraïbes-Pacifique.
CRIA	Centre de Recherches Industrielles et Agronomiques
CRNFB	Centre de Recherche de la Nature, des Forêts et du Bois
CSL	Central Science Laboratory, York, England
DG	General Department
DGA	General Department of Agriculture, Ministry of the Walloon Regional

Government

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DGRNE	General Department of Natural Resources and Environment, Ministry	MRL	Maximum Residue Limit
	of the Walloon Regional Government	NBS	Nationale Boomgaardens
DGTRE	General Department of Technologies, Research and Energy, Ministry of	OCDE	See OECD
	the Walloon Regional Government	OECD	Organisation for Economic (and Development
DIAGPRO	Diagnostic Protocols for Organisms Harmful to Plants	OEPP	See EPPO
DNF	Direction Nature et forêts	OILB	See IOBC
ECP/GR	European Cooperative Program for Crop Genetic Resources Networks	OIP	Organisme d'intérêt publ
EEC	Economic European Commission.	OMS PADD	See WHO Plan d'Appui Scientifique
ELISA	Enzyme-Linked Immunosorbent Assay		Politique de Développem
EPPO	European and Mediterranean Plant Protection Organisation	PCR	Polymerase Chain Reaction
ERDF	European Regional Development	PDV	Prune dwarf virus
	Fund	PepMV	Pepino mosaic virus
EU	European Union	PNRSV	Prunus necrotic ringspot vi
FAO	United Nations Food and Agricultural Organization	PPV	Plum pox virus (sharka)
FEDER	See ERDF	PSF	See SPS
FIWAP	Filière wallonne de la Pomme de	PSTVd	Potato spindle tuber viroid
	Terre	RAPD	Random Amplification of Polymorphic DNA
FUL	Fondation Universitaire Luxembourgeoise, Belgique	RGF	Fruit Germplasm
FUSAGx	Gembloux Agricultural University, Belgium	RpRSV	Raspberry ringspot virus
GIS	Geographic Information Systems	RW	Walloon Regional Govern
GLP	Good Laboratory Practices	SAU	See UAA
GTIS-CAP	GeoTraceability Integrated System for	SIG	See GIS
	the Common Agricultural Policy	SPP	Federal Office for Scientifi and Cultural Affairs
IBW	Instituut voor Bosbouw en Wildbeheer	SPS	Federal Science Policy Un
IIRB	Institut International de Recherches Betteravières.	TSWV	Tomato spotted wilt virus
ЮВС	International Organisation of Biological	UAA	Utilized agricultural area
1000	Control	UE	Union européenne
KUL	Katholiek Universiteit Leuven	UPOV	International Union for th of New Varieties of Plants
LChV-1	Little cherry virus-1	wнo	
LChV-2	Little cherry virus-2		World Health Organisatio
MA	Federal Ministry of Small Enterprises, Traders and Agriculture	WHOPES	World Health Organisatio Evaluation Scheme.
MAE	Agro-environmental measures		

L	Maximum Residue Limit
5	Nationale Boomgaardenstichting
DE	See OECD
D	Organisation for Economic Co-operation and Development
Р	See EPPO
В	See IOBC
I	Organisme d'intérêt public
S	See WHO
DD	Plan d'Appui Scientifique à une Politique de Développement Durable
R	Polymerase Chain Reaction
/	Prune dwarf virus
MV	Pepino mosaic virus
RSV	Prunus necrotic ringspot virus
/	Plum pox virus (sharka)
:	See SPS
Vd	Potato spindle tuber viroid
PD	Random Amplification of Polymorphic DNA
	Fruit Germplasm
RSV	Raspberry ringspot virus
	Walloon Regional Government
J	See UAA
	See GIS
•	Federal Office for Scientific, Technical and Cultural Affairs
;	Federal Science Policy Unit
vv	Tomato spotted wilt virus
A	Utilized agricultural area
	Union européenne
vc	International Union for the Protection of New Varieties of Plants
0	World Health Organisation.
OPES	World Health Organisation Pesticide Evaluation Scheme.



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1.MISSION STATEMENT

. The mission of the Quality Assurance Office (QA Office) is to improve quality procedures at the CRA-W. To this end the QA Office assists each department with putting in place and following up an appropriate quality system and harmonises procedures between departments.

. The QA Office reports directly to the CRA-W Management and is supported by quality correspondents within each department.

2 SCIENTIFIC PERSONNEL

Dominique Vrebos, Research Assistant (CRA-W) Blandine Gaurois, Research Assistant (CRA-W)

3. AIMS OF QUALITY ACTIVITIES

The aims of the quality procedures developed at the CRA-W, which are currently applied to analytical work and are being extended to experimental and research aspects, are: - to provide the guarantees required by our backers, the public and the scientific community, - to raise operator awareness of a new culture of rigour, responsibility and communication, - to achieve steady improvement in day-to-day practices.

4. SPECIFICATIONS, PROCEDURES AND NEW AREAS OF EXPERTISE

The quality systems in place or under development are mainly based on the ISO 17025 standard (general requirements for the competence of calibration and testing laboratories, which also provide a quality guideline for experimentation and research) and GLP (Good Laboratory Practice, as defined by the OECD) as regards studies of phytosanitary products.

Policies and procedures to supplement the good professional practice prevailing in the research field have been put in place in accordance with these standards.

Briefly:

organisational aspects:

drawing up functional organisation charts and defining duties and deputies for key posts;
control of documentation and records to provide a clear overview of operations and access to the full history of operations at all times;

 review of enquiries, invitations to tender and contracts with emphasis on how closely the service matches the client's explicit or implicit expectations;

 - complaints handling (external and internal);
 - control of non-conforming work and corrective and preventive action; - internal audits and management reviews to ensure that the quality systems and testing activities are always appropriate and effective.

technical aspects:

- management of personnel skills and continuous learning;

- more systematic management of equipment including calibration to ensure traceability of measurements to SI units (International System of Units);

-validation of test methods and measurement uncertainty assessment;

- test result quality monitoring by drawing up control charts, taking part in interlaboratory trials, etc.

5. STRONG POINTS

The organisation of the QA Office and the CRA W's resources with respect to the deployment of quality systems is characterised by three strong points:

- a team of fourteen qualified in-house auditors, drawn from all the departments, to ensure the necessary objectivity and independence in connection with internal audits that are essential if self-assessment of systems and activities is to be effective and a real aid to improvement;

- organisation of training by the QA office: requirements analysis and running of in-house training courses with external trainers for staff from various departments and liaising with the Walloon Regional Government Training Department;

- drawing on the expertise of the Unit of Biometry, data Processing and Agrometeorology with respect to the statistical approach and processing necessary for validation of methods and creating and monitoring control charts in a wide range of areas.

Qualified in house auditors

- Delcarte Jérôme (D5)
- Decock Valérie (D4)
- Fumière Olivier (D7)
- Galoux Michel (D4)
- Gaurois Blandine (D8-BAQ)
- Goffart Jean-Pierre (D2)
- Jacquemin Jean-Marie (D1)
- Jansen Jean-Pierre (D3)
- Mostade Olivier (D5)
- Reuter Véronique (D2)
 Sinnaeve Georges (D7)
- Steyer Stéphane (D3)
- Thilmany Françoise (D8-SSA)
- Vrebos Dominique (D8-BAQ)

6. PRIORITY AREAS AND PROGRESS REPORT

Quality Systems are implemented according to a schedule of priorities, in accordance with the legal (or contractual) accreditation obligations in the different areas.

In the case of some trials accreditation is or will ultimately be mandatory if the CRA-W is to maintain approval or official recognition by the food chain safety agency, AFSCA (statutory analyses), the Ministry of the Walloon Region (e.g. application of the nitrates directive), certifying bodies (labels, organic farming, etc.), laboratory networks (REQUASUD) and international organisations (EU, WHO, FAO, FDA, EPA, CIRAD and so forth). Relevant areas include microbiological analyses and milk composition, the breadmaking value of cereals, detection of antibiotics or meat and bone meal in cattle feed, GMOs (D7), mycotoxins, quarantine organisms (D3), pesticide residues (D4), sprayer testing (D5), virological testing of potatoes (SSA) and mineral nitrogen in soil (D2).

In other areas of testing and also for experimental and research purposes, accreditation is still voluntary, though likewise perceived as a sign of credibility of the laboratory's technical and organisational efficiency. These include for example determining the food value of forage and cattle feed (SSA), biofuel and fertilizer analysis (D5), potato and cereal variety identification, detecting harmful microorganisms in orchards (D3, D1), analysis of impregnated substrates for the World Health Organisation (D4), etc.

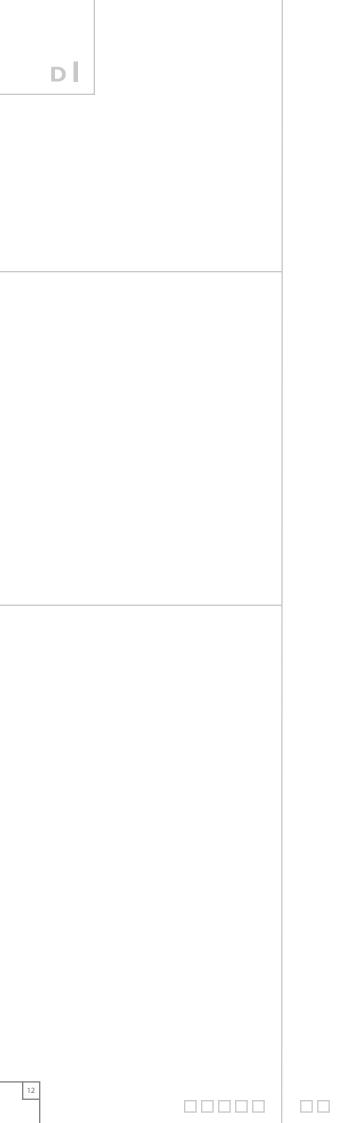
Q Y Е А ΙT А S S R Ν С Е L А С F F

Department	Benchmark certificates	Area of accreditation / certification
Pesticide Research (D4)	OECD/Louis Pasteur Scientific Institute of Public Health	GLP – Studies of plant protection product formulations and residues
		Analysis of pesticide residues in fruit, vegetables, cereals and other materials. Analysis of pesticides in treated seeds.
Biological Control (D3)	OECD/ Louis Pasteur Scientific Institute of Public Health	Ecotoxicology studies of plant protection products
Agricultural Engineering(D5)	Beltest	Testing of sprayers (nozzles and manometers) Chemical testing of solid biofuels. Physical testing of fertilizers.

The accreditation scope details (sample type, analytical item, analytical method) can be given at any customer request to the CRA-W Q.A office or the accredited department secretary.

> Quality builds together... BAQ Coordination : D1: D3: D4: D2: Dominique Jean-Pierre Michel Jean-Marie Stéphane VREBOS JACQUEMIN GOFFART STEYER GALOUX Blandine David Valérie DECOCK GAUROIS DANTINNE

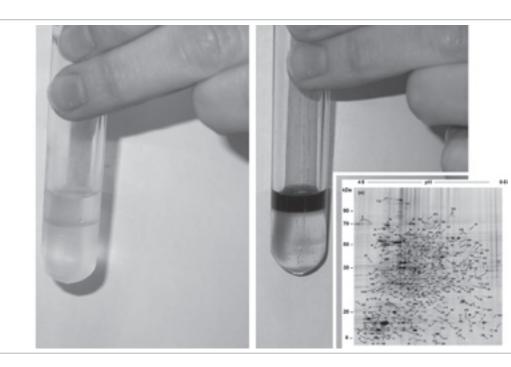
D5:	D6:	D7:	BDPA:	Farming Systems
Jérôme	Benoît	Véronique	Viviane	Françoise
DELCARTE	GREGOIRE	NINANE	PLANCHON	THILMANY
François	Nicole			
GIJS	BARTIAUX			



D

Department

BIOTECHNOLOGY



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e-mail: biotec@cra.wallonie.be



1. MISSION STATEMENT

. Molecular biological research in the areas of plant improvement in agriculture and fruit and vegetable horticulture, of plant pathology and soil microbiology.

. Research on the use of in vitro culture for plant propagation and on conventional propagation techniques in horticulture.

2. SCIENTIFIC PERSONNEL

6 permanent staff and 13 contract staff

2.1. Head of Department

B. Watillon, Inspecteur général scientifique

2.2. Scientists

- O. Arezki, Attaché scientifique (RW-DGA)
- A. Bultreys, Attaché scientifique (MRW)
- F. Delporte, Attaché scientifique assistant (MRW)
- Ph. Druart, Inspecteur général scientifique, Head of Section
- A. Dutrecq, Attaché scientifique (on career break)
- S. Farvacque Ingénieur industriel (RW AR258/APE)
- V. Gilbert, Attaché scientifique (RW-DGA)
- R. Gruselle, Attaché scientifique (RW- DGRNE/Interreg)
- M. Hajji, Attaché scientifique (MRW), till 17.10.04.
- J.M. Jacquemin, Directeur scientifique
- F. Legros, Ingénieur industriel (RW-DGA), till 17.10.04.
- H. Magein, Attaché scientifique
- S. Mauro, Attaché scientifique (MRW)
- D. Mingeot, Attaché scientifique (MRW)
- Y. Muhovski, Attaché scientifique (RW-DGA), since 01.08.04.
- J.-P. Misson, Ingénieur industriel (RW-DGA) S. Pieron, Chef de travaux (on career break)
- J.M. Terzi, Ingénieur industriel (MRW)

2.3. Ph.D. Students

G. Iwangou (CTB) C. Ugarte-Ballon (CTB)

3. NATIONAL REPRESENTATION

- APHW (Association pour la Promotion de l'Horticulture Wallonne), member of the board of directors

- Belgian Bioindustries Association, member.
- BPTCg (Belgian Plant Tissue Culture group),
- member of the board of directors - CEF (Centre fruitier wallon), consultative member
- of the board of directors - Commission Fruits de la Fédération horticole
- wallonne (FWH), member
- Comité scientifique du Centre technique horticole (CTH), member
- Comité scientifique du Centre d'essai maraîcher (CIM)
- Comité scientifique de la revue « BASE », secretary
- Comité technique pour la promotion de la fraise wallonne, member
- Comité technique du Jardin d'essai fruitier de Velm, member

- Conseil technique de la Station de Recherche de Gorsem, member

- Conseil du droit d'obtention végétale, member - Conseil Consultatif de la Recherche et du
- Développement, groupe de travail Horticulture (MA, DG6), member
- Conseil du Fonds budgétaire pour la production et la protection des végétaux et des produits végétaux (MA, DG6), member
- Conseil d'appel des Etablissements scientifiques de l'Etat (Min. Agric.), assessor member
- GRAGEF (Groupement Régional d'Amélioration
- Génétique des Essences Forestières), members - Conseil de la Filière Horticole Ornementale
- Wallonne, member and President
- Groupement des Fraisieristes Wallons, asbl, member
- Jardin d'essai fruitier de Merdorp (CEF), member of the board of directors
- Ligue pomologique des provinces de Hainaut et Namur : member of the board of directors, secretary
- OFB-BFO (Organisation Fruitière Belge -
- Belgische Fruit Organisatie) consultative member of the board of directors.
- PBB (Pépinière belge-Belgische boomkwekerij), asbl, organisme interprofessionnel responsable de l'ensemble de la filière pépinière belge, vice-President
- Comité Scientifique "Plantes transgéniques" du Conseil de Biosécurité a.i., members.
- Comité scientifique "Microorganismes géné-
- tiquement modifiés bactéries et champignons" du Conseil de Biosécurité, member
- Revue « Le Fruit belge», Directeur

4. INTERNATIONAL REPRESENTATION

- ASM (American Society for Microbiology),
- membre
- AUPELF-UREF, Réseau Biotechnologie et Génie Génétique, member
- Commission d'Evaluation Collective de l'Unité de Recherches INRA sur les Espèces Fruitières et Ornementales à Angers (France), expert
- European Fruit Research Institute Network EUFRIN, member
- Fraise de France, CIREF (Centre Interrégional de Recherches et d'Expérimentation sur la Fraise). representing european research in the scientific council
- IAPTC (International Association for Plant Tissue Culture), national representatives and members - ISHS (International Society of Horticultural Science), members of the Biotechnology and Fruit sections (Cherry and Plum groups)
- UE-DGXII, COST ACTION 843 « Quality Enhancement of Plant Production through Tissue Culture », Member of the Management Committee and national representative in the Working group 2 « Advanced propagation techniques ».
- UE-DG XII,-COST ACTION 836 (Integrated Research in Berries), national representative WG1 and member of the Management Committee. - UE-DG XII,-COST ACTION E28 ("Genosilva"), national representative in the Working Group 2.

5. RESEARCH TOPICS

During last years, the life sciences in general and the modern biotechnologies in particular were perceived like one of the key scientific disciplines for the future, being able to strongly contribute to the advent in Europe of an knowledge-based economy. The report of the capacity of these technologies to ensure, all at the same time, an economic growth (creation of goods and services) and an improvement of the living conditions for all led the European political authorities to affirm their will to support and promote the rise of biotechnologies. The Walloon agriculture, confronted today with many constraints and social requests, cannot miss taking advantages from opportunities and hopes raised by the recent developments of the life sciences. Turned towards the needs for the Walloon Region and its agriculture, the scientific work continued in our Department thus integrates the various biotechnological tools available today (such as the genetic fingerprinting technologies, the tissue culture methodologies or the genetic transformation techniques).

This approach appears through research projects aiming at objectives such as the development of plant varieties adapted to a more environmentallyconcerned agriculture, the support to emergent and promising sectors through technological innovation, or the definition of tools of certification and traceability.

6. RESEARCH REPORTS

- 6.1. Research on the use of in vitro culture for plant propagation and on conventional propagation techniques in horti culture.
- Development of new technologies for production of high-quality christmas trees. Misson J-P* (* RW-DGA)

Our project aims at supporting the industrial conversion of the Walloon production of christmas trees by the establishment of seed orchards and the development of technologies for the cloning of "elite" Abies nordmanniana lines. The first evolutionary seed orchard, established since 1996, currently comprises two hundred grafted plants divided into about fifteen clones; it is supplemented annually by new elite clones established by "in situ" grafting. It remains to fix about fifteen clones. A second orchard is in way of installation at Mussy-la-ville. The position of the trees located in Neufchâteau was determined using DGPS system (differential global positionning system). The use of individual variability by creation of seed orchards of controlled origin fits perfectly in a channel of certification for the plant material produced in Wallonia. In parallel, we seek the means of accelerating the flowering by the grafting of the elite clones on hyperfloral Abies cultivars. These tests are in hand since 1999 and the observations continue. If the

creation of so-called "synthetic" seeds within the seed orchards can reduce the heterogeneity of the individuals, it is only by cloning (i.e. by vegetative multiplication) that genetically homogeneous plant material could be obtained, thus offering the possibility of a completely rational exploitation. The first tests of cuttings for the Nordmann fir related to the time of taking away of the explants : the cuttings with wood which are carried out November at February, the herbaceous cuttings of June and those of October or semi-dormant. The cuttings taken in October-November and placed at cold (+4°C) for 4 to 8 weeks before their insertion, gave us 35 % of rooting starting from 12 years old mother plants. This type of cuttings seems interesting because they should be less prone to the polymorphism events often associated with others, but that remains to be confirmed.

The greatest hopes to solve the problems of mass vegetative multiplication of the selected clones of Abies nordmanniana rest however on modern technologies of "in vitro " multiplication and more particularly on somatic embryogenesis. In Nordmann, the propagation by cuttings will complement the modern techniques of multiplication, of which we know that the "in vitro" steps may induce juvenility. The process of somatic embryogenesis is controlled perfectly for several lines until the phase of maturation of the embryos. With the lines of reference, the conditions of germination are currently being optimized and the acclimatization of the first somatic embryos is in hand. In order to preserve the embryogenic competency of our lines, it was necessary to develop a technique of cryoconservation. Currently, fifteen lines are stored in liquid nitrogen.

Use of in vitro culture for studying flowering problems in woody fruit species. Ugarte-Ballon C.*, Magein H, Ph Druart (*CTB)

The production of certain fruit-bearing species is affected by adverse conditions to the floral transition. At the cherry tree for example, disturbances intervening during floral initiation lead to a later development of malformed fruits. A new study was undertaken in this direction within the framework of a PhD, on the basis of recent progress obtained with the in vitro flowering of Rosaceae. The prime objectives are to try to transpose on vitroplants, the conditions supporting the formation of floral buds by using, as references, several decorative species known for their facility of flowering and to determine in parallel the unsuited climatic factors on trees growing in Bolivia. The first flowers were obtained on a rose cultivar grown in vitro. This work is performed in collaboration with Professor P. Du Jardin (FUSAGx).

Development of true-to-type propagation methods in "Witloof" chicory and in artichoke. Hajji M.* (* RW-DGA)

The possibility of inducing the appearance of organogenic nodules on the leaves of in vitro cultivated Witloof chicory plants was used as a basis for the development of a method of micropropagation based on these nodules. Encouraging results could be obtained with this technique for all the lines tested, but its performances remain very variable among these lines. The multiplication via the nodules remains, in the current state, labour-intensive (in particular at the level of obtaining the nodules on the leaves). However, the possibility of obtaining a secondary nodulation process could be demonstrated, and makes it possible to consider a considerable reduction of the labour requirements for the multiplication by this way. Moreover, the nodules can be used for the in vitro conservation of the chicory lines to be maintained by a breeder : the nodules can be preserved, attached to the leaves, during more than 6 months without additional handling, then put to germinate to form secondary nodules which can in turn be preserved in vitro. The work undertaken on the axillary micropropagation of artichoke made it possible to strongly improve the performances of the method, by optimization of the culture media. In parallel, the "modeling" approach applied to this species led to the development of an extremely effective multiplication method (by the reduction of the labour requirements and the increase of the performances for proliferation), which could be applied to the various lines in test.

Creation of new Prunus and Malus genotypes by in vitro culture : organo genesis, somatic embryogenesis, protoplasts, genetic transformations, somaclonal variation. Ph Drugat

1. Study of competence to regeneration.

As opposed to what a review of the literature may seem to show, the control of regeneration still constitutes one of the main causes limiting the effectiveness of the protocols of genetic transformation in woody fruit species. A better knowledge of the circumstances of induction and initiation is required in the context of adventitious budding in apple tree and of somatic embryogenesis in Prunus. Following the repeated failures of transposition to other Prunus of the somatic embryogenesis method developed for Inmil (P. incised X serrula), we checked the behavior of the descent of this genotype. Zygotic embryos resulting from free pollination were thus put to germinate in vitro and sowings were propagated. Four of the five lines formed somatic embryos on the roots according to the procedure used at the origin for Inmil. The five lines appeared embryogenic on roots of second generation. A cytometric analysis performed on the various origins of somatic embryos did not reveal any genetic variation. It is to our knowledge, the first time that the hereditary feature of competency to somatic embryogenesis is shown in Prunus.

2. Protoplast culture in Prunus sp.

The protoclones of the rootstock' Inmil' (*P. incised X serula*) transferred in nursery appear phenotipically similar and behave like the control plants. The observations will continue until flowering. On the other hand, the regeneration of protocalluses of Damil (*P. dawyckensis*) failed. The tests were temporarily stopped.

3. Genetic diversity in apple tree aneuploïds.

The *in vitro* rescue of the aneuploïd embryos ensures the survival of these unstable genotypes while the *ex vitro* micrografting makes it possible to evaluate their behavior within the orchard before regarding them as potential parents. The tests carried out during these two years thus confirmed that, by using *in vitro* culture techniques, aneuploïd lines of apple tree could constitute a source of genetic variability that may be exploited through traditional breeding programs.

4. Somaclonal variation for fruit color and tree growth in apple tree.

Following the success of the variation of the color of fruit skin obtained with Jonagold, a first method for the screening of regenerants was developed on microcuttings raised *in vitro*. It would make it possible to distinguish a clone producing fruits with dominant red color from another clone whose fruits are predominantly green at the time of harvest.

A new program of somaclonal variation was engaged with the cultivar Pinova in order to check the conditions of induction of this type of somaclonal variation and to confirm the method of *in vitro* preselection. The main variant candidates were established by *ex vitro* micrografting on standard M9 KI 29.

The preliminary tests of adventitious budding were carried out with the cultivar" Boskoop spur " in order to observe the potential variation of the growth habit from the behavior of regenerants from leaves.

5. Genetic transformation by the intermediary of *Agrobacterium rhizogenes*.

The clones of Inmil (*P. incised X serrula*) transformed with the genes rol A, B, C of A. rhizogenes survive with difficulty more than three years when they are grown in pots in greenhouse. The problem is at the level of the root system. The resumption of growth is limited to the opening of vegetative buds.

Transformed roots of Inmil and *P. avium* " Summit " are subcultured *in vitro* for tests of regeneration. In addition, we were solicited to take part in the development of a process of genetic transformation in the horse chestnut tree (*Aesculus hippocastanum*) within the framework of a collaboration with Professor L Radojevic of the Institute for Biological Research "S. Stankovic" of Belgrade (Serbia-Montenegro). The objective is to obtain tissue-cultured lines having a great potential of growth in order to produce secondary metabolites largely used in pharmaceutical industry.



Strawberry, a model for the analysis of causes of persistent alterations of agronomical features induced by *in vitro* culture : proteomic approach of *in vitro* flowering in strawberry. *Mauro* S.* (*MRW)

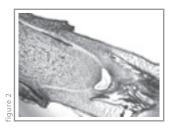
The *in vitro* culture conditions used for virus elimination or multiplication of agronomically important species are likely to modify the awaited behaviors ex vitro. In strawberry plants, the memory of the multiplication cycles (proliferation) carried out with high concentrations of benzylaminopurine is manifested by hyperflowering: the produced fruits have size features incompatible with their marketing. An in vitro system adapted to the constraints of a proteomic analysis is in phase of optimization. The choice of an in vitro system was determined by the facility, the homogeneity and the flexibility of the conditions of production of the plant material. Consequently, the development of a proteomic approach required the meeting of two preconditions:

- *in vitro* conservation of the capacities of perception and transduction of the signal determining the transition from the vegetative to the reproductive state;

- the possibility of isolating the meristems. The vitroplants of the strawberry variety Gento nova, produced under the standard conditions developed at the Department, preserve *in vitro* their sensitivity with respect to the photoperiod (Fig.1).



Floral apex (inflorescence) observed on strawberry plantlets after a 7-days induction with 8-hours photoperiod. (Fig. 1)



The isolation of the meristems was performed using laser-assisted microdissection (Fig.2).

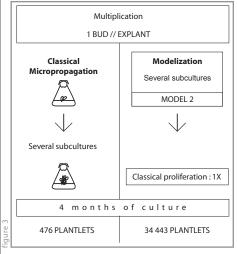
Laser-assisted microdissection. The dissection of the apical meristem is performed on fresh unfixated tissue.

This project is performed in collaboration with Sandy Jacobs (Lab. of Neuroplasticity and Neuroproteomics, K.U.Leuven. Naamsestraat 59. B-3000 Leuven).

- Dévelopment of innovative methods for in vitro culture.
 - Terzi JM*, Iwangou G** (*MRW, **CTB)

We continue the development of the concept of modeling at the laboratory: This concept can be defined and understood as the implementation and the combination of a set of propagation methods allowing a great flexibility of work, a major reduction in the production costs and proving particularly interesting for the large scale propagation of plants. The principal required assets are: very high proliferation rate, suppression of certain stages, reduction of the number of handling, long-term storage...

By using protocols and environmental conditions radically different from those generally used, we could bring interesting solutions to the *in vitro* multiplication of recalcitrant species (characterized by a weak rate of proliferation, bad rooting, high production cost, vitrification). The Pelargonium and the Cyclamen, having been the subject of work for several years, have been 2 examples. The work carried out so far, enabled us to highlight the potentialities of such a system. The diagram presented below illustrates the results obtained after 4 months of culture:



Comparison of the classical VS modeling techniques.

Within the framework of collaboration contracts with various public and private partners, this approach was extended to various species: - Pelargonium, New Guinea Impatiens: the results obtained for several years of research with modeling have currently made it possible to place at the disposal of the horticulturists a plant material being able to answer new criteria, in particular, miniaturization of the motherplants and increased production of cuttings. The established partnerships make it possible to have a data feedback from the horticulturist, likely to contribute to the evolution and the adaptation of the existing protocols. For Impatiens, a multiclonal of 13 genotypes is worked. In Pelargonium, we work on 10 different genotypes.

- Dipladenia: This species presents a defect of flowering following its culture *in vitro*. The plants originating from the laboratory being intended to become motherplants, the tools developed at the laboratory should allow an increase of productivity

and an increase of quality, while maintaining the aptitude of the plant for flowering.

- Vanilla: Prospective work, as well as an approach of the possibilities of modeling, is in progress - a multiclonal of diversified decorative species is worked within the framework of modeling: *Verbena*, primula, *Vinca*, *Calibrachoa*, *Dianthus*, *Lisiantus*, *Lysimachia*, *Plectranthus*, *Sanvitalia*, *Scaevola*.

Development of the culture of
 strawberry and woody small fruits
 in Wallonia.
 Farvacque S.*, Magein H. (* RW-AR258 / APE)

In Wallonia, the production of small fruits is primarily intended for the sale of proximity (farm sale, edge of road, ...) and for the transformation. Field trials for varietal, phytotechnical and of integrated protection features carried out within the experimental and demonstration infrastructure of the Department should supply the producers of strawberries and small fruits with additional knowledge about the monitoring of their complex cultures.

1. Integrated protection of the cultures

Several approaches for integrated protection against various pests of strawberry and small fruit cultures are being studied at the Department. A study related to a comparison between integrated protection and reasoned chemical protection against the thrips, the mites and the aphids in cultures of everbearing varieties. This study shows a good effectiveness of the auxiliaries introduced within the tunnel during the culture compared to reasoned chemical protection, where one observes a marked resistance of the pests towards plant protection chemicals.

2. Study of new varieties.

Several strawberry varieties (early, late and everbearing varieties) and woody small fruit cultivars (raspberry, currant bushes, blackcurrant and bramble) showing agronomic characteristics interesting for the production and marketing in Walloon area are being tested in order to widen the range of cultivars proposed to the producers.

Creation of intensive orchards. Magein H.

1.Extension of the varietal range for the cherry tree.

The evaluations of 11 varieties were finalized and continued for 35 other varieties. Grafting of 10 new selections of English (3) and

American origin (7).

We proposed a selection of 9 varieties advisable for the amateur based on a sorting independent of the fruit size.

Variety	Harvest delay Days after Burlat	Fruit weight (g)	Firmness	Cracking index (%)	Rotting index (%)	Suga (°Bri
Annabella	15	7,1	34,1	5,6	1,7	17,
Castor	21	9,1	21,4	16,1	2,7	17
Helshoven	11	5,5	19,7	6,6	3,2	17,
Regina	31	8,3	16,6	8,9	8,4	20,
Sam	16	7,5	17,8	10,8	3,6	16,
S. Ghijssens	26	8,4	10,2	10,7	5,6	19,
Star	18	7,5	18,4	8,9	2,5	17,
Ulster	17	7,8	13,7	19,7	3,4	17,
Kordia	24	8,8	10,7	15,8	7,5	18,

Variété	Flowering time Days after Burlat	Tree habit	Pollinators
Annabella	3	weeping	Castor, Schneider
Castor	3	1/2 erected	Corum, Early rivers
Helshoven	-2	weeping	Schneider
Regina	9	erected	Sam, Sylvia, Annabella, Schneider
Sam	6	1/2 erected	Hedelfinger, Sam, Schneider
S. Ghijssens	3	1/2 large crowned	Schneider
Star	6	Large crowned	Hedelfinger, Kordia
Ulster	- 1	Large crowned	Van, Burlat, Hedelfinger
Kordia	-2	1/2 large crowned	Regina, Hedelfinger, Schneider

able

2. Conservation of genetic resources in *Prunus*.

The *Prunus* collection of the Department currently counts 398 accessions, including 263 fruit-bearing varieties (acid and soft) and 135 ornamental varieties. The collection of ornamental *Prunus* whose first introductions go back to 1962 and fit within the program of the IBPGR (International Board for Plant Genetic Resources), is in the course of renewal; 67 varieties were regrafted on F12/1. 73 fruit-bearing varieties of cherry trees were also regrafted on Damil® with an aim at maintaining the integrity of the collection as well in the optics of conservation of the resources as in that of reference for certified identity.

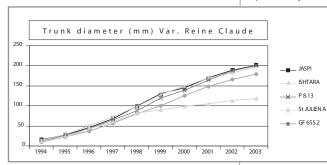
3.Widening of the range of the dwarfing rootstocks in cherry tree.

The evaluation of the growth and the fruit-bearing potential of the 28 potential candidates as new dwarfing rootstocks originating from the descent of Damil® and selected for their precocity of multiple bud formation, was continued.

4. Selection of dwarfing rootstocks in plum tree.

The first analysis of growth vigor of the descent of *Prunus bokhariensis* grafted in 1999 with variety R.C. of Althan was carried out in comparison with that generated by St Julien or Brompton. At the end of the third year, only three genotypes induce a vigor lower than that of St Julien and about half of the genotypes are more vigorous than Brompton. 21 additional genotypes resulting from the same descent were grafted into 2003 and 2004. Year 2004 concludes the observations within the

framework of the international test of rootstocks installed in our orchards in autumn 1993. The rootstocks Jaspi (France), Ishtara (France), P 8.13 and GF655/2 (France) were compared with St -Julien and were grafted with the varieties Reine Claude 1119 and Wignon.



The Ishtara rootstock displays a vigor lower (approximately 40%) than the control St Julien, while the other rootstocks show a variation of less than 10% compared to the control.

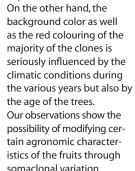
Ishtara also did not develop any suckers during the test while on the other rootstocks, the suckers appeared during 4th year and then spread gradually to the basis of every tree.. The fruits collected on the varieties grafted on Ishtara display, on the one hand, an increased earliness and, on the other hand, present an average weight 10 to 15% superior to those of the control. However the varieties grafted on Ishtara present all the year a clearer foliage and the trees obtained with this rootstock are very sensitive to the wind. Indeed, almost the totality of the trees grafted on Ishtara were lying at the end of the test, showing the weak anchoring of this rootstock. The cumulated outputs (kg/arbre) obtained during the period 1998-2004 for the variety Reine Claude also show that the production of the trees grafted on Ishtara is significantly weaker.

Phytotechnie des productions fruitières issues des techniques de biotechnologie Magein H.

1. Behavior of somaclonal variants in apple tree.

The evaluation of the variability of expression of the color of the fruits of lines corresponding to various events of regeneration of adventitious budding induced on the leaves of a mutant of Jonagold cultivated in vitro was continued. The first fruits were collected on the site of Gembloux on the 17 lines identified during the year 2000 as showing particular features and replanted in several specimens in order to determine the repeatability and the stability of these characters. The lines retained in particular for the precocity of the colouring of the fruit (appearing more than 2 weeks before the normal period of maturity of the cultivar of origin) confirmed this characteristic; their color appears red dark and strongly striated at the time of maturity and covers almost 100% of the surface of each fruit.

The characters of elongated shape, of plain color (not striated) and of firmness of the fruit have turned out to be maintained during years (1999-2004) as well as on the trees intended to study the repeatability and stability.



induced *in vitro* on foliar tissues. The observations of repeatability and stability will be continued for at least one year more.

According to the same technique, variants from the Pinova variety are in preparation.

2. Vegetative propagation of indicators for virus indexing techniques.

The trials of cutting propagation under fog were performed on 7 indicators and dealt with several periods of sampling (from April at August), starting from miniaturized plants originating from *in vitro* culture.

The pear trees rooted slowly; A20 and Doyenné du Comice however reach a rate of success of about 80% with very early cutting process. The cutting propagation of Shirofungen and Spy does not raise any problem anymore and succeeds with more than 80% whatever the time period. The sampling period is more important for the last indicators; for instance, Krikon reacts better to early cutting (92%), and the rooting of Pozzegazza reaches 60% as from June.

At the request of the administration in charge of the management of the river banks (MRW - DGRNE - Division of Water), a program of production of woody species used for the protection and stabilization of river banks was undertaken. The objective is to reach a good compromise between the resolution of the purely hydraulic problems and the safeguard of the biodiversity. The 3 species selected are, by descending order of priority, the alder (Alnus glutinosa), the willow (Salix sp.), and the ash (Fraxinus excelsior). In order to guarantee a maximum of biodiversity, the prospection and the fixing of various ecotypes of alder were undertaken and continue throughout Wallonia. On the whole, 26 sources of alder were fixed, each source comprising 5 to 15 clones. During winter 2003-2004, 20 sources of willow, each one comprising 10 clones at least, were collected in the form of dormant branches. After conservation in cold room, this plant material was propagated by cutting outdoor.

The fixing of the ash starting from suckers originating from stumps is more difficult. Approximately 10 clones resulting from 3 sources could be fixed by softwood cuttings. From the clones of alder fixed into 2003, a beginning of multiplication was undertaken during summer 2004.

The DGRNE is eager to widen the possible time periods for plantation. The transfer of the plants along the banks must be made when those are accessible and when the danger of flood is minimum in order not to mortgage the resumption of the trees. To this end, plank timbers of willow, and rooted cuttings of alder, willow and ash were preserved at $+2^{\circ}$ C and -2° C until the beginning of July. When transplanted on this date along the banks, the plant material showed an excellent recovery. The temperature of conservation does not seem to have an influence either on the rate of survival or on the later growth; complementary tests are nevertheless necessary. Since August 1 2004, this work continues in the

form of European project Interreg III Wallonia - Lorraine - Luxembourg. This project is managed by our Department. In alders, 4 Luxembourg sources and 1 French source are in the course of fixing in our greenhouses.

6.2. Molecular biological research in the areas of plant improvement in agriculture and fruit and vegetable horticulture, of plant pathology and soil microbiology.

Introduction of genes for disease resistance and growth habit modification in fruit tree species. Watillon B, Magein H.

The growth of the transgenic lines of apple tree expressing the KNAP1 gene (an homeobox gene with pertaining to the "knotted1" family) was observed on plants growing in greenhouse. Transformants were installed in pots under " insect proof" greenhouse and provided with an automatic system of irrigation. These observations made it possible to confirm the existence of significant modifications of the growth (in particular in term of average internode length and average length of the ramifications). Moreover, the formation of flowers and fruits could be obtained for two of the transformed lines. For one of those, the formed fruits present important deteriorations of the shape (development of a fleshy peduncle).

Ten transformed lines overexpressing, in a constitutive way, a gene coding for a "Hydroxy Prolin Rich Protein "-type cell wall protein from the family of the extensins were also obtained and observed as for their growth in greenhouse. In various plant species, the proteins belonging to this family would be implied in the response to the biotic and abiotic stresses. No significant anomaly of the growth and the development was observed for these lines. Their resistance to scab was measured compared to the original " Jonagold " variety, in collaboration with the team of the Department "Lutte biologique et ressources phytogénétiques" by inoculation in controlled conditions (inoculation bench). A limited but significant reduction of the sensitivity to the pathogen studied could be highlighted at two of these lines, and that in spite of the conditions of inoculation very favorable to the development of the pathogen. The experiment will be repeated during 2005.

Genetic transformation of wheat Delporte F*et Jacquemin JM (*MRW)

1.Genetic transformation of commercial wheat varieties with genes associated with disease resistance.

The control of viral diseases of plants is difficult, for there is not pesticide against the viruses. For a long time, the man selects varieties for their natural resistance to these agents. Biotechnologies offer an alternative way for the development of resistant varieties *via* the synthesis in the plant of antibody molecules which neutralize the viral infection, or at least decrease the accumulation of the viral particles, attenuate the incidence of the infection and delay the development of the symptoms.

Within the viral diseases, the group of the potyvirus includes the greatest number of phytovirus which infect the crop plants and cause important economic damages. We apply the technology of genetic transformation to introduce the sequences coding for antibodies specific of potyvirus in the wheat genome, in order to develop this alternative strategy for the control of the viral infections applied right now successfully in other plants.

2. Development of techniques of tissue culture and genetic transformation by the "biolistic " method for their application to the improvement of cereals.

The genetic transformation of wheat remains problematic and delicate. A second objective is the development of a simple and functional transformation method. The results achieved according to our process are promising as they are superior to those obtained according to the process described in the literature. Our model appears particularly attractive for the transformation of wheat as well as other cereals. Additional studies are necessary for the comprehension of the biological determinants of the aptitude for the transformation and for the "silencing " of transgenes, studies whose result will be of a great interest for any work of genetic engineering of the plants.

Biological and molecular identification and characterization of *Pseudomonas Syringae* and *Xanthomonas fragariae* A. Bultreys* (*MRW)

1. Study of the pyoverdins of fluoresceing *Pseudomonas*.

Following a collaboration with the laboratories of Professor Budzikiewicz of the University of Cologne and Wathelet of FUSA of Gembloux, the fine chemical structures of the lactonic forms of the pyoverdins originally produced by Pseudomonas Syringae and Pseudomonas cichorii was established. These two pyoverdins differ only by substitution from a serine by a glycine in the peptide chain. This substitution of a small neutral amino acid does not influence the recognition of these pyoyerdins by their respective membrane receptor for the two species incorporate each pyoverdine. On the other hand this small difference is detectable by the HPLC test developed at the CRA-W. This test made it possible to confirm on more than 500 strains the constancy of the pyoverdine produced within Pseudomonas Svrinaae.

2. Diversity of the strains of *Pseudomonas Syringae* isolated from orchards in Gembloux.

Pseudomonas Syringae was found in abundance, and in all its diversity, within some orchards of pear tree, soft cherry tree, acid cherry tree and plum tree of the area of Gembloux and Gorsem. Both principal pathovars of the species known on these cultures, the pathovars Syringae and morsprunorum, the two races of the pathovar morsprunorum, but also, in a more astonishing way, the strains representing unspecified pathvars from Pseudomonas Syringae and atypical Pseudomonas



viridiflava were found 169 times in these orchards. The pathogens showed a great diversity in the sometimes serious damage which they could cause, as well as apparently different specializations according to crops.

3. Characterization of quarantine bacterium *Xanthomonas fragariae* isolated in Wallonia.

Xanthomonas fragariae is a quarantine bacterium which entered into Belgium by way of contaminated strawberry plants coming from France and Netherlands. A collection of strains isolated in Belgium at the CLO and at the CRA-W was compared with strains of French, Dutch or varied origin, of which some belonged to defined genetic groups. The Belgian strains turned out to belong to varied genetic groups. Large genetic diversities were revealed among the strains of French and Dutch origin. Usually, rep-PCR does not show clear differences between the strains of both origins. The RAPD technique allows to follow individual strains, but it is not informative to determine the origin of an introduction because of the too specific profiles that are obtained and of the necessity to analyze additional French and Dutch reference strains. Results indicate multiple and frequent introductions of the quarantine pathogen in the European countries by way of contaminated plants.

Improvement of the control of bacterial diseases in Wallonia.

F. Legros*, A. Bultreys** (* RW-DGA, **MRW)

Optimized techniques for the diagnosis of Pseudomonas Syringae, Pseudomonas Syringae pv. Svringge and pv. morsprunorum race 1 and 2 as well as of Erwinia amylovora were developed and selected. On these bases, accelerated techniques of identification using notably Bio-PCR were designed to offer a successful and reliable service of diagnosis to the horticultural sector. Collections of 501 new strains belonging to the group of Pseudomonas Syringae and 41 new strains of Erwinia amvlovora were established following 235 samplings in fruit orchards throughout Wallonia. The analysis of these strains confirmed the diversities of strains and symptoms observed previously in Gembloux orchards and revealed other diversities. The potential involvement of Pseudomonas Syringae in several litigious cases in orchards was clarified. Finally, sensibilities of agronomically-interesting varieties were estimated by original or already developed tests. Some varieties appearing significantly more sensitive or more resistant were defined in several cases.

Détection et caractérisation de pathogènes bactériens en horticulture fruitière V. Gilbert*, A. Bultreys** (* RW-DGA, ** MRW)

The diseases caused by *Pseudomonas Syringae* and *Erwinia* will amylovora present important risks for several fruit-bearing cultures in Belgium. A study of the phenotypical and genetic characters of the Belgian stocks of these pathogenic in

fruit-bearing orchards (apple trees, pear trees, cherry trees, plum trees) is carried out in order to improve, to refine and accelerate their identification and their detection. Four hundred stocks of Pseudomonas Syringae were analyzed to date. The phenotypical characters taken into account were the phytotoxines, the sidérophores and the bactériocines. With regard to the genetic characterization, analyses of the type PCR revealed a hundred different profiles. These profiles make it possible to differentiate the stocks enters and within the pathovars. This shows on the one hand that it is difficult to fight against this species being given its diversity, and on the other hand that these profiles will perhaps make it possible to identify the most virulent stocks. Genetic analyses of type PCR, only used to date, of about fifty stocks of Erwinia will amylovora insulated in Belgium showed that these stocks are much more homogeneous.

Study of the involvement of the NpABC1 carrier in the response of Nicotiana plumbaginifolia to pathogens A. Bultreys* (*MRW)

Plants respond differently to the presence of a foreign organism according to the perception that they have from it. The various defence pathways that may be activated involve an important number of genes. The laboratory of the Professor Boutry (UCL) was interested in the role played by the NPABC1 ABC carrier from Nicotiana plumbaginifolia in the resistance of the plant. In this frame, various bacterial models were considered in the CRA-W to clarify which were the possible inductive bacteria and to help to clarify which were the induced responses. NPABC1 was led(inferred) in the presence of pathogenic and pathogenic bacteria of the tobacco, but the pathogenic tree stumps against which Nicotiana plumbaginifolia develops a reaction of sentimentality (HR) do not lead(infer) NPABC1. These observations and analyses realized in the laboratory of the Prof.. Boutry allowed to demonstrate NpABC1's implication in the defence pathway of the jasmonic acid. Attempts using transgenic plants and pathogenic Pseudomonas Syringae pv. tabaci, Erwinia carotovora and Botrytis cinerea showed that plants repressed in the expression of NPABC1 were more sensitive to at least one pathogen.

-the hypersensitive reaction (HR) and the induced systemic reaction (SAR). In their simplest presentation, these models associate the activation of the mechanisms of defence with the recognition of one of the proteins synthesized by the pathogen at the time of the initial phase of the infection. In the absence of this recognition, a compatible relation settles; it makes it possible for the pathogen to exploit the metabolism of the host. The methods of the metabolic adjustments related to colonization are documented little. In the case of the pathosystem Phytophthora infestans / Solanum tuberosum var Bintje, measurements of photosynthetic activities, carried out during the biotrophic phase, showed a reinforcement of the activity of the photosystems concomitant to a reduction in the capacity of oxygen release. These results suggest the installation of an alternative metabolic sink. The assumption of an activation of the metabolic ways related to the chlororespiration was tested and confirmed: an increased transcription of mRNA corresponding

to the subunit F of the chloroplastic NADH dehydrogenase (Ndh) is observed during the initial phase of colonization. This work is completed in collaboration with the Prof. Bartolomé Sabater (Departamento de Biología Vegetal, Universidad de Alcalá de Henares, Alcalá de Henares, 28871-Madrid. Spain).

Genomic approach of inulin metabolism in chicory.

Mingeot D*. Watillon B. (* MRW)

A project devoted to genomics of industrial chicory is led in partnership with the FUNDP, the UCL and the FUSAGX. This project aims to study the modifications of expression of the genome in the root of chicory during a season of culture. This study should allow to reveal mechanisms involved in the metabolism of sugars and to develop, from there, molecular tools for selection of highly efficient varieties in chicory. Samples of roots were taken in the field with regular time intervals. From these samples, ten cDNA libraries, representative of 10 different stages of development, were built. The partial sequencing of one thousand clones from each library is on the way: at the moment about 9000 ESTs are available (Expressed Sequence Tags). Proteomic studies are made in parallel from the same samples of chicory. The contribution of the CRA-W to the project is situated at the genomic level : Construction of libraries, participation in the sequencing. This project is realized in partnership with the following university teams of search: - FUNDP: Unité de recherche en Biologie cellulaire végétale

- FUSAGx: Unité de Chimie biologique industrielle - UCL: Unité de Biochimie physiologique

Varietal identification using molecular markers in major crop species present in Belgium (potato, strawberry, fruit crops...) Mingeot D.* (*MRW)

Within the framework of this project, the objective of the biotechnology department is to develop the effective and routine use of the molecular markers for the varietal authentification of crop species marketed in Belgium. From this point of view, the following points are studied: optimization of the protocols of DNA extraction, choice of the most adequate set of markers (reproducibility, capacity of discrimination) for each species, constitution of databases of genetic fingerprints in order to be able to identify by comparison the greatest possible number of varieties. Various projects are led, dedicated to various species. Thus, the use of AFLP and microsatellites markers in woody fruit species led to obtaining genetic fingerprints of a certain number of reference varieties as well in apple trees as in pear trees, plum trees and cherry trees. In the same way, varietal identification works are carried out out on potato by microsatellites markers, and on strawberry

by AFLP and microsatellite markers (in collaboration with the Section "Agricultural Systems"). Concerning the varietal authentification of potato by microsatellite markers, the laboratory aims at obtaining an accreditation according to the ISO 17025 standard.

Development of a methodology for obtaining molecular markers based on retrotransposons in apple. Arezki O.*, Watillon B. (* RW-DGA)

This project aims at the development of innovating techniques of molecular fingerprinting in apple tree. It relies on the preliminary highlighting of a retrotransposon of the "Copia" type in the genome of the apple tree. Two techniques were retained and adapted to obtaining polymorphic and reproducible genetic fingerprints in apple tree: the S-SAP (" Sequence-Specific Amplification Polymorphism") and the RE-MAP ("REtrotransposon-Microsatellite Amplified Polymorphism"). The S-SAP is primarily derived from the AFLPTM and relies on the use of primers corresponding to the conserved region of the retrotransposon (the "LTR" ends) at the time of the second phase of amplification of the DNA. The RE-MAP requires the use of pairs of primers targeting on the one hand the " LTR " region of the retrotransposon and on the other hand repeated sequences corresponding to "microsatellites".

These techniques were developed on DNA samples of the Jonagold variety and of its color mutants, in order to check if they could distinguish very closely related (from genetic point of view) cultivars, that are not easily identifiable by the other molecular marker techniques (such as the AFLPTM or the microsatellites). They were extended thereafter to a vaster panel of cultivated apple tree varieties (*Malus sylvestris*).

Molecular characterization of the genetic diversity in wild apple. Arezki O*, Watillon B. (* RW-DGA)

A research project carried out in collaboration by the CRA-W (D1 and D3), the CRNFB (Gembloux), the KUL (Leuven) and the CLO (Gent) aims at characterizing genetic diversity at the wild apple trees present in Belgium. Within the framework of this project, our team evaluates the interest of innovative techniques of molecular fingerprinting, based on retrotransposons (the S-SAP and the REMAP), for the analysis of genetic diversity. REMAP genetic profiles, consisting of multiple and polymorphic bands, are being obtained for a collection of several hundreds of apple trees (wild and cultivated) identified in Walloon Region. The genetic diversity underlined using this method will be compared with that detected by more traditional molecular markers (microsatellites). In parallel, a methodology allowing the detection of genetic markers related to genes potentially involved in pathogen resistance is developed. For this purpose, we developed a "gene profiling" technique based on the amplification of fragments between primers located on the one hand in the terminal region of a Copia-like retrotransposon (previously identified in apple tree) and on the other hand in resistance genes from the R gene family. The developed method will be applied to the collection of apple tree mentioned above.

Search for QTL involved in the adult resistance to septoriosis in wheat. Jacquemin J.M.,

The septoriosis is an important disease which strongly affects grain yield in our regions. To control diseases, a resistance more effective than specific resistance can be approached with the molecular and statistical techniques. Indeed, specific resistance to one pathogen may be quickly by-passed by the appearance of new physioraces that are insensitive to the resistance gene used. On the other, adult resistance is dependent on the expression of multiple genes and is of quantitative type (QTL). Varieties of winter wheat were cultivated in numerous places and maintained a very good level of resistance towards the septoriosis during numerous years; it is therefore estimated that these varieties harbor a durable-type resistance. Two populations were thus created by crosses between Oasis X Renan and Mobil X Hussard and they were fixed by Single Seed Descent (SSD). The mapping of the QTLs controlling this type of resistance has started. To reach this objective, the construction of genetic maps on these 2 populations has begun using microsatellite markers. The populations were also evaluated for their resistance towards the pathogen in the fields. The identification and comparison of QTLs for this resistance is on the way. This work is performed in collaboration with

Dekeyser A. (D3).

Genetic control of fusariosis in wheat MuhoVSki Y.*, Jacquemin J.M. (* RW-DGA)

The fusariosis of the ear is at present a major problem of food safety at the level of the Walloon, Belgian and European agriculture. Damages caused by the various types of fusariosis are for several years in net increase and, seen the importance of problems connected to the presence of mycotoxins (trichothecenes: deoxynivalenol-donation, nivalenol-NIV ...) causing severe human diseases, networks for monitoring and intervention were set up.

Various modes of control exist against the fusariosis; genetic resistance is one of the most effective and economic means to control this plague. However, the known sources of resistance were present in germplasms unsuitable for our regions. The creation and marketting of resistant Walloon varieties (Centenaire and Fourmi) allow to consider their generalized use in plant breeeding using molecular marker-assisted selection. Our project aims to localize loci involved in the adult resistance to fusariosis in wheat, by mapping populations obtained from crosses involving varieties Centenaire and Fourmi.. The existence of genetic linkages between molecular markers (microsatellites, EST, AFLP) and the quantitative loci (QTL) involved in the resistance to the fusariosis, has to be shown. We shall study and locate also candidate genes (carriers ABC ...) involved in the different defence mechanisms of plants, in order to understand the genetic determinism of envisaged quantitative characters. The discovery of molecular markers linked to these genes will allow to follow their introgression during the creation of new

varieties, and so to select quickly for this type of resistance and to control preventively the fusariosis.

This work is performed in collaboration with Ducourouble M (V. Jorion et fils) and Dekeyser A., Chandelier A., Detrixhe P. (D3).

Identification of cereals using molecular methods and EST markers Jacquemin J. M.

This project aims to estimate on cereals, mainly wheat, barley and spelt, the different techniques for molecular analysis of the plant genome, in order to consider the analysis of the UPOV criteria by these same techniques. Two molecular marker types were preferentially applied: microsatellites and AFLP® (AMPLIFIED FRAGMENT LENGTH POYMORPHISM). Based on the EST sequences (expressed sequence tags) identified in wheat and barley, microsatellite sequences were observed and used on various varieties. The EST sequences, which correspond to expressed characters, are therefore new and very interesting markers to monitor the UPOV criteria, which aim only at expressed characters.

Among the 3 UPOV criteria : distinction, uniformity and stability (DUS), we mainly studied distinction. Furthermore, genetic distances were calculated for the different varieties analyzed.

7. SERVICE ACTIVITIES

- Assistance with the technical accompanying of the strawberry producers of Wallonia (in collaboration with the Association of the Walloon Strawberry growers): sendings of semi-monthly technical letters, courses in winter, briefings and meetings in the field.

- Assistance with the technical accompanying of the Christmas tree producers : installation of a seed-orchard made up of elite clones of A. *nordmanniana*.

- Molecular analyses for the detection and the identification of the phytopathogenic bacteria in fruit cultures (*Erwinia*, *Xanthomonas*, *Pseudomonas*...)

- Molecular analyses for varietal certification in potato (for account of control services of the General Directorate of Agriculture and of private companies) and in apple tree (for account of nursery growers).

- Biochemical analyses of storage proteins of the cereal grains (for account of breeder companies).

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Subvention n° S 6081 du Ministère de l'Agriculture "Mise au point de méthodes de détection et de caractérisation des bactéries Pseudomonas Syringae et Erwinia amylovora en horticulture fruitière" – rapport d'activité.

Convention n°667 - Arrêté Royal 258 - Rapports d'activités

Convention RW-APE n° 001709 - Rapports d'activités

Subvention Région Wallonne n°2652 « Développement de la culture de fraises et de petits fruits ligneux en Wallonie » -rapports d'activités

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Subvention Région wallonne n° 2631 "Amélioration de la lutte contre les bactérioses en vergers fruitiers" - rapports d'activités

Subvention Région wallonne nº 2654 "Détection et traçabilité de pathogènes bactériens : amélioration du matériel de plantation en horticulture fruitière" – rapports d'activités

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Conferences/Posters

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breeding', 8-11 September 2004, Tulln, Austria.

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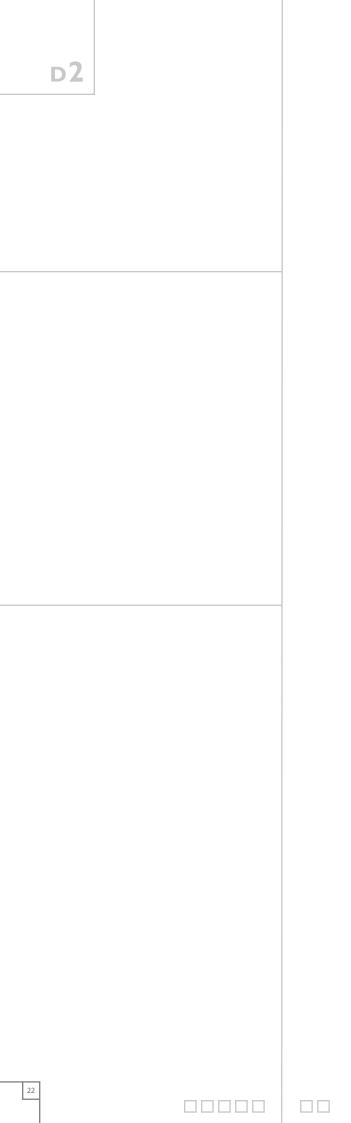
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1. MISSION STATEMENT

. The Department's general mission is to undertake research into crop production systems to promote the development of sustainable agriculture.

. The Soil and Fertilization Section carries out research into the structural state of the topsoil according to cultural practices, particularly tillage, and soil chemistry with emphasis on fertilization and in particular, the dynamics of nitrogen turnover.

. The Crop Husbandry Section studies production factors for main crops and crop diversification.

. The Plant Accession Section conducts research for the acceptance of new varieties for inclusion in Belgian and European catalogues and determines the characteristics of varieties in Belgian and EU catalogues in order to make recommendations to users and processors.

2. SCIENTIFIC PERSONNEL

8 permanent staff and 4 contract staff

2.1. Head of Department

M. Frankinet, Chef du département, direction

2.2. Scientists

- P. Courtois, Attaché scientifique (WR)
- L. Couvreur, Attaché scientifique
- M. Cuvelier, Attaché scientifique (WR) S. Dantas-Pereira (WR)
- J.-P. Destain, Inspecteur général scientifique, Head of Section
- J.-P. Goffart, Attaché scientifique
- J.-L. Herman, Attaché scientifique
- M. Olivier, Attaché scientifique (WR)
- S. Renard, Attaché scientifique (WR)
- V. Reuter, Attaché
- C. Roisin, Attaché scientifique
- K. Verdinne, Attaché scientifique

3. NATIONAL REPRESENTATION

- REQUASUD network (reference laboratory for nitrates)

- Nitrates Committee asbl

- Groupe de travail sectoriel "Agriculture" Plan wallon de l'air

- Comité pour l'élaboration du Catalogue national des variétés des espèces de plantes agricoles (MA DG4)

- Centre agricole des céréales et oléoprotéagineux (CADCO asbl)

 Centre agricole Betterave-Chicorée (CABC asbl)
 Centre agricole Pomme de terre (CAP asbl) and Centre Pilote Pomme de Terre (CPP)

- Agrisemza (Association des agriculteurs multiplicateurs de semences asbl)

- Agrenwal asbl

- Conseil de Filière Wallonne Grandes Cultures (CFGC-W asbl)

- Filière wallonne de la pomme de terre (FIWAP asbl)
- Conseil wallon de Filière Pomme de Terre asbl
- Fédération Wallonne Horticole (FWH)
- Office régional de promotion de l'agriculture et de l'horticulture (ORPAH) and Agence Wallonne pour la Promotion d'une Agriculture de Qualité

(APAQ-W) - Association pour la promotion des protéagineux et oléagineux (APPO asbl)

- Centre Interprofessionnel Maraîcher (CIM) -Maraîchage conventionnel
- Centre Maraîcher de Hesbaye (CMH) Légumes
- industriels

- Association belge des producteurs de semences (Semzabel)

- Groupement interprofessionnel belge des semences (Intersemza)

4. INTERNATIONAL REPRESENTATION

- Member of the Management Committee of COST-CEC D6 XII - 814 - Crop development for cool and wet regions and Working group Cost 832 - Eutrophication from agriculture - Coordination of Working Group 1 - Inputs of P from agricultural soils.

- EU Expert Group: EC Nitrates Directive
- European Association for Potato Research (EAPR)
 International Society for Horticultural Science (ISHS)

- International Soil Tillage Research Organisation (ISTRO)

- International Institute for Beet Research (IIRB)

5. RESEARCH TOPICS

In accordance with today's concept of sustainable development, agriculture has to meet society's expectations with respect to the environment, biodiversity, food safety, traceability and land use while remaining economically profitable so as to ensure its continued existence and thus its "sustainability".

Some research topics require long-term experiments, such as the study into the effects of organic manure and P K fertilizers on the physical and chemical status of cultivated soil. There are excellent reasons for continuing with this work. The same applies to reduced tillage practices, i.e. alternatives to ploughing. This research has revealed a need to supplement the description of the cultural profile with objective data to account for the quality of the structural state of the topsoil by means of a quantified indicator. The current method is based on data collected by penetrometry from a precisely determined volume of soil and interpreted in terms of the heterogeneity of that volume.

The European Nitrates Directive, which requires Member States to carry out policies designed to protect groundwater from pollution by nitrates from agricultural sources, forms the basis for several of the Department's research projects (the limit set is 50 mg NO3/litre). Our study of the dynamics of biomass and nitrogen compound formation, mobilisation and accumulation in winter wheat grains is the subject of some fundamental research involving in particular the use of fertilizer labelled with the 15N heavy isotope.

While decision support tools such as the AZOBIL software now enable crop nitrogen requirements and the soil mineral nitrogen supply to be estimated, adjustment of fertilization during growth allows *apriori* estimates to be fine tuned and corrected, using rapid non-destructive methods for evaluating crop nitrogen status (chlorophyll meter, reflectance). The expertise acquired in this area has enabled three projects to be carried out in connection with optimising nitrogen efficiency. These projects involve industrial vegetable growing, vegetable production for the fresh market and potato growing for both ware and industrial use.

With regard to potato crop husbandry, besides the nitrogen fertilization aspect our research is directed towards controlling potato scab by irrigation and reducing the risks of deformities and sprouting during growing by managing irrigation in conjunction with temperature maintenance at < 24-25 °C in the hill.

The benefits of using organic and organo-mineral fertilizers are assessed in terms of crop yield and quality.

Finally, the potential for low-temperature storage of different varieties is investigated to avoid the use of sprout inhibitors while preserving the intrinsic quality of the tubers (maintaining the non-sweet taste and avoiding browning during cooking).

The Department's body of agricultural knowledge is mobilised in order on the one hand to carry out an "agricultural areas" survey involving application and monitoring of nitrogen fertilizers in field crops on twelve mixed or crop-growing farms and, on the other hand, studying the spatial variability of fields with a view to better management of inputs in the context of precision farming.

Lastly, there are two special projects, one dedicated to winter wheat fertilization in organic farming and the other to studying crop combinations (grasses and dicotyledons) for wildlife set-aside.

6. RESEARCH REPORTS

6.1. SOIL AND FERTILIZATION

Long-term experiment on phosphoruspotassium and organic fertiliser application rates J.-P. Destain, C. Roisin, M. Frankinet, V. Reuter

The only observation to date in the trial established in 1967, comprising three levels of P and K mineral fertilizers (P0 or K0 - no input; P1 or K1 - replacement of the P or K removed; P2 or K2 - double the previous level) has been a substantial drop in beet yield with K0.

Significantly lower yields compared to the other two levels were obtained at both P0 and K0 in sugar beet in 2004.

Fertilization levels	Winter barley (2003) Grain yield (kg) at 15% moisture	Sugar beet (2004) Sugar yield (kg) /ha	
PO	7 537	12 545	
P1	7 631	14 128	
Р2	7 913	14 304	
КО	7 484	12 664	
K1	7 812	14 205	
К2	7 793	14 109	

Crop yield in the long-term PK trial

Year	Fertilization level		
	PO	P1	P2
1967	19.9	18.3	19.3
1981	16.6	16.7	17.6
1993	9.8	16.8	23.8
2003	7.1	12.8	19.6
	КО	К1	К2
1967	11.5	12.0	13.2
1981	11.5	18.1	25.9
1993	9.1	18.3	27.0
2003	10.0	15.8	19.6

table

Changes in soil P and K reserves determined by the AL method (mg/100g dry soil)

The available phosphorus and exchangeable potassium status of the soil now shows a marked difference. At level 0, P depletion seems to continue faster than K depletion. Level 1 results in larger P and K concentrations (>12 in the case of P and >15 K). Level 2 leads to enrichment of the soil. Values for levels 1 and 2 were lower in 2003 than in 1993, probably due to the drought promoting the adsorption of these nutrients on to the exchange complex.

Experiments with tillage techniques C. Roisin

Erosion control is attracting increasing attention in this part of the world. Reduced tillage practices are frequently mentioned in this context as one of the solutions to be adopted. In the case of some plants, however, such as beet and maize, it is still essential to loosen the topsoil to maintain their cropping potential. To be effective, this should be done using tools with spike teeth that can reach down at least to ploughing depth. This requires high tractive efforts and is not generally carried out on small to medium-sized farms. This observation prompted the Crop Production Department to set up a series of experiments aimed at investigating the feasibility of a planting method based on localised tillage concentrated in the area of the drills. The method involves decompacting the soil with an implement that has teeth with very short blades, to reduce the tractive effort, followed by sowing in the teeth tracks in order to site the plants in the places with the most favourable structural conditions. Over the past two years the technique of drilling directly in the rows loosened by the decompactor teeth has been tested using a machine with six teeth

50 cm apart and a precision seeder with six hoppers likewise 50 cm apart. The technique is based on a mechanical self-guiding system fitted between the tractor and the seeder which positions the seeder correctly with respect to a furrow made in the soil during decompacting.

Study into the use of penetrometer measurements to characterise agricultural soil structure C. Roisin

As regards cultural practices, maintaining soil fertility and environmental conservation are today regarded as essential components of decision-making processes. In the context of greater sustainability in agriculture, the structural guality of the topsoil is an important criterion in developing and assessing alternative, more soil-friendly cultural systems. Defining a quality indicator is thus a key

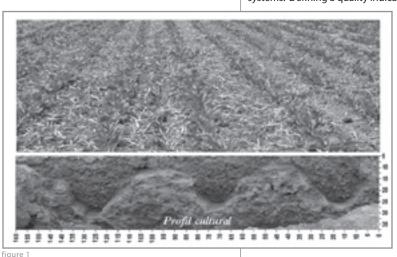
clumps, solid areas) created by agricultural ma-

chinery. The principle is based on recording and

processing bi-directional signals comprising 256

penetration resistance measurements collected

within a 75 cm square area and 5 cm apart in



task. A research project has accordingly been undertaken with the aim of devising a method to provide an objective description of sufficiently large soil volumes to take into account the complexity of the spatial organisation of morphological units (fine earth,

Alignment of beet rows with decompactor teeth tracks.

In crop husbandry terms, initial results with sugar beet are encouraging. Controlled positioning of the beet rows systematically improved the taproot shape, with fewer bifurcated beets, reduced the

soil tare at harvest and boosted crop yield. However, the differences observed with respect to random sowing are significant only in the experimental fields where the soil structure prior to decompacting was unsuitable for reduced tillage. In all cases, though, controlled positioning of the beet rows proved advantageous irrespective of whether the decompactor teeth blades were narrow or wide. Work therefore continues with the twofold aim of perfecting the technique

either direction.

for wider-scale application and measuring the benefits in terms of progress towards soil conservation based agriculture. As the soil is not disturbed between the rows it ought to have greater erosion resistance with enhanced soil bearing capacity in the interrows and less rutting at harvest, thus allowing reduced tillage to be continued the following season. In addition, the decompacting technique is expected to become financially more attractive and therefore accessible to more farmers.

Penetration resistance measuring equipment

Six data processing methods (autocorrelation, geostatistical analysis, Smith's model, spectral analysis, wavelet analysis and multifractal analysis) were tested and evaluated to establish their ability to supply relevant heterogeneity parameters consistent with soil diagnosis by the cultural profile analysis method. This study highlighted the advantages of multifractal formalism as a method for describing signals of the type recorded, which are frequently non-stationary and anisotropic.



Two heterogeneity parameters were defined on this basis, one to describe the fine structure and the other expressing possible partition of the profile due to the passage of tillage equipment. Their statistical properties (variability, normality, homoscedasticity, discrimination) were assessed by means of measurements made within an experimental design for comparing different soil management methods in loamy soils. The results obtained with this design showed the relevance of a structural heterogeneity index in agriculture. The method described above then underwent its first concrete application in a trial aimed at comparing different tillage techniques in maize. Some highly significant correlations were found between the mean coefficient of heterogeneity for the 0-15 cm layer and plant development by mid-July and also between the mean coefficient of heterogeneity for the 0-25 cm layer and the dry matter yield at harvest. However, the regression curves indicate that the effect of tillage techniques on the crop varies with the parameter taken into account (early growth or final yield), whereas the impact of the soil structure, assessed by the mean coefficient of heterogeneity, remains comparable irrespective of the parameter considered. Such results show that it is not the tillage technique as such but the resulting soil structure that should be studied when interpreting the results of tillage trials if the findings are to be suitably extrapolated and translated into practical advice for farmers. Work on this topic continues with the aim of relating the structural heterogeneity measurement to other, more conventional parameters such as density, porosity, cohesion, etc. The ultimate aim is by setting up and monitoring reference situations to arrive at an agro-environmental indicator to describe the overall quality of an agricultural soil structure and track its development over time.

- Study of the nitrogen cycle with the 15N heavy isotope
 - J.-P. Destain 1, V. Reuter 1, P. Luxen 2, B. Bodson 3, N. Boulelouah 3, I. Dufrasne4
 - 1 Crop Production Department
 - 2 Agra-Ost St Vith
 - 3 FUSA Gembloux 4 ULG Faculty of Veterinary Medicine

In our investigations into the evolution of nitrogen supplied as chemical fertilizer to winter wheat, close attention continues to be paid to the fraction applied at the last leaf stage and to the post-flowering removal and translocation of nitrogen in winter wheat. A doctoral thesis is being prepared on this topic.

As to the fate of nitrogen in animal waste, the study focused on slurry application periods in hay meadows and the evolution of synthetic urine nitrogen in permanent pasture.

The laboratory has also performed isotope analysis in connection with grassland experiments carried out by the UCL under a DGA subsidy, entitled "Temporary grassland for sustainable agricultural systems suited to mixed farming in central Belgium".

- Walloon Regional Government fertilization monitoring study
 - P. Courtois 1*, J.-P. Destain 1, V. Reuter 1, C. Vandenberghe 2, P.-Y. Bontemps 2, R. Lambert 3 *Financing: Walloon Regional Government DGAe.
 - 1 Crop Production Department 2 GRENERA FUSA Gembloux
 - 2 GRENERA F

As part of the Walloon Regional Government's sustainable nitrogen management plan, an "agricultural areas" survey is being carried out to establish the average level of potentially leachable nitrogen (PLN) in field crops. The aim of the exercise (12 farms selected within the network) is to show that by means of managed fertilization based on a projected nutrient balance, subject to adaptation during the growing period (notably according to the plants' nitrogen status), the environmental objectives of minimum PLN levels can be attained while ensuring optimum yields and crop quality. In concrete terms, the Department performs end-of-winter soil N profile analyses, collects plot management data, produces advice on fertilization (AZOBIL, White Paper) and carries out PLN measurement at three different times in autumn.

6.2. CROP HUSBANDRY

Potato husbandry

1. Managing nitrogen fertilization

Adapting the new strategy for managing nitrogen fertilization using a chlorophyll meter

M. Olivier*, J.-P. Goffart. (*WR, DGA – Convention n°2643/2)

During the preceding period (2001-2002) the strategy for nitrogen fertilization management developed in 2001, with conditional splitting of the nitrogen application, was validated. This involves applying 70% of the recommended nitrogen fertilizer at planting and monitoring the crop nitrogen status during the growing period with the aid of the Hydro N Tester (HNT) chlorophyll meter in order to establish whether the additional nitrogen is in fact necessary (30% initially withheld). The close match between the meter readings and the nitrogen absorbed by the crop, as well as its speed and ease of use, make it a valuable tool. As absolute HNT values cannot be effectively used due to the effect of the site soil and climate conditions, the threshold value system has been developed from relative HNT values by comparison with an unfertilized control area. The specification has initially been proposed and validated for the Bintje variety (specific factor). To increase its practical range of application the specification has to be adapted for other varieties. The strategy was validated by replication trials in 2003 for industrial varieties (Astérix, Agria and Felsina) and compared to the Bintje variety. The factor values were tested in a four-replication trial in 2004 with firm-fleshed varieties (Charlotte. Nicola and Merit). At the same time, the strategy was used and evaluated by some growers (parallel strip fields) with the Bintje, Victoria, Santana, Astérix, Lady Rosetta, Charlotte, Nicola, Merit and Franceline varieties.

The form of the supplemental nitrogen and conditions of application (three liquid fertilizer forms containing urea and solid ammonium nitrate) were studied in two trials with the Bintje variety. One form was found to be more effective than another (solid or liquid) but this varies from year to year. When applied in suitable conditions, the different types of nitrogen fertilizer are of similar significance.

The strategy was further refined by testing it in different contexts to those in which it was calibrated, with either a different soil texture or different organic manure application conditions. The strategy was extended in 2003 and 2004. Leaf colour measurements were made in 2004 with the aid of a colour analyser in order to ascertain the feasibility of making up comparative potato leaf colour charts and using them as a simplified indicative tool. Such variety-specific charts would be an alternative to the chlorophyll meter, thus making the technique affordable for any grower.

The 'Color Cue' spectro-colorimeter gives several types of numbers for a flat surface of uniform colour. The CMYK (Cyan–Magenta–Yellow–Black) system, used by printers, provides a smooth transition from potato leaf analysis using Color Cue to printing out coloured dots as close to the leaf colours as possible.

Field measurements with the colour analyser were made systematically at the same time as the measurements using the chlorophyll meter in two treatments with four varieties, the treatment without nitrogen fertilization and the treatment with 70% of the recommended nitrogen rate. In one plot, whereas the chlorophyll meter gives a single figure for 30 leaf measurements, the Color Cue gives 30 four digit numbers. The printout of the average colours obtained for each variety, treatment and measurement date shows visual differences according to time with the same treatment. The differences are less marked between the two nitrogen treatments studied on the same day. The matching of the Color Cue values with the HNT value has not yet been studied in detail.

Assessment of the potential of other crop nitrogen status measuring tools J.-P. Goffart

The strategy described above is based on the use of a chlorophyll meter to assess leaf chlorophyll levels in the field. Other tools for rapid, nondestructive measurement of crop nitrogen status are also available or are under development. Two such tools which measure light reflected by the leaves underwent field testing at Gembloux in 2004, namely the CROPSCAN model and the GPN model. The advantage of this technique over the chlorophyll meter is that it allows larger sampling areas to be assessed in the plots, yet is just as easy and quick to use. Moreover, the reflected light measurements appear to be sufficiently discriminating between different nitrogen fertilizations to permit practical use. However, before such tools can be incorporated into the nitrogen management strategy developed by the Crop Production Department, threshold values first have to be established as a criterion for crop nitrogen supplementation. Evaluation continues.





Measuring light reflected by potato leaves in the field with the GPN model (Grande Paroisse, Paris, France)

2. Mineral and organic nitrogen fertilization of potato crops

J.-P. Goffart (partly financed by Centre Agricole Pomme de terre, CAP asbl and private partners)

The increasing importance of the external and internal quality of tubers for marketing purposes (outward appearance, susceptibility to impacts, chemical composition affecting their cooking and technological qualities) and the need to minimise mineral nitrogen residues in the soil at harvest are now established. Fertilization has a major impact on these crop characteristics. Various forms of foliar fertilizers: organic, organo-mineral or with nitrification inhibitors or fertilizing substances are therefore proposed in an attempt to meet these requirements.

The Crop Production Department launched a set of field trials in experimental plots in 2004 to assess the value of some commercially available fertilizers or fertilizing substances.

Testing carried out on behalf of Rosier s.a. of amino-acid based liquid substances applied to the leaves during the season in combination with urea showed a marked positive effect on the yield and proportion of large tubers. Similarly, application to the soil of a complex liquid NPK (10/4/15) mineral fertilizer supplemented by humid acids had a positive effect on the proportion of tubers over 55 mm.

Two forms of fertilizer were tested for Derom, alone or in conjunction, but always as a supplement to a basic mineral nitrogen fertilizer applied to the ground in the form of ammonium nitrate before earthing up. The first of these was a complex pelleted NPK + MgO (8/8/12 + 4) organo-mineral fertilizer applied before earthing up at a rate of 25% of the total recommended N. The second consisted of complex solutions of nutrients containing N in ureic form, P2O5, MgO, Zn. Bo, chelated Mn and amino acids, spraved on to the leaves in three applications at 15-day intervals, starting at the beginning of tuberization. The organo-mineral fertilizer significantly increased the total tuber yield and the proportion of large tubers. These effects were magnified when used in conjunction with leaf solutions, with a slight drop in the tuber dry matter content. Two organic products were also tested in the field. The first, considered to promote the soil microbiological balance, assimilation of P2O5 and trace elements and good root development, is a concentrated liquid organic manure containing humic and fulvic acids mixed in water and sprayed on the ground at planting. This encouraged the production of large tubers with a lower dry matter content.

The second product, deemed effective in protecting tubers from black scurf and potato scab attacks as well as promoting better rooting, is marketed in the form of an inert water-soluble powder and contains *Bacillus subtilis* (an antagonistic soil bacterium). Initial examination reveals fewer external blemishes caused by parasites on the potato skins. On the other hand, the proportion of large tubers was perceptibly lower.

Finally, a pelleted mineral nitrogen fertilizer containing a new generation nitrification inhibitor (DMPP, dimethylpyrazole phosphate), considered more effective in terms of yield and tuber size and to lead to lower nitrate levels in the tubers and less mineral nitrogen residues in the soil at harvesting, was tested by application at planting. Observations confirmed these favourable effects on yield, large sized tubers and a lower dry matter content.

None of the products tested showed any effect in reducing the mineral nitrogen residues in the soil at harvesting.

3. Managing the risks of tuber deformities and sprouting during growing

J.-P. Goffart (partly financed by Centre Agricole Pomme de terre, CAP asbl and private partners)

A new field trial was undertaken in 2003 with the Bintje variety to investigate scope for controlling deformities and sprouting in ware potatoes. The risk are very high during the summer months, in dry weather and with temperatures in the hill around 25°C or over. Consequently, varying degrees of external tuber quality degradation can be observed at harvest, ranging from a low level of deformities to a high rate of vitreous primary tubers due to secondary tuber formation. The aim is to use irrigation as a means of maintaining the soil temperature in the hill below 24-25°C. The high soil temperatures that prevailed at times during 2003 did not lead to a high rate of deformed tubers in unirrigated plots. Moreover, there was no apparent difference with the level of deformities in irrigated plots. Our conclusion from this two-year trial (2002 and 2003) was that irrigation, which is restricting and difficult to carry out though it does maintain the soil temperature in the hill around 20°C in hot, dry weather, does not appear sufficient in itself as a means of reducing sprouting and the associated effects on tubers.

4. Low-temperature storage of potato varieties

J.-P. Goffart, A. Soete 1, D. Ryckmans2(partly financed by Centre Agricole Pomme de terre, CAP asbl) 1 Agricultural Systems Department, CRA-W, Libramont 2 Filière Wallonne de la Pomme de Terre

The research into potato variety storage at low temperatures (4° and 7°C, from November to May) begun in 2001 was continued in 2003 and 2004 by the Crop Production Department in cooperation with the Agricultural Systems Department at Libramont and FIWAP (Filière Wallonne de la Pomme de terre). The aim is to identify varieties suitable for storage at low to very low temperatures (around 4°C), to avoid using sprout inhibitors (chlorpropham-based) during storage, with no loss of intrinsic quality (in particular, a major risk of increasing the sweet taste of potatoes eaten fresh and browning of chips or crisps after cooking). The use of potato sprout inhibitors, a useful means of delaying the end of natural tuber dormancy and softening, is becoming increasingly controversial from a ware potato point of view and so alternative solutions need to be found. In the storage trials carried out since 2001 on varieties for industrial use, some high-yielding varieties on the market (Victoria, Ramos, Sempra, Artis, Lady Jo, Lady Claire) and some others not vet registered behaved well in storage, maintaining low levels of reducing sugars which promote a lighter colour after frying. As regards potatoes eaten fresh, the Charlotte and Amandine varieties showed a perceptibly smaller increase in sweet taste during storage than Nicola and Franceline, for example. This held true over several storage seasons.

Certain varieties therefore offer potential for very low temperature storage, thus avoiding the use of sprout inhibitors. These variety characteristics will undoubtedly be developed in future in view of the growing demand from supermarkets and the industry for potatoes stored without sprout inhibitors.

Nitrogen fertilizer in field and market garden vegetables

1. Field vegetable nitrogen fertilizer management

S. Renard*, J.-P. Goffart (WR, DGA, Project WR-1015)

This project, launched in January 2001 and scheduled for completion by the end of December 2004, is aimed at optimising nitrogen efficiency in rotations including field vegetable crops in Hesbaye. Vegetable crops have shallow root systems that prevent them from taking up nitrogen at depths of more than 60 cm. In the case of some vegetables, such as spinach, the short growth cycle (harvested before maturity) and high nitrogen demands prompt growers to apply a lot of fertilizer in order to ensure a constant nitrogen supply for the plant. In other cases, such as broad beans, French beans, etc., the sizeable amount of nitrogen-rich crop residues significantly increases the mineral nitrogen residue in the soil in autumn. The short growing time allows double cropping to be practised, that is, two vegetable crops in succession in the same year, which raises the question of rational nitrogen fertilizer management for the second crop. In addition, organic material such as manure and slurry are frequently applied. The resulting nitrogen contribution due to mineralization is difficult to estimate and manage with such short cycle crops.

Nitrogen management in the case of vegetable crops therefore has to be applied to the whole of the rotation if it is to be effective. The yield (quantity and quality), which is the grower's income, and minimising nitrate losses to the environment are the key parameters of this project, with a view to transferring results to the deep-frozen vegetable industry.



This threefold project comprises:

1) Determination of nitrogen requirements and plotting nitrogen response curves for vegetable crops (spinach/spinach, spinach/French beans, broad beans/spinach). Study of the AZOBIL decision support tool (*INRA*, Laon, France) from the point of view of meeting plant requirements as precisely as possible (no loss of yield; limiting nitrate losses to the environment) and providing advice to producers. Splitting nitrogen application, in particular with the aid of growing crop nitrogen status measurement tools (leaf chlorophyll levels using the HNT chlorophyll meter and Cropscan to measure light reflected by the foliage).



Broad beans 38 days after emergence (Darion, 02/07/01)

2) Impact of different vegetable nitrogen fertilization management techniques on the development of residual mineral nitrogen in subsequent wheat or a nitrogen-trap intercrop in the case of a spring crop following vegetables (beetroot, potatoes, carrots).

3) Determination of the effects of previous vegetables on the crop in the case of following wheat or spring crops (yield, product quality and residual mineral nitrogen in the soil).

The results of this research have enabled us to focus on the growing phases of spinach and French beans, these being the main crops where double cropping is generally practised. Spinach growth thus appears to be closely linked to nitrogen fertilization, unlike the French bean which can fix nitrogen from the air. Moreover, nitrogen fertilization management using the AZOBIL program enables a near-optimum yield to be achieved while limiting nitrate losses to the environment. The measurements made on growing crops show, in the case of spinach, that the HNT diagnostic tool is late in providing useful discrimination, too late for fertilization to be adjusted (very short growth cycle of 40 to 50 days). The plant nitrate level, on the other hand, correlates closely with crop nitrogen fertilization and could be a useful indicator for the nitrogen status of spinach at the early growth stage. Results with Cropscan are undergoing analysis. The intercrop trials (mustard, phacelia, radish, rye and ryegrass) have highlighted the usefulness of nitrogen-trap intercrops in all cases from an environmental point of view (less leaching in the interval). They also have an effect on the following crop, either in terms of post-harvest residues (richer beet post-harvest residues) or of yield (nitrogen released at the right time for potatoes). Rye emerged as the best compromise in all the cases studied.

2. Control of marginal necrosis *In fine* curly chicory

J.-P. Goffart (CIM/CRA W convention)

The Department has been researching marginal necrosis In fine curly chicory field crops since 2002, in cooperation with Centre Interprofessionnel Maraîcher (CIM). The aim is to examine the effectiveness of rational nitrogen fertilization management as a means of reducing the impact of this physiological disease on the plant, while still maintaining sufficient chicory production via appropriate nitrogen supply and reducing the often high post-crop mineral nitrogen residues in the soil. Splitting nitrogen fertilizer applications according to plant nitrogen status measurements is generally considered a good way of achieving these objectives. The usefulness of rapid leaf chlorophyll level measurement as an indicator of supplemental nitrogen requirements has been studied with a Hydro Agri chlorophyll meter (HNT model). Investigations continued in 2003 and 2004 via a trial with increasing nitrogen application rates. At this stage in the research it is clear that nitrogen fertilization of chicory has to be adjusted above all to the nitrogen supplied by the soil in order to attain the objectives. Other factors also come into consideration in limiting marginal necrosis. The chlorophyll meter appears to be a suitable tool for managing nitrogen supplementation in curly chicory. The measurements are sufficiently discriminatory in terms of nitrogen rates with regard to nitrogen removal by the crop. The measuring and decision procedure is also fairly straightforward for farmers to carry out. HNT threshold values for supplemental nitrogen reguirement detection still remain to be confirmed and validated.

Organic farming

Fertilization of winter wheat

L. Couvreur

The aim of this study is to measure the impact of spring fertilizer application on winter wheat grown in accordance with organic farming practices. In each of the past two years, increasing rates of poultry manure from organic farms and guanobased fertilizer were spread in March and/or April. Results in 2003 and 2004 show that March application of poultry manure equivalent to 160 nitrogen units increased the yield by around 700 kg grain per ha (20 to 26 % increase). In 2004, application of 40 and 80 nitrogen units in the form of organic fertilizer from guano, feather meal and cakes increased the yields by 755 and 1724 kg/ha respectively. March application always resulted in greater nitrogen efficiency irrespective of fertilizer type.

Precision farming

Study of spatial variability within fields aimed at efficient input management M. Cuvelier and M. Frankinet

Integrated management with a view to sustainability is the focus of current debate. Environmental policies are becoming increasingly restrictive, in particular as regards limiting agricultural inputs in cultivated fields. Against this background, precision farming technology seems to provide a response to better input management while maintaining production levels. The aim is to apply "the right rate at the right place". The object of this project is to develop advanced technologies for adjusting cultural practices as closely as possible to the plant's requirements according to intra-plot variations. As accounting for field heterogeneity factors is complex and varies from year to year, the CRAW has sought to develop an agronomic, rather than a statistical, approach to the problem, with the aim of identifying the areas of greatest homogeneity (production isopotential areas) within fields and studying their behaviour in response to input adjustment. The conditions developed allow response curves to such adjustments to be plotted for different points within the experimental field. The fields selected for the purpose of this trial are either known to be homogeneous or, on the other hand, apparently very heterogeneous.

From the data gathered for the different fields, maps have been produced of observed yield, probable maximum yield (assessed by interpolating yield data by fertilizer type or density), optimum fertilization or density and response to input adjustment.

Set-aside

Planting and monitoring mixtures for wildlife set-aside

L.Couvreu

A series of experiments was set up in 2004 under an agreement with the DGRNE/Department of Hunting and Fishing with the aim of selecting suitable cover plants for wildlife set-aside, with the ultimate objective of possibly adding to the list of permitted species on set-aside land. Results showed that some covers can fulfil the requirements of good agricultural practice while at the same time meeting the needs of set-aside for wildlife. In terms of agricultural practice, the main aim is to prevent weed invasion whereas for wildlife set-aside purposes, the land should provide year-round food sources, shelter in bad weather, protection from predators and breeding habitats.

Of the plants tried out, cereals offer the best choice from the point of view of both competition with weeds and the useful spaces between rows. Cereals could thus form the basis for a mixture to promote wildlife on set-aside land. Suitable companion plants would include cabbage, linseed, buckwheat and millet, which offer good food sources for birds, insects, hares, etc. without impeding their movements. Maize is another contender, but its inability to compete with weeds rules it out as a suitable plant for set-aside in a no-herbicide system. Another problem is that of sowing depth when maize is mixed with other grains requiring a shallower depth.

6.3 PLANT ACCESSIONS

Comparison of varieties for inclusion in the national catalogue and recommendations L. Couvreur, J.L. Herman

Trials for acceptance in the national catalogue of species and varieties have led to the listing of the following varieties:

- winter wheat: Landrel, Lieven, Tulsa, Anthus, Drees, Allié, Ephoros, Nemocart, Kansas, Anthem, Archipel;

- rye:-
- spelt: Stone;
- six-rowed winter barley: Palmyra, Mandy, Rosita, Jolival;
- two-rowed winter barley: Natival;
- triticale: -
- spring oats: -
- spring barley:-
- field pea: -
- horse bean: -
- potato: -

In parallel with official testing, the study of varieties has been widened to include varieties from the EU catalogue. Variety renewal over the past two years has chiefly concerned winter wheat.

Testing has revealed marked differences in terms of production, quality criteria and disease and lodging behaviour. While forage varieties have confirmed their greater productivity, there are nonetheless some varieties that combine good breadmaking quality with high yield, close on the forage varieties' level. Various trials have resulted in identification of the most suitable varieties for organic farming and recommendations have been made accordingly.

Variety description in terms of resistance or susceptibility to the various leaf and head diseases has enabled strategies to be devised for varietyspecific fungicidal control. 2004 was a good year for highlighting varieties' behavioural differences with respect to lodging.

In the case of winter rape, more varieties have been added to the range, in particular new variety associations and new hybrids that are more productive but also more susceptible to *Phoma* than conventional strains.

7. DEVELOPMENT AND SERVICE ACTIVITIES

7.1. Dévelopment

Demonstration and extension of the potato nitrogen fertilization management strategy - transfer of skills to field practitioners with a view to long-term extension M. Olivier* in collaboration with Fiwap asbl (*WR, DGA –

Convention No. 2643/2)

The strategy for split application of nitrogen fertilizer, using the chlorophyll meter as a decision support tool (cf. § 6.2.3), was demonstrated and disseminated in a joint project with Filière

Wallonne de la pomme de terre (Fiwap). Several demonstration fields were set up within the Walloon Region (four in 2003 and three in 2004), a letter was sent out to all Walloon growers with over 5 ha under potatoes, a clear, practical information booklet was produced and distributed and meetings at the demonstration sites provided an opportunity for growers and supervisors to see the conditional splitting technique at first hand and to gain experience in making measurements with the chlorophyll meter.

The growers were offered technical support with application of this new nitrogen management technique (in 2003 and 2004).

A skills transfer plan was put into effect in 2004 to ensure on-going support and guidance for growers using the strategy. Meetings were held in February when straightforward cooperation was proposed to field practitioners and technical advisers working in the area of potato growing. An agreement was signed with the external departments of the DGA, Department of Development and Extension, CARAH, Nitrawal, the agronomic departments at processing factories (McCain and Farm Frites) and several farmers' organisations (Tournai CETA, Thuin CETA, etc.). The FIWAP technician seconded to the project monitored fifteen fields in 2003. That number was increased to sixty in 2004 due to the skills transfer and the cooperative framework established.

CRA-W departments (seed production from own accessions, silage maize production, selection of new varieties, experiments with plant protection products, etc.).

- Participation in the testing network set up by the Flemish Region for registration in the national catalogue of varieties of grain maize, silage maize, catch crops (Westerwold ryegrass, mustard, fodder turnip, fodder rape, phacelia, vetch), pasture grasses (English ryegrass, Italian ryegrass, Westerwold ryegrass, meadow fescue and timothy), inulin chicory and fibre flax.

- Setting up national catalogue post-registration tests for winter wheat, spring wheat, spring barley, spring oats, winter rye, six-rowed winter barley, winter barley, winter oats, triticale, spelt, peas, rape and catch crops.

- Participation in the "rape yield" monitoring group for fixing of representative yields in non-food set-aside.

 Member of the Terra Nostra initiative technical support group promoting potatoes grown in the Walloon Region (specifications and traceability).

 Participation in the Potato Pilot Centre created in 2004 on the initiative of the MWR DGA.
 Member of Filière Wallonne de la Pomme de Terre
 FIWAP asbl (chair since May 2004).



Meeting with growers and other players in the industry at a demonstration site.

7.2. Service

- Running the 125 ha experimental site in accordance with the general farm regulations in force (CAP regulations, beet quotas, straw-manure exchange policy). This includes all the constraints inherent in an experimental site (soil regulation between trials) and also takes account of the specific requirements connected with the work of other - Member of Conseil Wallon Filière Pomme de Terre asbl, set up in 2003 on the initiative of APAQ-W (treasurer).

- Member of Fédération Wallonne Horticole (FWH).

- Supervision of training courses, dissertations and doctoral theses (Belgian and international students).

- Setting up of a Quality Assurance System to ISO-17025.



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GOFFART J.-P., RENARD S., FRANKINET M., SINNAEVE G., DELVIGNE A. and MARECHAL J. (2004). Leaf chlorophyll content measurements for nitrogen fertilization management of curled-leaved endives in open field. Poster and abstract (special edition of Italus Hortus, vol. 11, 3, 33-34) read at the ISHS Symposium "Towards ecologically sound fertilisation strategies for field vegetable production," Perugia, Italy, 7-10 June 2004.

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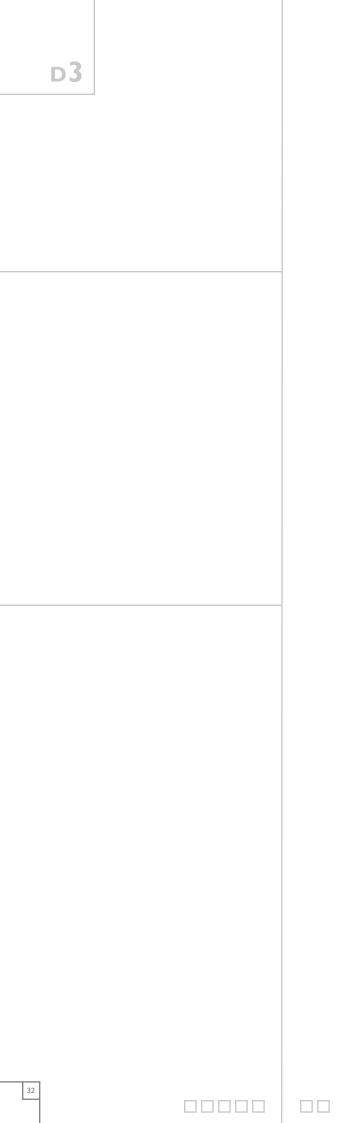
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1. MISSION STATEMENT

. Plant protection: identification of pathogens and pests, epidemiology, population dynamics of pests, development of integrated and biological control methods with the aim to turn out quality products in reducing the risks for health and environment.

. Management of agricultural biodiversity: conservation, characterisation and utilisation of fruit and cereal crop genetic resources. Designing spelt, wheat, barley and apple varieties meeting the sustainable agriculture goals.

2.SCIENTIFIC PERSONNEL

5 permanent staff and 13 contract staff

2.1. Head of Department

M. Cavelier, Inspecteur général scientifique

2.2. Scientists

- S. Abras, Attaché scientifique (DGRNE) from 01/02/04
- A. Antofie, Attaché scientifique (UE, SPP Politique scientifique)
- A. Barbier, Attaché scientifique until 31/10/04
- A. Chandelier, Attaché scientifique
- D. De Merlier, Attaché scientifique (DGRNE) until 31/01/04
- A. Dekeyser, Attaché scientifique
- P. Detrixhe, Attaché scientifique (SPF-Santé publique), taken up the
- post as research assistant on 01/02/03.
- C. Fassotte, Attaché scientifique
- H. Hautier, Attaché scientifique (SPF Santé publique), taken up the post as research assistant on 01/09/03.
- L. Jamar, Attaché scientifique (DGA)
- J.P. Jansen, Attaché scientifique
- M.H. Kestemont, Assistante (SPF Santé publique) until 31/01/03
- M. Lateur, Attaché scientifique
- B. Lefrancq, Attaché scientifique (DGA)
- T. Mahaut, Attaché scientifique (DGRNE) until 30/11/04
- A. Michotte, Attaché scientifique (DGRNE) from du 01/12/04
- A. Rondia, Attaché
- S. Schmitz, Attaché scientifique
- S. Steyer, Attaché scientifique

N. Viatour, Assistante (SPF Santé publiaue) until 30/08/03

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I. Villette, Attaché scientifique (RW-EU)

3. NATIONAL REPRESENTATION

 Agrobiopôle Wallon, Groupes de travail Horticulture et Agrosystèmes

- Centre Ágricole pour le Développement des cultures Céréalières et Oléo-protéagineuses (CADCO)

- Comité de Concertation Pépinières, RW (DGA) - Comité Régional PHYTO

 Comité de Biosécurité de l'Institut Scientifique de la Santé Publique - Sous-comité des écotoxicologues (SPF Santé publique)

- Comités de rédaction de BASE, de Parasitica et du Fruit Belge

- Comité Directeur « Convention sur la Diversité Biologique» (CBD)

 - Comités techniques du Centre Fruitier Wallon (CEF) et du Centre d'Essais Horticole de Wallonie à Ormeignies (CEHW) - Commission consultative arboriculture fruitière du Centre Technique Horticole (CTH)

- Conseil d'administration de l'asbl « Pépinière Belge » (P.B.B.)

- Conseil de Filière Wallonne des Produits Horticoles Comestibles (CFWPHC)

- Conseil Supérieur d'Hygiène
- Contrat de rivière Semois & affluents
- FUSAGx, collaborateurs scientifiques
- Groupement interprofessionnel belge des semences (INTERSEMZA)

 Groupe de contact « Stratégie nationale pour l'application de la « Convention sur la Diversité Biologique » (SPP Politique scientifique)
 Groupe de travail « Production intégrée en cultures fruitières » du Fonds pour la Production et la Protection des Végétaux et Produits végétaux (SPF Santé publique)

- Groupe de travail 'Pépinières' du Fonds pour la Production et la Protection des Végétaux et Produits végétaux (SPF Santé publique)

 Groupe de travail pour l'élaboration du catalogue national des variétés, section céréales (MA)
 Stichting Professor Albert Soenen, membre du jury.

4. INTERNATIONAL REPRESENTATION

- Association Française pour la Conservation des Espèces Végétales (AFCEV), Paris

- Centre International de Recherche Pomologique et de Documentation Fruitière, membre du conseil d'administration, Alès, France.

- Centre Régional de Ressources Génétiques de l'Espace Naturel, membre du Conseil scientifique, Lille, France.

- Commission des ressources génétiques pour l'alimentation et l'agriculture de la F.A.O., expert belge

 Commission Suisse pour la Conservation des Plantes Cultivées, expert en ressources génétiques fruitières.

- ECP/GR European *Pyrus* Database, co-responsible of the database

- Groupe de Travail « Pesticides et organismes utiles » de l'Organisation Internationale de Lutte Biologique (OILB)

- International Breeders Association (ASSINSEL)

- ISHS (International Society for Horticultural Sciences) et EUCARPIA (European Association for Research on Plant Breeding), member.

- National Focal Point of the National Plant Genetic Resources Inventory of Belgium, ECP/GR &

European Plant Genetic Resources Search Catalogue (EURISCO)

- OCDE, Task Force on Biological Resources Centres (BRC), National expert in plant genetic resources (SPP Politique scientifique)

 Organisation Internationale de Lutte Biologique et Intégrée (OILB), représentant du SPF Santé publique

 Point de contact national auprès de la F.A.O.
 pour la mise en œuvre de la CBD (Convention on Biodiversity)

- Programme Coopératif Européen pour les Réseaux de Ressources Phytogénétiques (ECP/GR) de l'IPGRI (International Plant Genetic Resources Institute), National Coordinator et membre du Steering Comity; Présidence du *Malus/Pyrus* Working Group et représentant national des *Prunus*, Barley et Wheat Working Groups

- UPOV, expert belge (SPF Santé publique) pour l'élaboration de principes directeurs pour la conduite de l'examen des caractères distinctifs, de l'homogénéité et de la stabilité pour les espèces *Malus* et *Pyrus*

5. RESEARCH TOPICS

Plant protection plays a key role in agriculture and environmental conservation, by limiting the impact of diseases and indigenous pests and controlling the introduction and spread of harmful organisms that are detrimental to wild or cultivated plants in agricultural and natural ecosystems. Identifying pathogens and pests and developing fast, reliable detection methods for the study of epidemiology and the dynamics of pest populations is a major part of the Department's work. This research leads to new control strategies for use in integrated crop management and organic farming based essentially on choosing diseaseresistant varieties, prevention and using the least environmentally harmful plant protection products, notably in terms of ecotoxicology and residues. These methods are put into practice by distributing advice and warnings to farmers, nurserymen and land managers, producing information sheets on diseases, pests and resistant varieties and drawing up pesticide selectivity lists with respect to useful arthropods.

The Department's work lies within the wider context of preserving and enhancing the genetic resources the diversity of which is the very foundation of sustainable agriculture and one of the keys that will enable farmers to adapt to the new CAP reform. In this regard it is the duty of public research to enable the agriculture of tomorrow to integrate the new socio-economic data. Accordingly, the Department emphasizes the creation and promotion of cereal and fruit varieties that require little input, with the twofold aim of ensuring farm profitability and environmental protection while at the same time meeting processors' and consumers' requirements. The use of these new varieties involves some deintensification of production and, consequently, a policy change as regards the use of certain inputs the need for which has to be questioned, not only from the point of view of product competitiveness on the markets but also for the social reasons mentioned earlier. The high level of technological sophistication in farming, mainly driven today by genetic progress, leading to a steady increase in yields has made this challenge possible. In this connection the assessment and description of collected plant material within the framework of European plant genetic resources opens up fresh prospects for development, encouraged by a policy of diversification of production sectors, including organic farming, where traceability is a determining factor in product quality management.

The Department is responsible for the development and extension of a large part of its research results and also maintains a network of public contacts through its service activities.

6. RESEARCH REPORTS

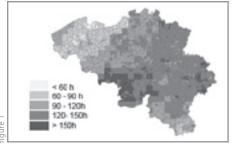
6.1. Biological and integrated pest management in phytopathology and applied zoology

Fungal diseases

1. Development of a winter wheat fusariotoxin contamination risk forecasting model

P. Detrixhe, A. Chandelier and M. Cavelier (in cooperation with the Biometrics, Agrometeorology and Data Management Unit and the Quality of Agricultural Products Department)

Toxic substances called mycotoxins, some of which are produced in the field by fungi belonging to the Fusarium head blight complex, can contaminate cereals. These metabolites are generally fairly resistant to processing and are therefore likely to end up in the finished product (bread, beer, biscuits, etc.). Health effects vary according to the quantities ingested and type of molecule. Some, such as T2 and HT2 toxins, are potential carcinogens. In response to concerns voiced by the sector (farmers, collectors and millers) and consumers and in order, also, to meet European regulations for maximum permitted levels in food, the Department's Mycology Laboratory, in conjunction with the CRA W Biometrics Unit, has since 2001 been investigating the problems of fusariotoxins (mycotoxins produced by Fusarium) as part of a project subsidised by FPS Public Health, Food Chain Safety and Environment. The work aims to draw up an inventory of winter wheat crop contamination levels with deoxynivalenol ("DON" for short), one of the commonest fusariotoxins, identifying the different species of toxinogenic Fusarium found in the grains and collecting meteorological and growing data in order to develop a wheat Fusarium head blight risk forecasting model. On going monitoring since 2001 shows that crops are not necessarily free from DON contamination and that the weather conditions at flowering play a major part in disease development. Four species of toxinogenic Fusarium generally occur in the grain, most commonly F. graminearum and F. avenaceum. DON contamination risk mapping, based on meteorological data, has been developed at national level. Data collected from weather stations (basic data) and from the IRM radar at Libramont (rainfall data) are processed for application to 1 km2 grids. Calibration reveals the multifactorial nature of the infection and the need to refine the risk calculation.



Mapping the DON contamination risk on the basis of meteorological data (number of hours when weather conditions favoured disease development during the wheat (Rowering period) – Data for 2003 – (map produced by D. Buffet, CRA W Biometrics Unit)

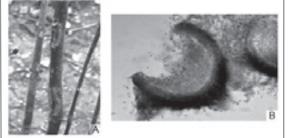
Through technical meetings with farmers the project also aims to draw up a list of "good agricultural practices" to reduce the risk of DON contamination. In this connection, a sample group of winter wheat varieties available on the market was evaluated in 2004 to establish wheat susceptibility to head blight and DON accumulation, by artificial inoculation with macroconidia of *F. graminearum*, *F. culmorum* and *F. avenaceum*. Initial results show significant variability of response between varieties, in terms of both severity of infection and DON accumulation.

2. Fungal diseases in ornamental nurseries S. Schmitz

Relatively few fungicides have been authorised for some "small-scale crops," such as ornamental or forest nursery crops, as their market share is too small to be interesting to plant protection product manufacturers. Consequently, unauthorised fungicides are often used without knowing their precise effect on the target pathogen or the minimum effective rate. The result is a large number of ineffective applications and systematic over-application, leading to pesticide build-up in ecosystems. Apart from limiting excessive use, the authorisation of more fungicides for small-scale crops would diversify use, thus reducing the risk of microorganisms developing resistance to the products used.

For these various reasons, one of the projects undertaken by the Mycology Laboratory involves setting up trials aimed at extending fungicide approval. This comprises first of all carrying out a preliminary *in vitro* test to select the most effective fungicides against the pathogen concerned. The most promising fungicides are then evaluated in a field trial. Work has been done on perfecting *in vitro* fungicide evaluation methods by measuring their effects on both mycelial growth and spore germination. The techniques developed have been used to identify some fungicides with *in vitro* effectiveness against *Botrytis cinerea*, *Pycnostysanus azalea*, *Verticillium dahliae* and *Chondrostereum purpureum*.

In this connection, some tests commenced during the previous two years have provided an *in vitro* and nursery assessment of various fungicides with respect to a *Phoma* fungus associated with ash seedling canker. Based on the results, the authorisation for Topsin M 500 SC (thiophanate-methyl) has been extended for control of this pathogen in ornamental tree and shrub nurseries.



Phoma exigua canker on Fraxinus excelsior

A : stem symptoms B : pycnidium releasing conidia

3. Silverleaf

S. Schmitz and M. Lateur

A resurgence of silverleaf caused by the pathogen Chondrostereum purpureum has been evident in Belgian orchards since 2003, most of the cases occurring in 'Conference' and 'Concorde' pear orchards and in 'Major' cider apple trees. There are no authorised active ingredients for the control of silverleaf in Belgium to date and only preventive measures, essentially based on removing sources of infection and care of pruning cuts, can be recommended. Several trials have therefore been undertaken to establish possible means of control using fungicides, antagonistic agents or less susceptible varieties. An experimental design has been set up in 'Conference' pear orchards to assess the effectiveness of fungicides preselected in an in vitro test against silverleaf. Some encouraging results are also to be found in the literature concerning biological control by protecting pruning cuts with antagonistic Trichoderma fungi. With the aim of developing a control strategy that combines chemical and biological means, a fungicide with only a slight inhibitory effect on T. harzianum growth has been identified as of interest for integrated pest management. Finally, variety resistance to silverleaf has been investigated by inoculating different varieties of pear and apple trees with C. purpureum.

4. Search for new means of biological control of *plant diseases* based on induced systemic resistance: application to fruit crops and field crops *B.Lefrancq, M.Lateur*

Besides introducing varieties less susceptible to disease and improving spraying techniques, another approach in our research into developing integrated pest management methods is to induce resistance in plants by using "elicitors". These can trigger the plant's own natural defence system by activating certain genes that would otherwise have remained unexpressed. We have thus moved on from direct control of pathogens to a means of biological control involving the plant's defensive reactions which are assimilated, by analogy, to the

vaccination principle. Induced systemic resistance (ISR) in plants has six interesting characteristics: (i) the possibility of using natural elicitors, i.e. relatively simple, inexpensive compounds; (ii) its adaptability and broad spectrum of action; (iii) its multi-site, nonspecific and therefore lasting action, making the

> emergence of resistant pathogens unlikely; (iv) its often systemic and enduring effect in plants, hence the name in general use, Induced Systemic Resistance (ISR); (v) as well as fungal diseases, the scope for controlling bacterial and viral diseases, against which little protection is available; (vi) the possibility of applying ISR to commonly marketed susceptible varieties. Despite the very promising prospects offered by ISR, many questions still

remain unanswered concerning the nature of the inducers, their effectiveness in the field, the timespan of the protection they provide, their



modus operandi, their harmlessness, the timing of applications and, lastly, the formulation, which determines their use in practice.

The project aims to identify natural substances that can trigger natural defence mechanisms against apple scab. The new compounds under consideration come from either plant and algae extracts, minerals or microorganisms. The dynamic interaction between the natural resistance level of some apple varieties and their defensive reaction in response to various inducers is a very interesting approach. In the first year of the project (2004), effectiveness screening of twelve elicitors, some known, some new, was carried out on young plants grown from seed, comparing them with substances with a similar action. In vitro testing was carried out with mineral compounds to establish their degree of fungicidal action with respect to scab (Venturia inaequalis). A preliminary experiment designed to study the cultivar-ISR interaction involved inoculating two elicitors into four varieties with a scab resistance gradient. Lastly, a seed trial is in progress to test the most suitable analytical methods to identify induced systemic resistance (measuring enzymatic activity, measuring total phenolic compounds or measuring oxygen released and chlorophyllian activity). A new orchard planted in winter 2004-2005 will allow elicitors to be tested in real conditions. The experimental design features a combination of biotic agents applicable to the soil and other elicitors to be sprayed on to the leaves. This project is subsidised by the DGA Research, Development and Quality Division.

5. Plant protection in organic apple growing

L. Jamar, M. Lateur

Most of the present commercial varieties are not suited to organic farming due to their susceptibility to fungal diseases and, in particular, scab caused by Venturia inaequalis. Furthermore, the number of active ingredients authorised for use in organic farming is very limited and their mode of action is purely preventive. The situation is likely to be further complicated by the possible ban on the use of copper-based plant protection products. The aim of this project, which is subsidised by the DGA Research, Development and Quality Division, is to study means of controlling scab in organic fruit growing. Various anti-fungal treatments are being tried out in an open-air experimental orchard optimising the main factors involved in the "orchard" ecological system, such as choice of variety, keeping the infection potential low, soil management to promote organic activity and creating an environment favourable to useful fauna. The underlying aim is to carry out a life cycle analysis for the system and to define the conditions for profitable fruit growing along organic lines. A two-hectare commercial-style experimental orchard was laid out in Gembloux in 2002 in a randomised block design (6 blocks). It contains eight apple tree varieties, old and new, with varying levels of scab resistance. Ten different scab treatment schemes have been tested each year since 2003. Annual monitoring of diseases and pests and fruit

quality appraisal are carried out according to the different treatment schemes. The treatments are applied with the aid of a spray tunnel in order to use modern technologies combining maximum effectiveness with maximum environmental soundness.

The general plant protection strategy involves applying barrier treatments when the ascospores that cause primary infection are produced. One particular result to emerge from the trials in 2003 and 2004 is that the spread of scab can be controlled in relatively susceptible varieties by applying smaller quantities of sulphur and copper precisely at the start of each infection period. This treatment scheme was not found to have any undesirable effects on a useful fauna indicator (*Typhlodromus pyri*).

Other tests were performed on potted grafts to examine the scope for cutting down the number of fungicidal treatments by utilising the varieties' genetic resistance. A number of effectiveness tests on apple seeds under controlled conditions led to the selection of new active ingredients to control scab. Specifically, the use of lime sulphur, bicarbonates and silicates was investigated as an alternative to sulphur- and copper-based products. This research incorporates innovative means of control that reinforce the plants' own active defence while also having a fungicidal action.



Spray tunnel with six small tanks (each containing a different product mixture), one large rinsing tank and one large mixture recovery tank (experimental prototype).

6. Study of *Phytophthora* ramorum A. Chandelier, M. Cavelier

A new disease called Sudden Oak Death appeared in California in 1993. A few years later, a disease caused by a Phytophthora was identified in Germany and the Netherlands, causing branch necrosis and brownish spotting of rhododendron leaves and buds. The cause of both diseases was found to be Phytophthora ramorum. In Belgium, this pathogen was first identified in 2002 on a viburnum plant (Viburnum bodnantense) from a nursery. P. ramorum infects leaves and branches. This pathogen was first identified in Europe on nursery Rhododendrons and viburnums. It has since been identified on other ornamental plants, notably Camellia, Hamamelis, Kalmia, Leucothoe, Pieris, Syringa, Taxus and Vaccinium. This is a heterothallic Phytophthora and only the A1 sexual type was present in Europe until recently, type A2 occurring in the USA. However, in 2003 one of the Belgian P. ramorum isolates was classified as European type A2.

In view of the large number of host plants, aerial

dissemination of the pathogen and the recent identification of the only European type A2 in Belgium, a study has been launched to assess the ability of Phytophthora (sexual types A1 and A2) to infect the forest species prevalent in the Walloon Region. Pathogenicity tests in the presence of the two sexual types identified in Belgium (type A1 isolated from Rhododendron and type A2 isolated from Viburnum bodnantense) have been carried out on young Picea abies, Pseudotsuga menziesii, Abies nordmannian, Fraxinus excelsior, Fagus sylvatica, Quercus robur, Q. petraea and Carpinus betulus plants. Characteristic symptoms of Phytophthora infection were observed on Q. petreae, Q. robur and F. sylvatica. The hornbeam (C. betulus) and ash (F. excelsior) plants did not develop bark necrosis. Among the conifers, the Douglas firs (Pseudotsuga menziesii) suffered some wilting of the young shoots, while the spruces (Picea abies) and firs (Abies nordmanniana) showed no symptoms. The pathogen was re-isolated from infected tissue in the case of Q. petraea, Q. robur and F. sylvatica. Around twenty strains of the pathogen isolated in the Walloon Region have been collected. Their sexual type (A1 or A2) will be determined and molecular analyses will be carried out to describe their genetic diversity.



Rhododendron branch necrosis caused by Phytophthora ramorum

7. Study of alder disease caused by *Phytophthora* sp.

A. Chandelier, S. Abras, M. Cavelier

1993 saw a wave of decay in common alder growing by rivers and streams in Britain. The infected trees had trunk necrosis and a bare crown. The pathogen responsible, *Phytophthora* sp., was identified for the first time in Belgium in 1999. An inventory of the entire network of waterways in the Walloon Region, carried out in 2002, revealed that most of the rivers and streams surveyed were affected, with over 25% of the trees being infected (Debruxelles, FUSAGx).

In an attempt to put in place some management measures to curb the spread of the disease, various research work was conducted at the Department's Mycology Laboratory under a DGRNE (Department of Non-navigable Waterways) subsidy. The first of these studies was designed to establish the diversity of alder Phytophthora isolates found in the Walloon Region. To this end, 24 isolates were collected from 13 sites throughout the Region and compared with reference isolates from other countries (Sweden, Germany, the Netherlands and Britain) by molecular analysis (RAPD, RAMS), morphological analysis of fruiting bodies and pathogenicity tests. These studies uncovered the existence of three groups of isolates corresponding to the three sub-species of alder



Phytophthora initially identified in Britain. A PCR test has also been developed specifically to detect alder *Phytophthora*.

Alder Phytophthora sporulation conditions in river water were examined. The research showed that the water temperature and the presence of bacterial exudates determined sporangium production. Experimental designs were also devised to demonstrate the role played by zoospores in the infection mechanism and the gateways into the host plant for Phytophthora. Root transmission was identified, notably via the root hairs, with stem infection generally being via the lenticels. As part of a new project concerned with setting up a network of woody ecotypes to rehabilitate river and stream banks (INTERREG III, ECOLIRI), common alder seeds were collected from trees. that had never shown any signs of wilting in the last three years despite being in a contaminated area. Sowing took place in 2005 and the level of disease resistance of the resulting plants will be assessed by various inoculation methods. Promising plants will be propagated and used in seed orchards to produce alder ecotypes for planting along the banks of contaminated waterways.

8. Phytosanitary study of woody species on the banks of walloon waterways

S. Abras, C. Fassotte, A. Chandelier, M. Cavelier

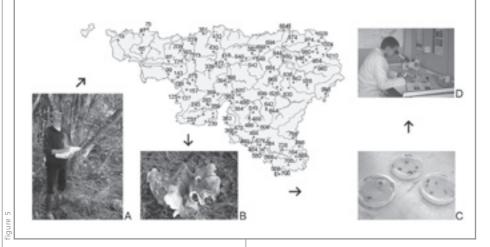
The river bank environment is subject to changes brought about by human landscaping and the direct or indirect effects of pollution. Such stresses impact upon the state of health of woody species and can lead to ecological disasters, as is indeed the case with alder disease (Phytophthora alni). This disease is already the subject of research but in order to get a clearer picture of the potential threats to plant health and to comply with European waterways and environmental management legislation, a phytosanitary study of the main woody species growing on river banks got under way in February 2004 throughout the Walloon Region. The main aims of this study, which is subsidised by the DGRNE (Department of Non-navigable Waterways), are to develop inventory methods, to identify potential plant health problems (diseases and insect pests) and to put in place a management support service for woody river bank species management.

Based on data collected by the FUSAGx in 2002 for the alder disease and riparian forest study, 105 small experimental plots have been selected in order to study 22 woody species throughout the Walloon Region. Four data and sample collection campaigns have been scheduled for 2004 and 2005, two in spring and two in summer/autumn. Most of the plant health problems encountered in spring 2004 were caused by insect pests and in the main consisted of leaf damage by colonies (examples: Agelastica alni Coleoptera, Yponomeuta and other Lepidoptera). The other insect pests present generally occur in small numbers and present very little danger to the tree. Few if any pathogenic fungi were observed at that time of year. In summer and autumn 2004, the proportion of pathogenic fungi occurring increased considerably. The most prevalent diseases were either leaf pathogens (such as powdery mildew and rust) or wood-destroying fungi (e.g.: Armillaria). Alder disease, meanwhile, is still fairly prevalent in alders along the banks of rivers and streams in the Walloon Region. The most numerous insect pests at that time of year were sawflies (Hymenoptera) and miners (Lepidoptera or Diptera), which attack the leaves.

2. Cereals

S. Steyer

In connection with CADCO (Cereal and Oil Seed Plant Development Centre) warnings about *Barley yellow dwarf virus*, RT-PCR detection of different



Location of experimental plots for the phytosanitary inventory of woody riverbank species in the Walloon Region (Maps from MRW, DGRNE). A: Field symptoms. B: Sample (Meripilus giganteus). C: Planting. D: Laboratory determination.

Plant virus diseases

1.Biological and molecular characterisation of cereal mosaic diseases and their vector *Polymyxa graminis* in Belgium

A. Barbier, S. Steyer (in cooperation with the UCL Phytopathology Unit)

The aim of this project, which is subsidised by the DGA Department of Research, Development and Quality, is to describe the agents responsible for cereal mosaic diseases in the Walloon Region and curb their spread.

During the first two years of this project the CRA - W team focussed mainly on studying the diversity and variety specialisation of the P. graminis populations and viral pathotypes occurring in the Walloon Region. Around a hundred Walloon soil samples were collected from fields thought to be contaminated with the barley mosaic virus. Our explorations also revealed, for the first time in Belgium, soil contaminated by the wheat mosaic virus. By growing reference varieties on these soils it should be possible to establish the diversity of the viral and vector strains present. This work is being carried out under controlled conditions, on the basis of symptom observations and FLISA and RT-PCR analyses, having first defined the symptominducing conditions in growth chambers. Variety susceptibility trials using different cereals (barley, wheat and rye) are conducted in reference fields in order to select genotypes with good resistance. These genotypes still have to be tested under controlled conditions on the soils that have produced the various isolates found in the Walloon Region. Genotypes resistant to a number of pathotypes can then be recommended for use in breeding programmes.

The effects of mosaic diseases on the performance of varieties currently grown is also measured in yield trials designed also to investigate the process and dynamics of infections. BYDV isolates directly from aphids collected in the field is in the process of finalisation and ought to significantly shorten insecticide treatment decision times (2 days instead of a week).

3. Hops S. Steyer

With a view to the new outlets developed by Biodynamics NV (Ostend) for hops in Belgium (capsules containing hop extracts), micropropagation of hop varieties with high levels of hopeine or 8-prenylnaringenine is continuing.

4. Fruit trees

S. Steyer

The fruit certification development programme run jointly with FUSAGx has focussed on developing simple, reliable tools for identifying the virus disease complex specific to the cherry tree. Little cherry disease, caused by at least two different viruses (LChV-1 and LChV-2), has been found at several growers in Belgium. Epidemiological monitoring of the orchards ought to enable the vector, the apple mealy bug (*Phenacoccus aceris*) to be located and specific solutions proposed in the field in an attempt to check its spread.

5. Greenhouse crops

S. Steye

The Laboratory is increasingly being consulted in connection with virus disease problems in ornamental and vegetable crops, affecting for examples tomatoes, chrysanthemums and geraniums.

6.Quarantine viruses

S. Steyer

Following on from the DIAGPRO project, a fresh international cooperative link-up called PORTCHECK, instigated by CSL, York, aims to identify the PSTVd directly in the field, based on a common design using real-time PCR techniques.



Entomology

1. The sinuate pear tree borer, Agrilus sinuatus Ol. (Coleoptera Buprestidae), in fruit and ornamental nurseries

C. Fassotte

The sinuate pear tree borer, *Agrilus sinuatus* OI. (*Coleoptera Buprestidae*), causes severe damage to pear tree nurseries producing standard fruit trees and ornamental trees and shrubs. The insect larva hollows out a barely visible downwardly spiralling gallery in the intergraft which cuts the sap flow, causing partial or complete withering. Ultimately, a depressed black canker appears, marring the appearance of the tree and also offering a way in for pathogens. The borer is therefore a serious threat to nurseries and also to the plant marketing and pear growing sectors, as this pest is hard to detect in the early stage of development.

In cooperation with the Walloon Regional Horticultural Testing Centre (CEHW, Ormeignies) the flight of this pest was monitored in fruit and ornamental plant nurseries in the west of the country (Lesdain) in 2003 and 2004. Observations were carried out twice weekly in a heavily infested reference plot and comprised 80 trees in 2003 and 114 in 2004. The whole surface of each rootstock was inspected and the flight holes left by the adults counted. This allows pinpoint detection of the first borer emergences, enabling warnings to be issued to growers throughout the Region. The first emergences took place on 16th May 2003 and 21st May 2004. In 2003, no more insects emerged after 20th June, whereas in 2004, emergence continued strongly until late June, with a few isolated specimens still being observed in late July and even late August. The mass emergence predicted for 2003 did indeed take place and resulted in heavy infestations of this insect in 2005. However, the summer 2003 heatwave seems to have stimulated borer development and shortened the cycle, leading to an unexpected sizeable emergence in 2004, creating a greater risk for nurseries.

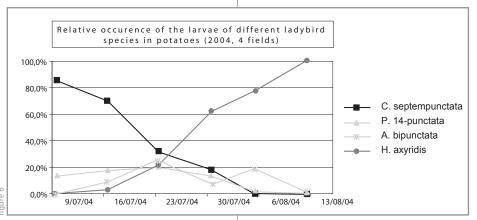
2.The sinuate pear tree borer, Agrilus sinuatus Ol. (Coleoptera Buprestidae) in pear orchards C.Fassotte

As indicated by our own observations and according to various sources, the sinuate pear tree borer, *Agrilus sinuatus* Ol. (*Coleoptera Buprestidae*), is currently spreading through the Walloon Region's active pear orchards, probably originating in the nurseries. An inspection of 1,563 pear trees in a plot in the Eghezée region in 2004 revealed a 15% trunk damage rate and evident mortality in some trees. Both old and newer exit holes were found. This plot, in the centre of the Walloon Region, will in future be used as a reference for refining our warnings to fruit growers and nurserymen in the Region.

3. The horse chestnut leaf miner, Cameraria ohridella D. & D. (Lepidoptera Gracillariidae) in ornamental nurseries.

C. Fassotte

Observations of the horse chestnut leaf miner, *Cameraria ohridella* D. & D. (*Lepidoptera Gracillariidae*), detected in 2002 in ornamental nurseries in the west of the country (Lesdain), were carried out in 2003 and 2004 jointly with the Walloon Horticultural Testing Centre (CEHW, Ormeignies). Twiceweekly monitoring of miner flight was conducted The knocking method proved more effective than emergence traps as a means of detecting first flights and siting any phytosanitary treatments as accurately as possible. Repeated pyrethrum spraying was unsuccessful in controlling P. oblongus, whereas a single Indoxacarbe® treatment was sufficient to check weevil infestation during the flowering period.



from mid-April through to late September, using synthetic pheromones not yet on the market, on the one hand, at Lesdain with four traps in 2003 and three traps in 2004, and secondly at Gembloux with just one trap. These trapping sites then served as a reference location for our preventive control warnings to Walloon growers. Three flight periods were recorded: from mid-April to mid-May, from mid-June to early August and from early August to late September, the latter two periods partly overlapping.

4. leaf-eating weevils in apple and pear orchards (Coleoptera Curculionidae) C. Fassotte, M. Lateur, B. Lefrancq

A study of leaf-eating weevils (*Coleoptera Curculionidae*) in apple and pear orchards was carried out in 2003-2004 within the framework of a student dissertation (E. Moreau). The aim was to draw up an initial inventory of weevil species occurring in orchards in the Namur region, describe the phenology of the leaf-eating species responsible for perifloral damage to fruit trees and devise a control strategy.

A systematic study based on an existing collection of 5,018 weevils collected since 1986 in the course of integrated pest management research in five orchards in the Gembloux and Namur region showed there to be 29 species, 17 of which are relevant to fruit growing. The two commonest species are *Phyllobius oblongus* L. (59%) and *Polydrosus sericeus* Schall (33%).

Weevil population checking by the knocking method in the Department's "breeding" orchard revealed *Phyllobius oblongus* to be the dominant species (80%). At the same time, five emergence traps captured this species in a similar proportion to the total, but with greater variability per trap.

5. Study of the biodiversity of specific aphid predators in ware potatoes J-P. Jansen

Ware potato sampling in 2003 highlighted the diversity of aphid predators, comprising eight species of syrphid, six species of ladybird and two species of green lacewing. *Episyrphus balteatus* DEGEER was the commonest syrphid, with *Chrysoperla kolthoffi* (NAVAS) clearly dominant among the green lacewings.

As to ladybirds, besides the prevalence of *Coccinella* septempunctata (L.), *Propylea quatuordecimpunctata* (L.) and *Adalia bipunctata* (L.), the Asian ladybird *Harmonia axyridis* PALLAS, which comes from South-East Asia, was found for the first time in Belgian field crops, in the egg, larval, nymphal and adult stages. Deliberately introduced in 1997 to control aphid populations in crops grown under cover, this species seems very well suited to Belgian conditions. Besides its spectacular proliferation in city trees, it also finds field crops a suitable environment.

Ladybirds were the subject of a specific study in 2004 to investigate in greater detail the growth of the Asian ladybird, regarded as an invasive species and a potential threat to other ladybirds. Results indicate that this species is later than native species and does not synchronise well with aphid infestations, arriving too late to be an effective means of control. It does appear, however, to be synchronised with the growth of other ladybird species and, in view of its particularly aggressive larval behaviour, it is perhaps a more likely ladybird predator than aphid predator. A question mark hangs over the survival of indigenous species in the medium term and the effectiveness of aphid control by predators in field crops.

Ecotoxicology

Drawing up selectivity lists for plant protection products with respect to beneficial fauna within the framework of developing integrated pest management specifications for field crops and market gardening

J-P. Jansen, L. Hautier, N. Viatour

(in cooperation with the FUSAGx Analytical Chemistry and Pesticide Research Unit)

This project, subsidised by FPS Public Health, involved assessing all the plant protection products authorised for use with potatoes in terms of selectivity to the three arthropods most representative of the beneficial fauna, namely an aphid parasite hymenopteron, a ladybird and a syrphid. The results of this work and ten years' observations of the phenology of beneficials in potatoes have culminated in selectivity lists based both on normal periods of product use, their intrinsic toxicity to beneficials and the exposure risk according to phenology. The lists have been completed and distributed, in French and Dutch, to farmers and to the industry. Results indicate that phytosanitary protection for the potato which is both cost-effective and safe for beneficials is indeed possible. While the choice of insecticides used may sometimes need to be adapted, as none is sufficiently selective to all three species, fungicidal protection, on the other hand, does not present any special problems based on the products currently authorised. The lists will be updated in 2005 to take account of data for recently authorised products. A similar exercise is now under way for outdoor market garden crops. Products used on carrots have been specifically investigated in the first phase with respect to the three species of beneficial previously studied and two others that are more crop-specific, namely a rove beetle, Aleochara bilineata GYLL., which is a carrot fly parasite and a predatory ground beetle, Bembidion lampros (HERBST). Initial results indicate that many insecticides, notably all those used to control the carrot fly, are highly toxic to ground beetles and rove beetles, making it difficult to include such products in integrated pest management schemes targeted at the carrot fly.

Control of water-specific pests

1. Optimising control of waterspecific pests

T. Mahaut

Work within the framework of the research agreement entitled "Optimising the control of water-specific pests (muskrats, brown rats (norway rat), coypu and black-fly)", financed by the DGRNE (Department of Non-navigable Waterways), continued in 2003 and 2004 with the main emphasis on investigating the environmental impact of the control methods employed.

With regard to muskrat control a programme of tests designed to assess the risk of secondary poisoning of predators or carrion feeders preying on muskrats that have eaten carrot bait treated with chlorophacinon showed that the risk to mammals scavenging poisoned carcases was in fact very low. Things would no doubt be different if a second-generation anticoagulant were used, as has been demonstrated by trials conducted in animal houses with difenacoum or bromadiolone, in relatively severe exposure conditions (brown rats fed on nothing but contaminated meat for three days). Chemical analysis is currently being undertaken to look for chlorophacinon residues in the organs of poisoned muskrats (in cooperation with the Pesticide Research Department) and the results should supplement the information obtained from the secondary poisoning tests. As regards the problem of blood-sucking black-fly (Diptera, Simulidae), tracking of black-fly larva populations occurring on water crowfoot in Semois has continued from March to October each year, both in the section of river usually treated with Bti (Bacillus thuringiensis var. israelensis) downstream of Herbeumont and in the untreated upstream section. Based on the results of counts carried out in early spring for several years in succession and having gradually reduced the area usually treated, it was decided not to apply a biological larvicide (Bti) in 2004. Observations will nevertheless continue in 2005 to verify that stopping treatment does not significantly affect larval population levels the following spring.

2. LUTANUIS: a cross-border project to control the muskrat

T. Mahau

In cooperation with Muskrat Trapping Department at the Water Division (DGRNE), the Department is playing an active role in the LUTANUIS project financed by the European Regional Development Fund (ERDF) as part of INTERREG III (France - Walloon Region - Vlaanderen). Launched in November 2002, this project aims to promote cooperation and exchange of information between muskrat control bodies in the 38 districts covered by the project, in France's Nord department, western Flanders and the western part of Hainaut (notably Comines-Warneton, Mouscron, Estaimpuis, Pecq and Celles). The relevant scientific expertise built up by the Department over a number of years is thus directly accessible to all the partners (CRA W being the only research institution participating in the project). The medium-term aim is to harmonise the control methods used, in compliance with the laws and regulations in force on either side of the borders. So far the project has already led to the recruitment of more trappers (including one for the Walloon districts). The Walloon Region player has also undertaken to develop a bilingual project Website.

6.2. Plant genetic resources and plant breeding

1. Wheat and spelt breeding programme A. Dekeyser

Work on winter wheat and spelt selection continued in accordance with the objectives laid down in 1991 for the creation of lines compliant with the new constraints generated by the Common Agricultural Policy. In this context maximum gross crop yield is no longer the main aim as it is no longer the key to maximum financial return. A series of experiments begun in 2002 aims to identify within varieties the characteristics suited to "low input" farming. Of seventeen winter wheat varieties grown according to four cropping methods representing conditions ranging from extensification (no treatment, low N input) to intensification (three fungicidal treatments, two applications of regulator and high nitrogen input), the varieties with the highest disease resistance, high tillering capacity and a fairly tall straw were found to offer the best financial return in the market conditions prevailing in 2003 and 2004. The Fourmi and Tourmalin varieties, both obtained from the same cross and developed by the wheat-breeding group at Gembloux, rank top in terms of gross margin. That maximum is reached by applying agri-environmental measures (200 cereal plants/m², azobil-30 U, fungicide, no regulator), even disregarding the subsidy which this cropping method attracts. The result is clear confirmation that the plant breeding choices made over ten years ago were correct.

The impact of the cropping technique on breadmaking quality was also considered, bearing in mind that nitrogen fertilisation levels are 25% lower with low input. Contrary to expectation, the protein levels measured in the 17 varieties differ by less than 0.5% between the extreme growing techniques and the W Chopin values are not significantly different. This is due to the marked reduction in input of the first nitrogen split application and the difference being carried over to the last leaf stage.

The winter of 2003 brought cold of a severity rare in the past few years. Crops suffered considerable frost damage. The Gembloux wheat and spelt lines already registered or undergoing breeding programme performed more than satisfactorily in the conditions.

Behavioural observations of several CRA W breeding lines with respect to brown rust (*Puccinia recondita*) confirm the partial and multigenic nature of the selected resistances.

In 2004, in association with the Department's Mycology Laboratory, a wheat *Fusarium* head blight susceptibility assessment was carried out on more than 200 varieties and lines. The rated symptoms will be examined with respect to mycotoxin production (DON).

During the past two years the Allié winter wheat variety, which comes into the "low input" category, was admitted for inclusion on the Belgian list of varieties. This is a forage variety which is resistant to leaf and head diseases and to lodging. A further five lines were submitted for registration in 2003. Three of them were accepted for a second year of experimentation. One of these lines is

characterised by its value for breadmaking in addition to its yield potential and disease resistance. This line has a Zeleny index of 48, a Zeleny/protein ratio of over 4 and a W Chopin of 250. This clearly points up the move in breeding programme towards globalisation of characteristics, including those with a complex determinism.

The Stone spelt variety entered the Belgian varieties list in 2003. Stone is characterised by high disease resistance, lodging resistance, a breadmaking quality comparable to the reference variety, Rouquin, and above all a hulling rate at threshing under 1%, making this the most "spelt" line in the current range.

Cautious use of old spelt germplasm has continued by means of retrocrossing.



2. Winter barley breeding programme *A. Barbier*

Breeding work has continued with the aim of developing winter barley varieties (2 and 6 rowed) with resistance to lodging and to the main fungal and viral diseases. The Department has acquired expertise in assessing the field resistance of barley varieties to viral diseases such as mosaic diseases (soil-borne *Barley yellow mosaic virus*, BaYMV-1 and -2 and *Barley mild mosaic virus*, BaMMV) as well as the aphid-transmitted *Barley yellow dwarf virus*, BYDV. Within this framework the Department is involved in the Winter Barley Genetic Resources working group (RGOH), coordinated by GEVES (France).

3. Genetic resources and disease resistance in local fruit trees M. Lateur, I. Villette

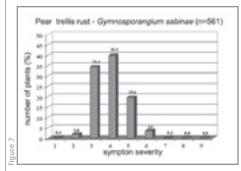
Work on safeguarding our local fruit tree genetic resources has been on-going since 1975, with considerable input from a keen general public. Over the past two years the Department has attended 50 exhibitions and fairs and given 22 public talks on the subject of old varieties, why they matter and how they can be developed.

Our collections currently number 3077 introductions, comprising 1,568 apple trees, 996 pear trees, 341 plum trees, 76 cherry trees, 47 peach trees and 49 vines. This is one of the most important collections in Europe in terms especially of the diversity and originality of the material (varieties mainly of 'farm' origin) and above all, the extensive evaluation, description and development that have been undertaken with respect to our fruit-growing heritage. Apple and pear varieties from campaigns or old collections in untreated orchards are examined each year to assess their disease resistance and main agricultural characteristics. Assessment of genetic resources enables the material to be identified, on the one hand, and the diversity of the characteristics to be highlighted, while at the same time serving as a basis for selection of promising varieties for use either directly, through specific development, or indirectly, as parents in breeding programmes. All the observations made are fed into a database which operates as a collection management and rationalisation tool. The intention is to double it by setting up a Walloon network of conservation orchards, to ensure the survival of this unique collection.

To cope with the thorny problem posed by the many alternative names for varieties, a variety family grouping program has been written as part of the European database of pear (Pyrus) genetic resources (ECP-GR/IPGRI) managed by the Department in cooperation with the CRA W Biometrics, Data Management and Agrometeorology Section. The last few years have seen a significant resurgence of pear rust (Gymnosporangium sabinae). The following figure gives the 2004 breakdown of the 561 pear tree introductions by pear rust susceptibility class, showing that sources of resistance are very rare but so are highly susceptible varieties. Varieties in classes 1 to 4 are easy to grow without fungicidal treatment, thus providing a big selection basis for other characteristics.

In contrast, 2004 was marked by a sizeable epidemic of pear tree fire blight (*Erwinia amylovora*), the severest in our conditions since 1991.

Among commercial nurseries the range of original varieties ('RGF' varieties) that are disease-resistant and diversified is expanding all the time. Around ten pear varieties have been identified as being of interest and are now the subject of further research with a view to their future distribution through our network of commercial nurserymen. Arrangements have been made with some nurserymen to create a new "custom grafting" sector for the main varieties local to the Walloon Region that are not in the catalogues.



4. Development of fruit genetic resources for industry and small and medium enterprises.

I. Villette, M. Lateur

The agreement with the DGTRE expired in the second half of 2002 but analysis of results continued into 2003. The aim of the project was to identify, from the diversity of fruit genetic resources in the collection, those varieties with good disease resistance and sufficient productivity to be of interest for further development by industry and small and medium enterprises in the Walloon Region. The three target sectors were commercial nurseries, table fruit tree growing and the processing industry. Among commercial nurseries the work involved testing the "microsatellite" molecular labelling method with the ultimate aim of checking the variety identity of plants sold at nurseries. Work also focussed on identifying some pear tree varieties ('Canada', "Sans Etiquette", 'Angora' (Virus Free), Pv-RGF30 and Pv-RGF03) as potential replacements for 'Carisi' as an interstock that would also be less susceptible to the sinuate pear tree borer (Agrilus sinuatus). These varieties will now go forward to a larger-scale trial to establish their behaviour, resistance and graft compatibility with the main varieties on the market.

Another aspect of the research carried out under this agreement comprised determining analytical methods for the nutritional value of fruit. Being armed with facts on the nutritional quality of old varieties is an additional weapon for their development with respect to the consumer, grower and processor, adding diversity to the varieties on sale and opening up new markets. Besides analysing the fibre and vitamin C content, flavonoids were studied. Of the sample of varieties selected for this trial, 'Judeline', 'Jonagold' and 'Radoux^{RGF'} contain quercitrin and its glycosylated forms at levels over 600µg/g dry matter, compared to under 400µg/g DM in other varieties. These substances are important antioxidants and have "anti-cancer" properties. They occur mainly in the epidermis of the fruit.

The 'Président H. Van Dievoet^{RGF'} variety stood out from the other varieties tested in a stewing trial. This variety has very good technological and taste properties, as demonstrated by cooking tests and tastings using sensorial analysis methods. It has the further advantage of being disease resistant, enabling it to be grown with little or no fungicide, which can only be good for the environment. Finally, a survey of apple juice producers confirmed that smaller firms are keen to develop new products from the region's traditional fruit resources and to put in place quality procedures in line with market requirements.

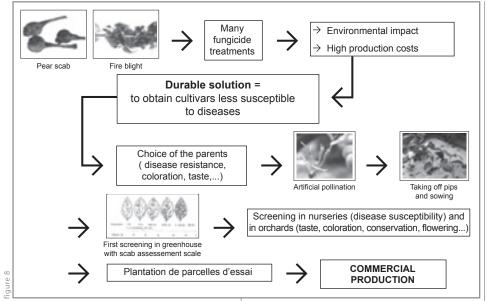
5. INTERREG III – Cross-border fruit heritage and biodiversity

I. Villette, M. Lateur

The Walloon part of this project, which is financed by the European Regional Development Fund (ERDF), got under way in early 2004 and is part of the European INTERREG III programme for the management and enhancement of fruit biodiversity in the Franco-Walloon Region. The project makes official the partnership with the Regional Genetic Resources Centre at Villeneuve-d'Ascq, France, part of the Regional Nature Reserves.

Work during 2004 concentrated first of all on creating the structure of the unique database for more efficient management of the cross-border partners' respective collections. The database has been built in cooperation with the CRA W Biometrics, Data Management and Agrometeorology Section and is intended to improve traceability of all the introductions in the collection, to optimise the use of past and future knowledge and to rationalise the collections.

A new pear tree breeding programme is now under way, within the context of restricted variety diversity on the market, aiming to create new commercial varieties that look different and are not susceptible to disease. Preliminary experiments involving artificial inoculation of clonal seeds from several varieties were carried out using pear scab (Venturia pirina) spores. The populations obtained from the seeds were then classified according to scab susceptibility as a guide to selection of future parents based on their resistance characteristic transmission rate. In the second stage, a series of 26 crosses were made in the spring of 2004 with the aim of obtaining novel new varieties with good disease resistance. This work involved fertilizing 6,500 flowers by hand. Just under 3,700 pips were taken from the resulting fruit and the plantlets grown from the seeds will first of all undergo scab susceptibility testing before selection on the basis of productivity, novelty of the fruit, taste and storage properties. The work will then be handed over to the Regional Genetic Resources Centre at Villeneuve-d'Ascq for nursery assessments.



Pear tree breeding programme designed for low susceptibility to disease, principally pear scab (Venturia pirina).

6. Development of new varieties of pome fruits suited to the Walloon Region in line with quality arboriculture M. Lateur and 'X'

The aims of this project are, firstly, to create new, disease resistant commercial apple varieties to liven up the current range, which has declined markedly in recent years (with 'Jonagold' and its mutants having cornered over 70% of the market), and secondly, to develop an experimental network in the Walloon Region for new varieties of apples and pears to suit our economic conditions. The idea is to develop a multi-tiered structure for objective experimentation with the Department's new accessions, comparing them with new varieties on the market. The research is directed at creating commercial varieties with good taste quality that are suitable for commercial arboriculture in line with the integrated pest management approach and that have either lasting resistance or low susceptibility to scab and powdery mildew. so that the resulting level of tolerance allows the number of fungicidal treatments to be substantially reduced. Any attempt to create totally diseaseresistant varieties for growing with no treatment at all would, however, be merely utopian and misguided. The exclusive use of total resistance sources like the resistance gene Vf from Malus floribunda 821 has in fact illustrated the limits of this strategy, with a new strain of scab managing to bypass this specific resistance mechanism. Despite the ending of subsidies for the apple tree breeding project in 2002 and 2003, a considerable amount of work was nevertheless done, comprising artificial pollination of 7,936 flowers and removal of 776 pips, sowing of 28,298 pips obtained from artificial pollination between 1999 and 2003 and early scab susceptibility testing by inoculating plantlets, selection and grafting of 493 elites from 5,980 seeded plants, further development of an early canker inoculation test and, finally, testing the sustainability of resistance to a new strain of scab in potted plants.

This project has been funded by the DGA Research, Development and Quality Division since 2004. Seventy-four crossings have been done, involving hand pollination of 21,031 flowers, not counting the 2,095 flowers pollinated as part of a methodological crossing technique improvement test; the phenological stage and flowering intensity were recorded for 4,246 elite and evaluation hybrids; and, lastly, harvesting and tasting and storage tests were performed on over 1,800 hybrids planted in a selection orchard. In the second part of this project, seven new pear varieties and 17 new apple varieties from all over the world which looked promising for our growing conditions have been planted along with 17 hybrids from our own breeding programme in three orchards, one of which is at the Profruit Centre at Cerhexe-Heuseux. All of this material underwent an initial evaluation in 2004.



Bypassing Vf gene scab resistance at Gembloux

7. Production of high quality, disease resistant apples within the framework of sustainable agriculture

(High-quality Disease Resistant Apples for a Sustainable agriculture – HiDRAS, projet européen : QLK5-CT-2002-01492, Directorate - General for Research - Quality of life and Living Resources Management Programme M. Lateur, A. Antofie, A. Rondia & R. Oger

The HiDRAS project comprises five main objectives: (1) Identifying genetic factors controlling fruit quality and sustainable disease resistance with the general aim of reducing fungicide use; (2) Equipping broaders with new molecular tools

(2) Equipping breeders with new molecular tools

for improving breeding techniques based on the use of efficient QTL (Quantitative Traits Loci); (3) Making European growers more competitive with respect to the non-European countries which currently hold a large share of the market, especially in summer when European produce has to compete in quality terms with imports from the southern hemisphere; (4) Improving consumer choice and assessing consumer preferences with regard to apples as part of a healthy diet and (5) Enhancing the status of European research in the genetic sector by finding new ways of reducing the use of plant protection products in commercial orchards.

The results of the HiDRAS project should include the development of pedigree creation and QTL identification software. These software programs will be based on the use of a central database comprising all the phenotype and genome data The HiDRAS project is coordinated by the University of Milan (Italy) and brings together ten partners from seven EU countries plus Switzerland: DCA-BO (Italy), PRI (Netherlands), INRA (France), ETH-FAW (Switzerland), HRI (UK), BAZ (Germany), CRA-W and FUSAGx (Belgium), SGGW and RIPF (Poland). CRA-W's role concerns phenotypical evaluation of the different quality traits of the fruit of a set of cultivars used as parents in breeding programmes. The work involves carrying out sensorial analysis and conventional chemical and physical analysis throughout the fruit storage period. The ascorbic acid (Vitamin C) and total polyphenol content is analysed with the assistance of FUSAGx (Prof.G. Lognay). A sample of disease resistant varieties was compared to standard varieties in order to establish the quality parameters that rank highest among European consumers. CRA-W (R. Oger and A. Antofie) is also responsible for creating the structure of the central database and for database management. The intention is to build a specific database for apple breeders to facilitate management of the mass of genetic and phenotypical information and also to make better use of the wide spectrum of varieties stored in European germplasm collections.

8. Study of the genetic diversity of the wild apple tree: scope for conservation and use of genetic resources

M. Lateur, A. Antofie, B. Watillon & O. Arezki

The wild apple tree (*Malus sylvestris* Mill.) is a native species scattered throughout Western and Central Europe. Due to the loss of suitable habitats it is now regarded as an endangered species, especially in Flanders where it now only grows in a few very small areas. The idea is to set up a long-term conservation programme to guarantee the continuing existence of this species and to maintain a big source of genetic variability that may be useful for forestry management and cultivated apple breeding.

This research project is financed by the Scientific Policy Federal Public Programming Service (SPP). Six institutions are taking part: four Dutch-speaking (KUL, CLO-DvP, IBW, NBS) and two in the Walloon Region (CRA-W and CRNFB), the latter two focussing primarily on regional material.

The main aims are: (1) Assessment of the genetic



diversity of the wild apple tree in Belgium and apple tree cultivars using a combination of conventional techniques and molecular labelling: (2) Assessment of the extent of hybridization between the wild species and cultivars and development of tools to distinguish pure and hybrid varieties; (3) Establishing management principles to conserve the wild apple tree in its natural environment (conservation in situ) and in the form of plantations (conservation ex situ); (4) Drawing up development plans to optimise management of the genetic variability of cultivar collections. At the time of writing, all the forest districts managed by the WR Department of Nature and Forests have been covered with the assistance of the forestry staff, enabling us to identify and describe 400 trees in the Walloon Region. Tree numbers in Flanders are lower at only 200 trees. All this material is listed, georeferenced by GPS and described in situ. Of this set of 400 trees, 271 have already been reproduced by grafting and the remaining 129 are currently being worked on. These grafted plants are choice material for the creation of a seed orchard representative of the intra-species variability of this species in the Walloon Region. Alongside this study of Malus sylvestris, work is also going on into Belgian cultivated apple tree germplasms (M. domestica), in terms of phenotypical and genetic diversity. A database containing all this information is currently being built and will be partly accessible by the end of this contract.



Distribution of the wild apple tree (Malus sylvestris Mill.) in Belgium (2004)

7. SERVICE ACTIVITIES

5. Schmitz, A. Chandelier, M. Lateur, M. Cavelier

In 2003-2004, the Mycology Laboratory performed over 600 analyses , 40% of them for commercial concerns and 60% for private customers. Within the framework of our working relationship with cider makers Stassen, around ten consultations took place in commercial cider apple orchards, prompted by a significant new epidemic of silverleaf caused by the basidiomycete fungus, *Chondrostereum purpureum*. This disease has also occurred to an alarming extent in several tens of commercial orchards growing 'Conference' and 'Concorde' pears.

Increasingly frequent attacks by wood-destroying fungi, in particular Armillaria mellea and Meripilus giganteus, have occurred throughout the period. Special attention has therefore been focussed on urban arboriculture techniques for preventing, assessing and effectively managing problems of this kind. Vascular wilt caused by Verticillium dahliae has also been observed across a wide range of host plants (woody and non-woody ornamental plants and small fruit crops). Root rot caused by different species of Phytophthora or Fusarium is also prevalent. Five new data sheets describing fungal diseases affecting ornamental and forestry nurseries have been produced for reference by nurserymen (Botrytis cinerea in spruce, Guignardia aesculi in chestnut trees and damping-off, Phytophthora ramorum and Verticillium dahliae in ornamental plants).

Since 2003 the Laboratory has been taking part in the survey launched by the Plant Protection Department (Federal Food Chain Safety Agency, AFSCA) aimed at detecting *P. ramorum* in ornamental nurseries and parks. This pathogen is detected by real-time PCR. More than 80 samples were analysed during the two years, mainly rhododendron and viburnum. Moreover 24 analyses were performed for detecting *P. ramorum* and/or *Fusarium* as well as viruses in hop root (7 reports).

7.2. Mycotoxins

P. Detrixhe, A. Chandelier

The ELISA technique was used to determine the vomitoxin content in 915 wheat grain samples in the framework of different monitoring contracts with the private sector or trials conducted by the Pesticide Research Department.

7.3. Virology

S. Steyer

The Department is the AFSCA reference laboratory aimed at detecting plant quarantine viruses. Therefore the following analyses were performed during the last biennial: 471 analyses for the detection of Pepino mosaic virus (PepMV) on leaves and fruits of tomato (77 reports), 110 analyses for the detection of rhizomania (Beet necrotic yellow vein virus, BNYVV) in potato soils (16 reports), 64 analyses for the detection of the Tomato spotted wilt virus (TSWV) on chrysanthemum leaves (9 reports), 49 analyses for the detection of diverse ornamental plant viruses (15 reports), 413 analyses for the detection of Sharka (Plum pox virus, PPV) on leaves of diverse Prunus (45 reports), 186 analyses for the detection of Potato spindle tuber viroid (PSTVd) on potato leaves (32 reports).

7.4. Entomology

C. Fassotte

The entomology advisory service received some 150 requests for insect identification from private individuals and commercial operators during the two-year period. Most of these relate to ornamental and fruit plant pests in private gardens and planted public areas (74 to 78%), the remainder being arthropods in private homes or storage premises (22 to 26%).

For the ornamental sector 2003 was marked by spectacular invasions of the spindle ermine moth (*Yponomeuta cagnagella* Hübner), occurring mainly in the province of Hainaut, along motorways and in private gardens. Some unusual pests appeared, such as the leopard moth (*Zeuzera pyrina* L.) on ash and bay tree jumping plant lice (*Trioza alacris* Flor) on *Laurus nobilis*. In fruit trees, eriophyids (*Phytoptus pyri* Pgst. in pear trees and P. similis Nalepa in plum trees) were the main source of concern for private gardeners.

In 2004, huge clusters of the mycetophagous Orange ladybird Halysia sedecimguttata L. were reported on ash trees at a nursery. Several enquiries concerned a pest never seen before, the pine adelges (*Pineus strobi* Hartig) on *Pinus strobus*. Among domestic arthropods, the biscuit beetle (*Stegobium paniceum* L.) still dominates in domestic premises, to the detriment of starchy food and woodwork, followed by moths. However, the heatwave year 2003 was marked by a proliferation of face flies (*Musca autumnalis* De Geer), massing on house gables in autumn and spring.

7.5. Ecotoxicology

J-P. Jansen, T. Mahaut

Forty-two expert reports were produced on behalf of the Ecotoxicology Subcommittee (FPS Public Health, Food Chain Safety and Environment), the body responsible for considering applications for pesticide authorisation for agricultural use (unintentional effects of pesticides on birds, wild mammals and useful arthropods).

7.6. GLP studies

The laboratory of ecotoxicology has been certified GLP for carrying out studies on useful arthropods. During the last biennial, 13 studies were performed following the Good Laboratory Practices (OECD).

7.7. Warnings J-P. Jansen, S. Steyer

In cooperation with CADCO and PAMESEB, in the framework of the warnings sent to farmers, populations of cereals and potato aphids were monitored in a number of reference fields to avoid unnecessary insecticide treatment and to target more precisely the periods when the treatment is necessary.

7.8. Rodent control

Within the framework of rodenticide authorisation procedures 16 trials designed to assess the effectiveness of new products against brown rats and mice were conducted in individual cages and pens on behalf of various Belgian and foreign companies. This work is carried out in cooperation with the Pesticide Research Department, which is

specifically responsible for chemical analyses and appetence tests to study product behaviour over time (ageing tests).

7.9. Maintenance of lines and varieties A. Dekeyser

In connection with distribution of winter wheat and spelt varieties registered by the Department, lines are maintained by progeny selection. Between 400 and 1,000 ear lines are sown and monitored, according to the stage of variety development. The seeds produced are made available to the Crop Production Department, which propagates them for distribution to traders and farmers.

7.10. Safeguarding and developing fruit genetic resources M. Lateur. I. Villette

Distribution of budwood from 17 old "RGF" fruit varieties selected for disease resistance and novelty is continuing, with steady demand from commercial nurserymen. Parent stocks of these varieties have been grafted in order to start a 'nuclear stock orchard' in cooperation with the Walloon Horticultural Testing Centre (CEHW). The reference plot contains all the varieties of apples and pears marketed by all the nurseries in Belgium, so as to guarantee the identity of nursery-grown material.

Our advice service to assist with identification of fruit varieties is used by commercial nurserymen, regional natural heritage associations and a number of private individuals. A total of 1,534 fruit samples have been analysed in connection with 373 different cases.

The advice service linked to the setting up and management of a network of conservation and teaching orchards is developing so rapidly that it will be treated as a project in its own right.



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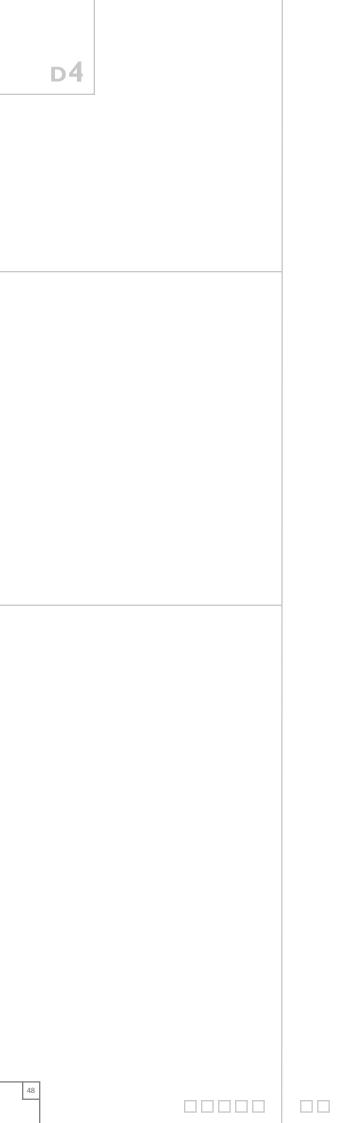
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PESTICIDES RESEARCH



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Department PESTICIDES RESEARCH



1. MISSION STATEMENT

. Research into the chemical and physicochemical characteristics of pesticides, their evolution in plant products and their environmental impact. Development and validation of analytical methods for active substances, impurities, formulations and pesticide residues. Involvement in establishing international specifications to guarantee the quality of products put on the markets.

. Study of the agricultural characteristics of new or little-known pesticides and development of reliable, economical and environmentally compatible crop protection methods suited to the specific features of Belgian agriculture, horticulture and forestry.

2. SCIENTIFIC PERSONNEL

5 permanent staff and 10 contract staff

2.1. Head of Department

P. Meeùs, Inspecteur général scientifique

2.2. Scientists

- F. Ansseau, Attaché
- A. Bernes, Attaché
- F. Cors, Attaché scientifique (MA) M. De Proft, Directeur scientifique
- B. De Ryckel, Attaché
- P. De Vos, Attaché scientifique
- M. Galoux, Inspecteur général scientifique, Head of Section
- N. Ducat, Attaché scientifique
- F. Henriet, Attaché scientifique
- J.-M. Moreau, Attaché scientifique
- O. Pigeon, Attaché scientifique
- J.-F. Salembier, Attaché scientifique (until 1/8/2004)
- S. Vandecandelaere, Attaché
- B. Weickmans, Attaché scientifique

3. NATIONAL REPRESENTATION

- Agence Fédérale pour la Sécurité de la chaine Alimentaire (AFSCA)
- Commission d'Ethique en Expérimentation animale
- Comité d'agréation des pesticides à usage agricole belge
- Comité Régional Phyto (WR)
- Centre Agricole de Développement des cultures de Céréales et des Oléo-protéagineux (CADCO)
- Centre Agricole Betterave-Chicorée (CABC) - Centre de Recherches Vétérinaires et Agro-
- chimiques (CERVA- VAR), Tervuren
- Centrum voor Landbouwkundig Onderzoek (CLO), Gent
- Comité scientifique plantes transgéniques du Conseil de Biosécurité
- Conseil Supérieur d'Hygiène
- Comité Scientifique de l'Agence Fédérale pour la Sécurité de la chaîne Alimentaire
- Faculté Universitaire des Sciences Agronomiques
 Gembloux (FUSAGx) (Gembloux Agricultural University)
- FIWAP

- Institut Royal Belge pour l'Amélioration de la Betterave (IRBAB)
- Institut Scientifique de Santé Publique « Louis Pasteur », Bruxelles
- Katholiek Universiteit van Leuven (KUL), Laboratorium voot Fytopathologie en Plantenbescherming,
- Leuven - Koninklijk Opzoekingsstation van Gorsem,
- Sint-Truiden - Laboratoire Fédéral de l'Alimentation, Liège
- Laboratoire Federal de l'Alimentation - LISEC – Genk
- LISEC Genk
- Provinciaal Proefcentrum- en Voorlichtingscentrum voor Land- and Tuinbouw, Rumbeke
- Provinciaal Proefcentrum voor de Groenteteelt, Kruishoutem
- Rijksuniversiteit Gent (RUG), Laboratorium voor Fytopharmacie, Gent
- Service Public Fédéral Santé Publique, Sécurité de la Chaîne Alimentaire et Environnement
- Université Catholique de Louvain (UCL), Faculté d'Ingénierie biologique, agronomique et environnementale, Louvain-la-Neuve
- Université Libre de Bruxelles (ULB): Laboratoire de Biologie Animale et Cellulaire

4. INTERNATIONAL REPRESENTATION

- Agroblu Srl, Italy
- Agrisearch UK Ltd., UK
- Agrolab, Greece
- Agro Research International B.V., Netherlands
- Association Française de Protection des Plantes (AFPP)
- Association of Official Analytical Chemists (AOAC)
- Center for disease control (CDC), Atlanta, USA
- Comité européen de normalisation (CEN)
- Central Science Laboratory (CSL), York, UK
- Centre de Coopération Internationale en Recherches Agronomiques pour le Développement
- (CIRAD), Montpellier, France - Centre Technique Interprofessionnel de la Vigne
- et du Vin (ITV), Epernay, France
- Collaborative International Pesticides Analytical Council (CIPAC), UK
- Codex Alimentarius pour les Résidus de Pesticides - Den Haag - Netherlands
- Europe Africa Caribbean Pacific Liaison
- Committee (COLEACP), Brussels, Belgium
- DEFITRACES Brindas France - Food and Agriculture Organisation of the United
- Nations (FAO)
- Deutsche Gesellschaft für Technische Zusammenarbeit GmbH, (GTZ) Eschborn, Germany
- Hellafarm S.A., Greece - Huntington Life Sciences – Eye Research Centre
- Suffolk UK
- Institut de Recherche pour le Développement, laboratoire des Insectes Nuisibles (IRD-LIN),
- Montpellier, France - International Institute for Beet Research (IIRB)
- Institut national de la Recherche agronomique (*INRA*), Paris, France
- Institut Tropical Suisse, Geneva, Switzerland - John Hopkins University, Baltimore, Maryland, USA
- Laboratoire interrégional de la Direction de la Concurrence, de la Consommation et de la Répression des Fraudes, Massy, France
- Ministry of Agriculture, Fisheries and Food (MAFF), York, UK

- Pesticide Safety Directorate (PSD) York UK
 Plant Protection and Soil Conservation Service, Hungary
- PROMO-VERT, France
- European and Mediterranean Plant Protection Organization (EPPO), France
- World Health Organisation (WHO) Expert and
- Official Collaborating Centre
- Recerca Agricola, Spain
- SPF GAB Biotechnologie, Germany
 - SPF GAB Italia srl Poggio Renatico Italy -TNO prins Mauritzs Laboratory – Rijswijk, Netherlands; Organisation for Applied Scientific Research - European Union (EU) – Directive 91/414/EEC
 - Residues and active substances working groups
 UNICEF Copenhagen, Denmark
- World Health Organisation Pesticide Evaluation Scheme (WHOPES), Geneva, Switzerland

5. RESEARCH TOPICS

Like other areas of agricultural sciences, pesticide research has changed in the last few years. From being primarily concerned with improving yield, the focus has now shifted to take account of society's expectations in terms of quality and food safety.

Also, in view of the economic context, errors or waste adversely affecting farm profitability are rare nowadays and so the industry itself is keen to see production cost reduction techniques introduced.

To this end it is essential to develop crop protection methods that are less aggressive towards the environment, economically profitable for users and that guarantee quality products for the consumer. In response to this challenge the Department's chemists and biologists are pooling their efforts to improve their knowledge of the chemical and biological properties of the new products developed by the pesticide industry. These products have to be described and their potential assessed and to do this, we need to devise, adapt, develop and validate suitable analytical and experimental methods for use in the field.

The complementary nature of the two disciplines also allows programmes to be undertaken in such areas as the distribution and fate of active substances, residue analysis (particularly in smallscale crops of little economic significance to the industry), improving the quality of treatments and drafting treatment quality standards.

To ensure the objective, independent traceability and integrity of its results, the Department has put in place a "Quality" initiative.

The Department achieved GLP (Good Laboratory Practice) certification in 1994 and ISO 17025 certification in 2002. Certification brings the Department recognition and opens the way to collaboration with authorities, industry and the major international organisations. One such example is recognition of the Department as an official WHO Collaborating Centre for chemical analysis of pesticides for public health and agricultural use.

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6. RESEARCH REPORTS

6.1. Chemistry and physicochemistry of plant protection products

Development of analytical methods A. Bernes, B. de Ryckel, O. Pigeon, M. Galoux

The ever-increasing pressure brought to bear on agriculture and specifically on environmental control of chemical inputs requires reliable, reproducible, validated analytical methods to be developed that meet at least the international criteria laid down by the European Union (EU), the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA).

Validation covers not only the analytical methods for active substances and their representative impurities but also the formulated products. The specificity, linearity, repeatability and recovery of the methods used have accordingly been determined in respect of a number of active substances and different types of formulation. The laboratory has taken part in collaborative studies of d-allethrin (TC, MV), bioallethrin (TC, MV), S-bioallethrin (TC), deltamethrin (TC, SC, WG, EC, TA), dinetofuran (TC, WP, SG), esbiothrin (TC, MV), glyphosate (TC, SL), parathion-methyl (CS), d-phenothrin (TC, AE), prallethrin (TC, AE), novaluron (TC, EC) and SZI-121 (TC, SC). These studies have not only resulted in approval and publication of these methods by CIPAC but have also put the laboratory's work on a very good footing compared to others worldwide.

In collaboration with DAPA, German standardisation organisation, and NEGAG, the Netherlands counterpart, new methods of physico-chemical analysis for pourability and rinsing of SC and emulsion stability for EC, EW and ME have been developed. The laboratory is also involved in the analysis of formulations containing more than one active substance (possibly in various forms), their impurities, isomers and enantiomers, additives, etc.

New types of formulation (soluble bags {*SB*}, suspo-emulsions {*SE*}, emulsifiable granules {*EG*}, etc.) are also the focus of detailed research.



Phytotoxicity testing of new formulations under controlled conditions

Preparation of physico-chemical dossiers for registration

A. Bernes, B. de Ryckel, O. Pigeon, M. Galoux

Due to its involvement in drafting the European Directive, its contribution to standardisation of analytical methods (CIPAC) and its work within the FAO and WHO in connection with specifications and hence product quality; but also in view of the OECD "GLP" quality system introduced in 1994, confirmed in 2004, the Department is an obvious first contact for pesticide manufacturers who have produced active substances or formulations, under or out of patent, and is one of the leading laboratories in this field today.

The Department uses standardised methods to determine the active substance content in technical compounds (TC) and formulations, and manufacturing impurities, or validated analytical methods on new formulations, mainly by gas and liquid chromatography.

The Department also evaluates the physicochemical characteristics of formulations according to CIPAC published or approved methods and recommendations contained in the European Directive.

The principal methods are: appearance, water content, flash point, acidity, alkalinity, pH, viscosity, surface tension, density, wettability, foaming, suspension stability, emulsion stability, spontaneity of dispersion, stability of dilution, sieving (dry and wet), particle size distribution (sieving or laser diffraction), pourability, rinsability, physical and/or chemical compatibility, long-term stability (6 months, 1, 2, 3 years, etc.) at ambient temperature, accelerated storage (14 days/54°C, 8 weeks/40°C, 12 weeks/35°C, 18 weeks/30°C), cold stability and thermal cycles.

Establishing of specifications M. Galoux, O. Pigeon, A. Bernes, B. de Ryckel

The concept of appropriate quality criteria to ensure the effectiveness and harmlessness of chemicals entering the environment gave rise to the drafting of specifications for pesticides used in agriculture and public health. Over time, these specifications have become essential tools for the international trade in pesticides and their approval by states which have put relevant legislation in place.

Belgium and the Belgian Registration Committee, the states of the European Union and the United States use these specifications today within the framework of their pesticide registration (authorisation) legislation. Many other countries that do not as yet have approval systems in operation also use these specifications for pesticide quality control purposes.

On the basis of its experience in analysing pesticides for agricultural and non-agricultural uses, the Pesticide Research Department has for over 25 years been represented on the group of experts (FAO/WHO) appointed to evaluate and establish specifications. These specifications have been common to the FAO and WHO since 2002. In connection with the new (2002) specification establishing procedure (which now takes account not only of the active substance but also of the manufacturing process), the Department has been asked to evaluate active substances such as dicamba (herbicide) and deltamethrin (insecticide) and to draw up specifications for technical compounds (TC) and formulations such as water-soluble liquids (SL), wettable powders (WP), suspension concentrates (SC), water dispersible granules and tablets (WG - WT), emulsifiable concentrates and granules (EC - EG) and ultra low volume liquids (UL). The Department has also been involved in evaluating and drafting specifications for active substances and formulations containing: azimsulfuron, bifenthrin, chlorothalonil, chlorpyrifos, cymoxanil, cyphenothrin, diflubenzuron, dimethoate, fenthion, icaridin, imidacloprid, iprodione, malathion, novaluron, paraquat, picloram, pirimiphos-methyl, prochloraz, propanil, propoxur and transfluthrin.

Deltamethrin marketed by generic manufacturers will be evaluated in 2005 to ascertain whether these technical compounds are equivalent to the former out of patent product with its well-known toxicological properties.

As an official WHO Collaborating Centre, and in collaboration with WHOPES, the Department was involved in harmonising the criteria for FAO and WHO specifications throught JMPS (Joint Meeting on Pesticide Specifications), a counterpart to JMPR (FAO-WHO collaboration), better known for many years in the area of pesticide residues.

6.2. Evolution of plant protection products in agricultural crops, treated substrates and the environment

Study of pesticide residues in fruits, vegetables and cereals O. Pigeon, N. Ducat, P. De Vos, S. Vandecandelaere, M. Galoux

1. Development and validation of analytical methods

In the course of the past two years over 25 analytical methods have been developed for determining pesticide residues in fruits, vegetables and cereals. These methods cover a whole series of insecticides, acaricides, fungicides, herbicides and growth regulators in a variety of crops: apples, grapes, plums, kiwi fruit, clementines, cabbages, Brussels sprouts, cauliflowers, leeks, spinach, celery, parsley, radishes, mushrooms, onions, potatoes, maize, wheat, industrial chicory, sunflowers, ground nuts and cocoa beans. The methods were optimised at each stage of the analysis: pesticide extraction using a suitable solvent, purification of the extract [filtration, centrifuging, liquid-liquid partition, solid phase extraction (SPE), gel permeation chromatography (GPC)] and chromatographic determination: gas chromatography-mass spectrometry (GC-MS), gas chromatography with electron capture detection (GC-ECD), gas chromatography with specific nitrogen or phosphorus detection (GC-NPD) and high performance liquid chromatography coupled with mass spectrometry detection (LC-MS/MS) or diode array detection (HPLC-DAD).

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The analytical methods were then successfully validated for specificity, linearity of response, reproducibility (repeatability), accuracy (recovery yields), limit of detection (LOD), limit of quantification (LOQ) and pesticide stability in solvents and extracts.

Liquid coupled mass spectrometry (LC-MS/MS), an advanced technique recently introduced in the laboratory, has led to the development of analytical methods for active substances that are particularly hard to identify and quantify. Examples include determination of sulfonylurea and triazolopyrimidin herbicides in maize, residues of asulam and its metabolites in celeriac and spinach and carbamate insecticide residues in leeks. Due to its sensitivity and specificity this technique opens up interesting new prospects for the future, notably for the newer herbicides and active substances which because of their physico-chemical nature are difficult to analyse by gas chromatography. A new liquid-liquid extraction (SSLLE) purification technique has also been implemented by the laboratory. This works by loading the (aqueous) extract on a diatomeous earth column and eluting the pesticides with a non water-miscible solvent. Compared to conventional liquid-liquid extraction in a separating funnel, the new method offers greater precision and more efficient extract purification. It also has the advantages of being faster and using less solvent. The new technique has been used for triazole and strobilurin residues in cereals and celery and within the framework of a multi-residue analysis method using GC-MS to determine pesticide residues in eluates and substrates of biofilters.

As part of the "Walloon Region potato quality audit", project run by the CRA-W Farming Systems Section, a GC-NPD multi-residue analytical method has been developed and validated for determination of chlorpropham and fungicide residues, insecticides and nematicides residues in potatoes.

2. Residues trials for registration

In 2003 and 2004, around 150 pesticide residue trials were carried out to obtain the scientific data needed for registration and to work out maximum residue limits (MRL's), notably within the framework of the Plant Protection Product Directive (91/414/EEC). Tests have been conducted with various insecticides, acaricides, fungicides, herbicides and growth regulators in fruits, vegetables and cereal crops. Some of these tests were carried out entirely (from test plot selection to treatment, harvesting and finally, laboratory testing) by the CRA-W Pesticides Research Department, which notably combines both agronomic and chemical aspects of pesticide research. The rest were carried out in cooperation with other European research institutes in Belgium, France, Germany, United Kingdom, Netherlands, Italy, Spain, Greece and Hungary. The Department has thus come to specialise in running multi-site residue trials with the field and laboratory components being carried out at two different institutions, in accordance with the application of the OECD principles of Good Laboratory Practices (GLP) to the organisation and management of multi-site studies. Tests

have been carried out on field crops or "major" crops, such as wheat, maize, sunflowers, grapes, apples, beans, carrots, lettuce, tomatoes, peppers, cucumbers, melons, cabbages, leeks, onions, plums, clementines, etc. and also on small-scale or "minor" crops, such as celery, corn salad, radishes, mushrooms, spinach, parsley, chives, raspberries, kiwi fruit, and so on.

Study of the quality of seed treatments O. Pigeon, P. De Vos, P. Dardenne, G. Sinnaeve, M. De Proft, B. de Ryckel, A. Bernes

Good quality seed treatment with pesticides requires, on the one hand, the average active substance concentration on the seed to be as close as possible to the target rate (registred rate) and, on the other hand, the active substance to be evenly distributed over all the seeds in a lot. Chromatographic methods (gas chromatography and high performance liquid chromatography) can be considered benchmark methods for determining (identifying and quantifying) plant protection products on seeds, in terms of both average rate and seed-by-seed rate. These methods are specific, precise, accurate and sensitive. During 2003 and 2004, some new GC and HPLC methods have been developed and validated to determine beta-cyfluthrin on beet seeds, diniconazole and imazalil on barley seeds, silthiopham and bitertanol on wheat seeds and thiram on linseeds. These methods have been used to analyse lots of treated seeds to provide scientific data for use in pesticide registration or in treatment quality control on commercial lots. The work begun in 1996, in collaboration with

Quality of Agricultural Products Department, on developing near-infrared spectrometry (NIRS) for the determination of pesticides on seed has continued, culminating in a doctoral thesis (O. Pigeon) presented at the Agricultural University of Gembloux in May 2003. The work demonstrated NIRS's potential for determining the quality of seed treatment with plant protection products and showed that NIRS, a fast, non-destructive and relatively inexpensive method, permits guantitative determination of the average active substance content of a sample of treated wheat, barley or beet seeds and also provides qualitative information on the treatment distribution. This new application for NIRS opens up fresh prospects for the quality of seed treatment. The work is now continuing with a new insecticide on beet seeds.

Insecticide persistence on mosquito nets, tarpaulins and treated walls O. Pigeon, N. Ducat, P. De Vos, M. Galoux

The World Health Organisation (WHO) has for many years been concerned with protecting people from insect-borne disease (malaria, etc.) On the basis of its analytical experience with pesticides for agricultural and non-agricultural use, the Pesticides Research Department has been appointed an official WHO Collaborating Centre (renewed for a further 4 years in 2004), in partnership with the Collaborating Centres in charge of biological assessment (effectiveness). In this capacity the Department is responsible for quality control of public health products proposed by the WHO (DDT, malathion, fenthion, deltamethrin, etc.) and for analysing pyrethroid insecticides in treated materials such as wall scrapings and mosquito nets that have been soaked or impregnated in the fibre. Insecticide stability and persistence are also investigated and set alongside biological efficiency observations in the field and the type of formulation used. Much research is still being carried out into mosquito nets and tarpaulins where the active substance is incorporated into the matrix rather than deposited on the surface. The main active substances studied are deltamethrin, permethrin, lambda-cyhalothrin and alpha-cypermethrin. The laboratory also performs quality control on behalf of UNICEE of insecticide treatment of the mosquito nets available on the market.

Study of plant protection treatments of storage potatoes

S. Noël, B. Huyghebaert, O. Pigeon, B. Weickmans, P. De Vos

Since 2001 the Agricultural Engineering Department has been conducting a programme of research into the heterogeneity of chlorpropham distribution (CIPC) in stored potatoes, this being the principal cause of occasional excesses of sprout inhibitor on the potatoes, ultimately creating a risk of excessive residues when the potatoes reach the table. The various treatment methods have been assessed: quality of application (distribution), effectiveness, ease of use, and so forth. A monitoring and follow-up network has been set up involving a number of potato growers/storers. During 2003-2004 the different formulations (DP, EC and HN) and the associated application methods have been classified according to efficiency, residue levels, environmental impact and implications for user safety. The Pesticide Research Department has worked on the development and validation of the GC-MS and GC-NPD analytical method for CIPC determination in potatoes and has analysed over 1,500 samples. The results of this work have contributed to the approval file for "splitting of the rate applied" by thermonebulisation as well as providing information to the Registration Committee and users on the pros and cons of the technique.

Several European studies have highlighted the part played by occasional losses in the problem of water pollution by pesticides. One source of such losses is sprayer filling and cleaning. Prompted by this discovery, the Veterinary and Agricultural chemical study Research centre, VAR, launched an initiative aimed at developing "biofilters" to treat effluent from occasional losses after collection. Following a shift in the emphasis of its research work, VAR has since given up this study. CRA-W was keen to continue the research in order to build on the knowledge gained and develop the systems for large-scale use.

Development of biofilters for treatment of sprayer rinsing and washing water C. De Vleeschouwer, O. Pigeon, F. Cors, B. Weickmans, P. Meeus

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A biofilter is a filter comprising a substrate made from a mixture of earth, straw and vegetable mould (1/2/1) which can adsorb the active substances on its constituent organic material and break them down. Initial results after two years of observation have shown, in the case of the herbicides studied, adsorption or degradation capacities of over 90% in all cases (with one biofilter actually reaching 99%). Since 2004, 10 new biofilters have been installed in the Walloon Region, covering several different types of farm (field crops, fruit, market gardening) as well as highways maintenance departments. Some of them were installed in pairs to compare the efficiency of different substrates. This assessment of bio-filter efficacy has also been extended to other chemical herbicide families, such as sulfonylurea, aryloxyalcanoic acids, chloroacetanilides as well as insecticides and fungicides from various chemical families (pyrethroids, carbamates, dicarboximides, phenylamides, triazoles and strobilurins). Two analytical methods have been developed, one using GC-MS and other HPLC-DAD, and optimised in order to study these different active substances in substrates and waste. Measurement of the microbial activity of the substrate also got under way in 2004 for the purpose of describing and studying the biofilter efficiency trend.

Pesticide residues in ACP countries' agri cultural products

O. Pigeon, M. Galoux, I. Tamba, N. Ducat, P. de Vos

The review of active substances and the harmonisation of maximum residue limits (MRL's) for pesticides in the EU, coupled with growing consumer demands with respect to food safety, relayed by the major distribution chains, are posing big problems for ACP (Africa, Caribbean, Pacific) countries that export fruits and vegetables to the EU. If the ACP countries are to keep their European export market and if we are still to have access to these products, the production and marketing criteria for exported ACP fruits and vegetables will have to be brought into line with the new constraints. To achieve this, changes will have to be made to systems all along the chain, from producers in the ACP countries to importers in the EU. Crop protection methods in ACP countries will need to keep in step with phytosanitary legislation. Growers will have to use new plant protection products that are more environmentally compatible and make changes to their cropping methods. The agricultural chemical industry and the research institutes have a key role to play in the search for sustainable alternatives. Exporters in ACP countries and the European importers will need to set up a traceability and guaranteed phytosanitary guality system for exported fruits and vegetables. Finally, European regulations will have to take greater account, when setting MRL's, of imports into the EU and phytosanitary constraints associated with crops and working conditions. Fixing MRL's or import tolerances for pesticides used on tropical fruits and vegetables, on the basis of scientific data in accordance with European legislation, is one possibility. The Pesticide Initiative Programme (PIP), substantially financed by the EU and implemented by COLEACP (Europe – Africa – Caribbean – Pacific Liaison Committee) can provide the necessary means of bringing about this change. Due to its pesticide residue experience, the Pesticides Research Department was recently appointed by COLEACP as an official European laboratory to carry out analyses and provide advice on pesticide residues determination in fresh fruits and vegetables exported by ACP countries, with a view to ensuring compliance with European regulations. The Department is at present assisting with the setting up of a phytopharmaceutical consultancy (ACPhyto Consulting), a CRA-W spin-off, to act as an intermediary between CRA-W and the ACP countries. This project is supported by the Namur Provincial Economic Office (BEP) within the framework of the Namur Entrepreneurship Center and the Walloon Export Agency (AWEX).

6.3. GPL certification and ISO 17025 accreditation

M. Galoux, O. Pigeon, N. Ducat, P. De Vos, S. Vandecandelaere, A.Bernes, B.de Ryckel

The laboratory of technical and formulated products has been recognised as adhering to the OECD principles of Good Laboratory Practice (GLP) since January 1994. Pesticide residue research (scientific and analytical aspects) for registration purposes has likewise been carried out in accordance with these principles since 1998. Certification was confirmed in March 2004 (certificate C04). Extreme rigour is required in our work, from setting up the field trial through to the laboratory, including sampling, transfer and receipt. During 2001 the Department also put in place a new Quality Assurance system, in collaboration with the CRA-W QA Office, and obtained ISO 17025 accreditation for pesticide residue analyses in fruit, vegetables, cereals and other products and pesticide analyses in treated seeds. Certification denotes full validation of all the methods developed according to official standards. The accreditation certificate (no. 250-T) was issued by BELTEST in May 2002 and confirmed in 2003 and 2004, covering the following areas: carbamate, organophosphorus, pyrazole and pyrethroid insecticide/acaricide residues on plants; azole, benzimidazole, dicarboximide, dithiocarbamate and phenylpyrrole fungicide residues on plants, chlorpropham and propamocarb residues in potatoes, pyrethroid insecticide residues in impregnated substrates (mosquito nets), anthraquinone, fludioxonil, tefluthrin and imidacloprid on seeds and amide, nitroaniline and triazopyrimidine herbicide residues in plants. The officially accredited scope of work can be viewed on the CRA-W and BELAC Websites

6.4. Control of Plant Diseases

1. Establishing the effectiveness spectrum for fungicides and optimising their use

<u>Cereals</u>

The main focus of attention in the 2003 and 2004 seasons was the emergence of strobilurin resistance in *Septoria tritici* (leaf spot) and its

spread through a large part of Western Europe. Strobilurin-resistant strains of Septoria were in fact discovered in several areas of Belgium in 2003. Impaired performance is therefore to be expected from the active substances in this family, as was only too clear during the 2004 season. This sudden, widespread emergence of strobilurinresistant Septoria greatly complicates the making of recommendations on disease control. A number of experiments with winter wheat were accordingly set up in an endeavour to find strategies for dealing with this new situation. These comprise evaluating the residual effectiveness of strobilurins on Septoria and a comparison with the performance of compounds from other chemical families and determining the value and conditions of use of strobilurins in protection programmes.

At the same time, a trial programme launched in 2002 was also continued to investigate scope for controlling the development of wheat *Fusarium* head blight and its effects on the grain mycotoxin concentration. The products were applied in trials carried out in both natural and artificial infection conditions. The main products studied during the 2003 and 2004 seasons were tebuconazole, metconazole, epoxiconazole, dimoxystrobin and prothioconazole, applied alone or in a mix.

Finally, metrafenone and cyflufenamid, two specific anti-oidium products, and boscalid are three new active substances produced by the plant protection products industry that were also studied in cereal crops in 2003 and 2004.

<u>Potatoes</u>

Two new anti-blight active substances, currently coded, were studied during the 2003 and 2004 seasons, along with some new combinations of the active substances already on the market.

<u>Beet</u>

Trials during the past two years were aimed at assessing the effectiveness of a number of fungicidal strategies in controlling the pressure of *Cercospora beticola* (Cercospora leaf spot) which has been increasing in the last five years. These revealed differences between products currently approved and available on the market but, above all, highlighted the difficulty of choosing the right time for application during the season. An *in vitro* study of the susceptibility of a *C. beticola* strain collection to triazoles and strobilurins is also in progress.

Carrots

A test series was organised each year to find the best way of controlling carrot black rot and powdery mildew. The pattern of disease was very different in the two years under review, with 2003 being mainly characterised by powdery mildew, with yield losses of up to 40 t/ha, whereas black rot alone prevailed in 2004. Treatment programme start dates and application rates were studied with respect to compounds containing triazoles, strobilurins, boscalid or coded products, applied on their own or in a mix.

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Other market garden crops

Tests were performed on leeks and spinach in response to demand from the chemical industry (study of specific products) and growers (research into effective techniques).

2. Study of stimulants or boosters for natural plant defences

Laminarine, a brown alga extract, and harpine, a protein, are currently described as stimulating plants' natural resistance to pathogens and are already on sale with that claim in other countries. They are not thought to possess directly antimicrobial properties. A product called Siliforce, containing potassium and boron silicate, is described as strengthening plants' natural resistance. All of these have been included in various trials set up each year in wheat and carrots to assess their capabilities in Belgian field crop growing conditions. Although the tests were designed to suit the experimental products (preventive treatments repeated at specified intervals), results so far have been inconclusive.

3. Relationship between rates of fungicide application to wheat and effectiveness against leaf diseases

The Department aims to supply appropriate scientific and experimental bases to assess the relevance of an approach using smaller quantities of fungicides and strategies adapted to variety susceptibility. Two sets of trials have been organised annually, jointly with the UCL Phytopathology Unit, to study the risks of selection of fungicideresistant pathogens as a result of reduced product use. Another programme, launched in 2002 and due to run for several seasons, aims to describe the relative effectiveness against leaf diseases of 100%, 50% and 25% of the approved rates of application of the principal cereal fungicides. Lastly, in collaboration with the Crop Production Department, two annual sets of trials have investigated different approaches to fungicidal protection with respect to four wheat varieties with widely varying susceptibility to wheat glume blotch and brown rust.

4. Study of the distribution and evolution of fungicides in wheat plants and their impact on the control of fungal diseases

F. Henriet, J-M. Moreau

Much debate currently surrounds the optimisation of fungicide use in cereals, in terms of the number of applications and when they should be carried out. Current advice to growers is based on the development of the different diseases during the season. Lack of data on the exact behaviour of these products is proving an obstacle, however.

The research project undertaken aims, firstly, to quantify the distribution, redistribution and persistence over time of two fungicides from different chemical families (triazoles and strobilurins) in wheat plants, in natural conditions and using chromatographic techniques, and secondly to determine, in standardised conditions, whether the quantities of fungicide thus determined are sufficient to control fungal diseases such as leaf blotch.

After a season of spraying in the field and leaf sampling, the distribution of fungicides sprayed at different stages of wheat growth and their persistence up to 40 days after application were ascertained.

Standardised conditions for obtaining known quantities of fungicides in seedlings grown under glass and inoculum production methods were also established.

6.5. Control of field and forest

pests

M. De Proft

1. Insecticide effectiveness assessment with respect to various species of potato aphid

Several new aphicides have been studied in potatoes. Experiments during 2003 showed that some insecticides applied at the start of infestation (mid-June) could lead to late infestation (end July) by large numbers of aphids, whereas the aphids had all but disappeared from the untreated plots, probably due to the effect of the products on aphid parasites and predators. This effect was particularly marked in the case of carbaryl. The effectiveness assessment of possible new aphicides was largely unsuccessful in 2003 due to insufficient infestation.

Three trials were set up in 2004 when high infestation levels by a number of aphid species, notably *Aphis nasturtii* and *Aulacorthum solani*, occurred. Efficiency was very successfully assessed. As spraying took place early in the season, monitoring of aphid populations after treatment provided some useful information on the effect of the products on natural enemies. The results can be used in connection with registration dossiers for the different products concerned.

2. Protecting potatoes from wireworm, Agriotes spp

Three new trials were carried out in 2004 in wireworm-infested potato fields. These experiments are part of a quest for alternative solutions to soil treatment with all-purpose insecticides such as lindane, which are now banned. To avoid widespread soil treatment, the tests focused on the use of toxic bait. Very good results were achieved with some types of toxic bait, much better than with the reference treatment, i.e. ethoprophos sprayed and incorporated into the soil prior to planting. A comparison of application methods also yielded some useful information.

This new treatment concept should soon be extended to other soil pests, such as some species of cockchafer currently infesting the south of the country (particularly Gaume), which have defied all solutions tried to date.

3. Protecting sugar-beet from the beet cyst nematode *Heterodera schachtii* Schmidt

The risk of aldicarb shortly being withdrawn from the market, coupled with beet seed producers giving up work on genetic resistance projects, is likely to leave beet growers facing problems due to the beet cyst nematode. Infestation is expected to reach harmful levels in about fifteen per cent of beet fields.

Our experiments in 2004 took a threefold approach: assessment of new nematicide active substances applied as seed dressing; assessment of variety tolerance and the benefits of nematicide protection; and evaluation of a combination of oxamyl and carbofuran as an alternative to aldicarb. The last of these has been tested not only against the beet cyst nematode but also on other beet pests, such as the pygmy beetle.

4. Protecting sugar-beet from millipedes (Blaniulus guttulatus and Archiboreoiulus pallidus) and other soil pests

Protecting sugar-beet from millipedes (*Blaniulus guttulatus* and *Archiboreoiulus pallidus*) and other soil pests

The efficacy of the new insecticide products was assessed against some soil pests that have been fairly marginal until quite recently but are now a growing source of problems, most notably millipedes, wireworms and crane flies. Some useful results have been obtained with new active substances, principally neonicotinoids, sometimes combined with pyrethroids.

Millipedes attacks in particular are hard to predict and difficult to control by means of seed treatment alone.

5. Controlling lawn pests: Hoplia philanthus and Subanguina radicicola

We are increasingly being contacted by individuals and parks managers troubled by lawn pests. Some sports fields belonging to a Walloon football club have been under surveillance for the last two years. Infestation by Subanguina radicicola, a root-knot nematode, is indeed causing poor root systems and patchy withering. Regular analyses are performed to measure the effect of treatments applied and the infestation trend. Damage, in some cases very striking, can also be caused to lawn turf in light soils by several species of cockchafer. Different insecticidal treatments were compared to an application of Heterorabditis, an entomoparasitic nematode. Early results indicate that some insecticides could prove fairly effective in controlling this pest. However, the most appropriate treatment periods remain to be established.

6. Warning system for cereal pests

Observations and warnings were a fairly straightforward matter in 2003. In 2004, in contrast, the





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exceptional wheat infestation by *Sitobion avenae*, followed later on by *Rhopalosiphum padi*, which are particularly viruliferous, in young winter cereal crops put the CADCO unit under unprecedented pressure.

As well as routine coordination of observations, drafting of 24 recommendations for farmers and setting up validation trials for summer aphids in wheat and aphids carrying *Barley yellow dwarf virus* in winter barley and wheat, the unit was also kept busy dealing with a flurry of questions by telephone and e mail from growers, distributors of pesticides and all the players in the plant protection chain.

Gall midge (*Sitodiplosis mosellana*) flight measuring trials were once again carried out with the aid of limed panels. The resulting capture levels proved insufficient, as the big evening flights produced only a few captures. The concept is worth retaining, however, as a specific gall midge pheromone is now available. The refined concept will be studied in 2005.



ure 2

Gall midge (Sitodiplosis mosellana) trapped in lime

7. Ambrosia beetle in beech woods

The 2003 studies of ambrosia beetle infesting beech woods and causing unprecedented damage were aimed, on the one hand, at developing our knowledge of the harmfulness of the different species and, on the other hand, perfecting our trapping techniques.

Our approach in the first case took the form of experiments to establish the attack distribution by species (standing trees, lying timber, lineatin and ethanol traps) in two stands suffering from "beech disease". The beetle holes were counted in the different substrates over large areas (7.5 hectares per stand). It was found that Trypodendron domesticum was by far the commonest species. This species was also found to be the most likely to attack standing trees and thus the most harmful. This study carried out at sites where traps had also been set also provided a measure of the potential impact of trapping on a forest insect population. A threefold approach was taken to perfecting trapping: selectivity with respect to non-target species, feasibility and scope for combined trapping of several species, some conifer dependent and some broad-leaved dependent.

8. Trapping Diabrotia virgifera virgifera

In 2003, for the first time, the Western corn root worm was the subject of a national detection campaign organised by the Federal Food Safety Agency, AFSCA. This comprised setting limed traps using specific pheromones around the most likely entry sites. Some tens of individuals were captured near Zaventem Airport in September. The detection campaign was repeated in 2004. The CRA-W's role in these two detection campaigns consisted in setting traps in the east and south of the Walloon Region. The Centre was also involved in designing these detection campaigns and in the emergency plans set up to eradicate emerging focuses.

6.6. Weed control

F. Ansseau

Weed control in field crops

1. Cereals

Various formulations were compared in the course of different studies aimed at improving the formulation or composition of certain products (bridging studies).

Firstly, we looked at sulfonylureas herbicides formulated as water-dispersible granules (WG), used in spring on cereals (principally metsulfuronmethyl, tribenuron - methyl and thifensulfuron-methyl), which regularly present problems for farmers due to product residue build-up inside the spray booms (even after thorough rinsing). These residues are then washed out when treatments are applied at the same time of year to other crops, mainly beet, using products often formulated as emulsifiable concentrates (EC), the solvents of which can reactivate the sulfonylureas traces, severely damaging the crop subsequently sprayed.

Accordingly, various tests were carried out in the past two seasons to compare products formulated as water soluble granules (SG), where the active substances are completely solubilized and thus washed out during treatment without any residue problems, with products currently on the market presented in the form of water-dispersible granules (WG), in terms of selectivity and effectiveness. In spring 2004, some treatments using flupyrsulfuron and metsulfuron-methyl combined with diflufenican were also trialled from a selectivity and effectiveness point of view. The selectivity trials were carried out on winter and spring barley, spring wheat and spelt and the effectiveness trials mainly on winter wheat.

A product containing only diflufenican, an active substance with a mainly dicot weed spectrum, was trialled in both seasons on winter wheat and on spring wheat and barley as part of a series of selectivity studies. The product was tested on its own and also in various mixes with either another dicot-targeted herbicide or a grass killer, with the aim of developing full treatment schemes, i.e. for control of both broad-leaved weeds and grasses.

The selectivity of two new bromoxynil octanoate based NPE-free products was compared to that of

products with the same bromoxynil concentrations but also containing nonylphenols.

The selectivity of a product containing flufenacet combined with diflufenican, presented in suspension concentrate (SC) form, was compared in winter wheat and barley to that of the product currently on the market, which contains the same active substances at the same concentrations, but is presented in the form of water-dispersible granules (WG).

Selectivity trials were also conducted in spelt to study the selectivity of two coded products with a grass control spectrum.

Again in spelt, various trials were carried out to compare the selectivity and effectiveness of two coded products based on different concentrations of metsulfuron-methyl to that of the product currently on the market, which contains the same active substance but the patent for which is soon due to lapse.

Spelt was chosen for the purpose of these trials because very few authorised products are at present available for this crop, which is nevertheless gradually gaining ground in Belgium.

Effectiveness trials targeted mainly at grasses (*Alopecurus* and *Apera*) were also carried out to compare the efficiency of two products, one containing mesosulfuron, iodosulfuron-methylsodium and mefenpyr-diethyl and the other containing iodosulfuron-methyl-sodium and mefenpyr-diethyl, presented in liquid form (SC and OD - oil based dispersion, respectively) with that of products containing the same active substances but presented in water-dispersible granule (WG) form.

Specific blackgrass control trials were undertaken to compare the effectiveness of treatment schemes comprising autumn applications, spring applications or a combination of both. These trials were carried out on hard to control blackgrass populations, either in no-till growing conditions or where resistance was thought to have developed. In the experimental conditions the double treatment (autumn and spring) proved most efficacious, also producing the best yield. These trials will be repeated in the 2004-2005 growing season.

2. Maize

The work begun in 2002 on comparing the current nicosulfuron formulation, a suspension concentrate (SC), with a new formulation as water-dispersible granules (WG) was continued and developed during 2003 and 2004. Various selectivity and effectiveness trials were carried out during the two years. The 2004 trials concluded this programme.

Trials were conducted to establish the selectivity of some new products containing dimethenamid - P, the isomeric form of dimethenamid, with the aim of reducing active substance application per hectare, the isomeric form of the active substance being more active than the racemic form.

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Another product containing tritosulfuron and dicamba was also investigated in terms of both selectivity and effectiveness.

A separate study looked at the effectiveness for grass and broad-leaved weed control of two new products, one containing foramsulfuron and the other containing a coded new active substance in combination with the same plant protector, isoxadifen-ethyl.

3. Beet

The only study topic for beet was the selectivity of various products containing desmedipham, phenmedipham and ethofumesate, two of them also in combination with metamitrone. Split applications were carried out under the FAR system (Belgian sugar beet low dose system).

4. Chicory

In a collaborative link-up with Centre Agricole Betterave Chicorée (CABC), a selectivity trial was organised in order to extend dimethenamid P and ethofumesate authorisation from beet to chicory, with the aim of finding complementary solutions for more efficient control of some weeds which are still a problem with this crop, notably mercury, wild chamomile, gallant soldier, fool's parsley, sowthistle and fumitory.

Split applications of the treatments were carried out, on the same principle as using post-emergence herbicides with beet.

The selectivity of a new, carbetamide-based product was also compared to that of a product containing the same active substance currently on the market.

Weed control in "minor" crops

1. Lupins

The spring lupin is attracting increasing interest in this country as a new plant protein source for cattle feed.

Weed control remains one of the critical points at the moment, as the lupin is a poor competitor and rather susceptible to competition from weeds, especially at the early stages of growth. As no herbicides have so far been authorised for the lupin, solutions need to be found as a matter of urgency if this crop is to be successfully developed. Against this background, a number of active substances were tested during the year. The most selective with respect to the crop were diflufenican for broad-leaved weeds and haloxyfop-r-methyl for grasses.

2. Christmas trees

Christmas trees are becoming a significant crop in the Ardennes area of Belgium. However, as in the case of lupins, few products have been authorised for use and weed control is often one of the most critical points for growers. The plots planted with Christmas trees are generally in fact poor soil colonised by a variety of species, often hard to control. Some species of fir, such as *Abies nobilis*, can be highly sensitive to herbicides, especially at the early growth stage.

Trials have therefore been set up to study the selectivity of a new active substance recently authorised for non-selective herbicide use, flazasulfuron.

7. SERVICE ACTIVITIES

7.1 Appraisal of biological dossier
 of plant protection products on
 behalf of the registration committee.
 F. Cors, B. Weickmans

The unit, which works in close cooperation with other services of the Department, was created in 1994 for the purpose of handling one of the Department's main assignments: making recommendations to the Registration Committee on the efficacy of pesticides submitted for Belgian sales authorisation. The unit plays an active part in drafting Belgian and European rules for certification drawn up at national or international meetings and in this connection is involved in drafting new Belgian standards and EPPO directives. Managing the Department's pesticide database is another of the unit's tasks.

7.2. Compilation of analytical dossiers comprising the chemical and physico-chemical aspects of active substances and their formulations for the purposes of registration or drafting specifications. A. Bernes, B. de Ryckel

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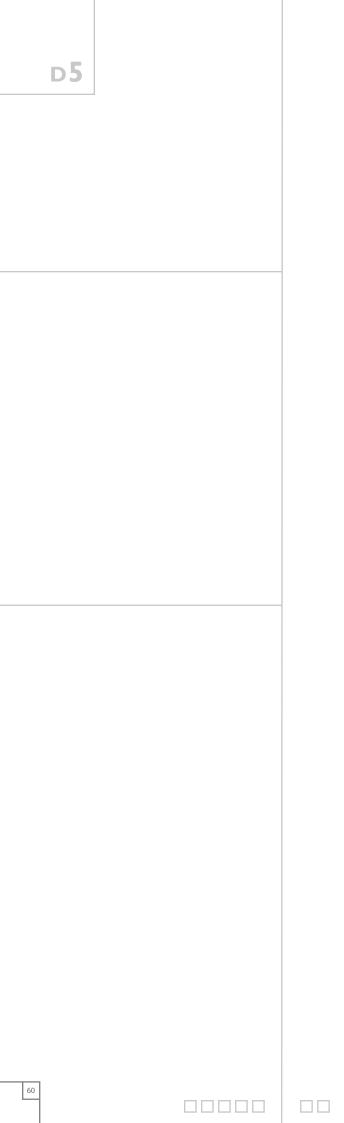
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Departement PESTICIDES RESEARCH



Department

A G R I C U L T U R A L E N G I N E E R I N G

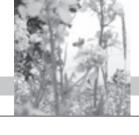


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1. MISSION STATEMENT

The Agricultural Engineering Department carries out basic and applied research and provides quality scientific services on the techno-economic analysis of agricultural devices and the techniques relating to energy and industrial utilization of biomass. Special attention is paid to the environmental and socio-economic impact of agricultural mechanisation and the utilization of biomass, as well as to the professional development of techniques and their transfer to other regions of the world (Central and Eastern Europe, Africa, Asia, Latin America).

2. SCIENTIFIC PERSONNEL

4 permanent staff and 13 contract staff

2.1. Head of Department

Y. Schenkel, Inspecteur général scientifique

2.2. Scientists

- A. Berrio-Smith, Attachée scientifique (RW DRI, DEE)
- R. Crehay, Attaché scientifique (RW DGTRE, CE DGRECH)
- C. Delaunois, Attachée scientifique (until 31/03/2003) (RW DGA)
- J. Delcarte, Attaché scientifique
- B. Huyghebaert, Attaché scientifique (CRA-W)
- S. Loyen, Attaché scientifique (CRA-W: various conventions)
- D. Marchal, Attaché scientifique (RW DGA, DGTRE)
- O. Miserque, Attaché scientifique (CRA-W: various conventions)
- O. Mostade, Attaché scientifique (CRA-W)
- S. Noël, Attachée scientifique (FDMP)
- F. Rabier, Attachée scientifique (CE DGRECH, RW-DGA)
- M. Temmerman, Attaché scientifique (CRA-W)
- J.-F. Van Belle, Attaché scientifique (CRA-W)
- D. Vander Stricht, Attachée scientifique (RW DGA)
- R. Vankerkove, Attaché scientifique (ERBE)
- O. Vitlox, Attaché scientifique (until 31/05/2004)

3. NATIONAL REPRESENTATION

- Agrobiopôle wallon
- Comité régional Phyto
- Fedagrim/Cote
- Comité Directeur pour le contrôle des pulvérisateurs
 Comité technique pour l'application des produits phytosanitaires
- Centre agricole betterave-chicorée
- Centre agricole pomme de terre
- Commission « Machines & Produits »
- CTA Libramont
- Société de Génie rural
- Groupe « Lubrifiant d'origine végétale »
- Association pour la Valorisation de la Biomasse (ValBiom)
- Association Belge pour la Promotion des
- Energies Renouvelables (APERE)
- Belgian Forum for Agricultural Research and Development

4. INTERNATIONAL REPRESENTATION

- CIGR: International Commission of Agricultural Engineering
- European Committee of Standardization TC 144 (Agricultural devices), TC 260 (Fertilizers), TC 335 (Solid biofuels)
- EurAgEng
- European Network for Testing of Agricultural Machines (ENTAM)
- Institut de l'Energie et de l'Environnement des Pays Francophones
- International Energy Agency/Bioenergy Agreement : Comité Exécutif, Biomass Production for Energy from Sustainable Forestry, Biomass Combustion and Co-Firing
- International Standardization Organisation TC 23
 OECD: Organisation for Cooperation and Economic Development

5. RESEARCH TOPICS

Today, agricultural research needs to be directed not only towards farmers, but also towards the general public. Historically, it focused mainly on increasing crop yield and animal productivity and on making farmers' work easier. This approach is not appropriate any longer: the expectations of society need to be integrated into agricultural research. Such research is of interest now to the general public, to politicians and to economists. In particular, agricultural research must focus on priority topics such as the quality of food products, the competitiveness of agriculture, the sustainable utilization of natural resources, rural development, and the development of agricultural systems compatible with environmental sustainability.

The Agricultural Mechanisation Unit has conducted many research and development projects on soil tillage, the application of agrochemical products, the application of mineral and organic fertilizers, as well as on mechanisation.

The Unit of Energy and Industrial Use of Biomass conducts research and development activities on the mobilization and inventory of natural resources, biomass conditioning and bioenergy.

6. RESEARCH REPORTS

6.1. Agricultural mechanisation

Traction and soil tillage

1. Soil-machine-tractor interactions

- O. Vitlox, S. Loyen (*)
- (*) CRA-W contrats divers
- Soil-machine interaction

Many studies have been carried out using the penetrometer, in order to assess the impact of this device on soil structure. The number of measurements for a given area was increased in order to produce a three-dimensional view of areas with the same compaction levels.

Soil-tractor interaction

A 3-day training course was organised for the European, African and Asian delegates of a multinational company manufacturing tyres. Different types of tyres were tested on a dynamic test rig with different inflating pressures, loads and traction coupling. The results focused on the landslide, traction effort and damage caused to soil structure.

2. Development of a horizontal penetrometer

- S. Loyen (*)
- (*) CRA-W contrats divers

In order to conduct penetrometric tests continuously and extend the analysis to the size of the plot, a horizontal penetrometer is being developed. Three penetrometric shafts are mounted on a coulter, itself fixed to a parallelogram frame. This equipment makes it possible to vary the measurement depth while ensuring that the penetrometric shafts remain horizontal when moving forwards.

3. Natural regeneration in the Sonian Forest

- S. Loyen (*)
- (*) CRA-W contrats divers

The impact of soil compaction on the regeneration of beech trees in the Sonian Forest was studied. After a series of measurements aimed at defining the soil compaction state in the forest, tests were carried out on the surface. The technique which proved to be the best was the use of a plough equipped with discs passing over the soil before the beechnuts fell. Besides a good levee, an on line seedbed is observed, which is favourable to a mechanised management for the next operations. Exploitation systems were also compared to assess damage caused to the soil. A thorough study was conducted to assess the three-dimensional soil structure in the plots on which a plough equipped with discs had been used, as well as in the model plots. An international seminar was organised to disseminate all the findings. Collaboration: Instituut voor Bosbouw en Wildbeheer. DNF, AMINAL, IBGE .

Application of agrochemical products

Research on the application of agrochemical products sought to gain a better understanding of the quality parameters of the application techniques, a reduction of the impact of plant protection products (PPP) on the environment and the improved safety of the user.

1. Comparative study of the nozzles wear

B. Huyghebaert (*), O. Mostade (*) (*) CRA-W

Since 2002, several sets of spraying nozzles used in the field have been analysed. Their wear dynamics (increase in the flow rate, deterioration of the spray pattern, evolution of the droplet spectrum)

and their performance were studied and compared to the results obtained in the laboratory. This large-scale comparative study focused on recent models of flat fan nozzles: anti-drift nozzles with calibrating disc, air injection nozzles, and mirror nozzles.

Collaboration: Agricultural University of Lublin (Poland)

2. Study of the heterogeneity of Chlorprophame treatments used to prevent potato germination

S. Noël (**), B. Huyghebaert (*)

(*) CRA-W

(**) FDMP

Since 2001, the Department has conducted research on the heterogeneity of CIPC distribution in potato stocks, the main cause of occasional overapplication of sprout inhibitor on tubers, increasing the risk of there being too many residues in potatoes served up to the consumer. Different treatment techniques were assessed, including quality of the application (distribution), efficiency, and ease of utilization. A monitoring network of those who produce and stock potatoes was established. In 2003–2004 we classified the different formulations (DP, EC and HN) and the associated application techniques according to efficiency, residue content, environmental importance and user safety. The results endorsed the technique of splitting the applied dose by thermonebulisation; we made the standardization committee aware of the advantages and disadvantages of this technique.

3. Study of the efficiency of anti-drift nozzles for the treatment of fungicides on cereals

O. Mostade (*), B. Huyghebaert (*) (*) CRA-W

In collaboration with the Pesticides Research Department, comparative biological tests were carried out on the use of different types of nozzles in combatting fusariose on wheat ears (Beaufort variety). The tests sought to establish whether the anti-drift nozzles were more efficient. The tests were based on sprayings of 300 l/ha. The observations, to be treated with caution, showed that, compared to the model (untreated), efficiency increases in this order: flat fan nozzle vith drift reduction > classical flat fan nozzle > air injection flat fan nozzle > turbulence nozzle > twin jets nozzle. Additional tests are necessary to complete the observations made in 2004.

Chemical application O. Miserque (*)

(*) CRA-W – contrats divers

1. Fertilizers

Blended fertilizers are obtained by mixing basic fertilizers. The technique is flexible and economic but mistakes arising from the chemical composition, such as the components tending to segregate, are common. A study is being carried out to identify the parameters influencing this segregation, focusing on mixtures of two components. Raw materials differ from each another in size, shape or density. The mixture is subjected to various stages of handling: from flowing out and the loading up of a trailer to spreading. At each stage, the segregation of both components is measured. Depending on the differences between components, physical and/or chemical segregation of varying degrees appears. Within this framework, a study was carried out for the European Blenders Association (EBA). Typical mixtures were manufactured and were subjected to monitoring of the segregation before being used to validate tests of two components mixtures.

Along with this research, the laboratory analysis of the physical characteristics of fertilizer particles was developed and is now accredited according to the standard ISO 17025. *Collaborations : UIg, EBA*

Tests of machinery

1. Tests of chicory harvesting machinery O. Miserque (*)

(*) CRA-W – contrats divers

Tests of chicory harvesting machinery continue. In addition to harvesting, chicory sowing is a delicate step that has a strong influence on the crop yield. The seeds and seeding elements are tested just before seeds are distributed to farmers. The testing procedure was validated and makes it possible to detect problematic sets of seeds.

6.2. Energy and industrial use of biomass

Biomass resources supply JF Van Belle (*) (*) CRA-W

1. Inventory of resources

Research on the characterization and inventory of resources involved developing a methodology for using predictive models to assess harvestable biomass, including local factors (ecology, growth conditions) and cultivation and harvesting practices (technical itineraries). The methodology was tested on Picea abies (L.) Karst on 25 stations in the production area in Wallonia (Ardennes and Gaume) of this crop and Quercus spp (Condroz). The models make it possible to assess biomass, its energy potential, the exportation of mineral elements and the reduction potential of greenhouse gas emissions (CO2). These studies were carried out with the support of the DGRNE (General Directorate of Natural Resources and the Environment) and the Walloon Region.

Collaborations: FUSAGx, HEPHO, ERBE

2. Supplying logistics

Various studies were carried out in the field to assess the productivity and techno-economic profits of harvesting methods. A harvesting test unit was set up, making it possible to study continuously the parameters (e.g., couple, speed, power, productivity, granulometry, gas emissions) influencing harvesting, so as to create models. Tests were carried out with different biomasses(*Picea abies* (L.)*Karst*, *Betula pubescens Ehrhart*, *Prunus avium L.*, *Populus sp.*, *Phyllostachys vivax*, *Ph. aureosulcata 'spectabilis'*, *Ph. Praecox*, *Quercus robur L.*). The work is part of an international collaborative project with the Energy International Agency.

Collaboration: HERS, UPM-Spain, CEPLAC-Brazil

3. CO, emissions and energy balance

A conceptual model for the calculation of CO_2 balances of biomass energy projects was set up. The work focused on the supply chain and the results were used to determine CO_2 coefficients for the calculation of the green certificates issued by the Walloon Commission for Energy (CWAPE). *Collaboration: HERS*



4. External activities

Experimental equipment was set up in collaboration with the University of Liège, in order to study the effect of spreading combustion ashes on production plots. The first results showed a perceptible improvement in fertility using several methods of spreading and concentrations of the spread. In collaboration with the South of the State of Bahia, a study was conducted to quantify the biodiversity gradient of potential carbon wells linked to cocoa bean production (Theobroma cacao L.) by using ants as bio-indicators. *Collaboration: Ula, HERS*

The research of the biomass conditioning unit focuses on three main topics related to solid biofuels: methods of analysing the physical and mechanical properties, reducing conditioning costs and improving the quality, and the utilization of biofuels in developing countries.

1. Methods of analysis of the physical and mechanical properties of biofuels

The units is conducting measurements of properties such as the moisture content of the volumic mass (net or rough) of solid biofuels, the durability of pellets and briquettes, and the size and granulometric distribution of the particles.

Biomass conditioning



BioNorm (Pre-normative work on sampling and testing solid biofuels for the development of quality assurance systems) F. Rabier (**), M. Temmerman (*) (*) CRA-W

(**) CE-DGRECH

The role of the unit in this project is to compare the physical and mechanical tests carried out on solid biofuels (measurement of moisture content, bulk volumic mass, particle size, distribution of net volumic mass, and durability of pellets and briquettes).

The results of this work will be integrated into a quality assurance system for biofuels covering the whole supply chain. The results will also be integrated directly into the ongoing standardization work of the CEN TC 335 on 'Solid biofuels'. *Collaboration: consortium of 39 partners, coordinated by IEE - Leipzig*

2. The reduction of conditioning costs and the improvement of the quality of solid biofuels

M. Temmerman (*)

(*) CRA-W

Considering the importance of the moisture content of matter in terms of its energy utilization, our research is focusing on the drying of biomass and, in particular, on the relationship between the granulometry of a matter and its resistance to air flow.

3. Utilization of biofuels in developing countries

Wood charcoal and fire wood remain the main fuels used for domestic purposes in developing countries. Their replacement by fuels manufactured from agricultural or forest residues reduces the pressure that forests are subjected to in these countries. Three projects are being developed to address this issue.

Setting up a production pilot unit to produce fuel balls by agglomeration of rice husks in Ross Bethio, Senegal M. Temmerman (*), A. Berrio-Smith (**) (*) CRA-W (**) RW – DRI

The agglomeration technique developed by the Agricultural Engineering Department makes it possible to use different types of biomass, including residues from forests and the food industry. Using the simple and inexpensive production unit developed by the department, biomass is mixed with clay and water to produce 'fuel balls'. Their behaviour when burning is similar to that of wood charcoal and their utilization on a large scale would reduce wood charcoal consumption. A pilot unit, established in January 2004 in Ross Bethio in northern Senegal, is producing balls from rice husks. It was set up to study the production and acceptability of the fuel. It allows us to gather information needed to assess it technical and economic merits and its industrial use with other biomass sources or in other regions lacking traditional fuels.

Collaboration: PROGEDE, CERER, SAED, CRAT (Senegal)



boulets combustibles

The two other projects on this topic are:

Support for the development of the bioenergy sector in Cuba. Project focusing on wood artificial drying using sawmill by-products as a heat energy source A. Berrio-Smith (**) (**) RW – DRI

Collaborations : IFF, MINVEC, GEAM (Cuba)

Bamboo Thematic Network (BTN

M. Temmerman (*) (*) CRA-W Collaboration: consortium of 17 partners, coordinated byO**pri**ns Plant, N.V. (B)

Energy use

J. Delcarte, Y. Schenkel

1. Slow pyrolysis

Wood used for building is treated in order to improve its durability. Among the preservation agents, CCA (Cu-Cr-As) is replaced by the more environmentally friendly CCB (Cu-Cr-B). But any treatment of materials raises the problem of contamination when demolishing the structure. The behaviour of wood treated by CCB salts in slow pyrolysis (carbonisation) was the focus of this research. Slow pyrolysis is a way of reducing the waste volume to be treated while obtaining homogeneous matter. To the considered temperatures, the three elements (Cu, Cr, B) concentrate in wood charcoal.

Collaboration: ULB, FUSAGx

2. Combustion

The Agricultural Engineering Department and its Swiss partner, Verenum, obtained IEA (International Energy Agency) financing in 2004 for research on determining the energy efficiency of automatic installations of biomass combustion and on the relationship between energy efficiency and atmospheric emissions at different engine speeds. The engine speeds being investigated were 100, 50, 30% and a value < 30% of the nominal power. The characteristics of a boiler (yield, emissions) are always measured at the nominal power level. In real conditions, a boiler rarely works full time at full speed. We therefore considered it appropriate to analyse these intermediary speeds. *Collaboration: Verenum (Switzerland)*

6.3. Decision support for public and private sectors

The Agricultural Engineering Department is responsible for technological development monitoring, which includes the task of making public and private agencies aware of technological developments and helping them make decisions on the applications of these technologies.

1. Standardization activities

O. Mostade (*), B. Huyghebaert (*), M. Temmerman (*) (*) CRA-W

The 2003-2004 biennial Over the past 2 years much research has been done on spraying techniques. The Department is actively involved in the formulating ISO and CEN standards, especially in relation to problems of spraying drift and the cleaning and rinsing of sprayers.

As for biofuels, the Department is involved in the technical commission CEN TC 335 on 'Solid Biofuels', which includes work on terminology, sampling, physical analyses and chemical analyses.

2. Study of methods for reducing the spraying drift

B. Huyghebaert (*), S. Noël (**), O. Mostade (*) (*) CRA-W (**) FDMP

The aggregation committee assesses the accreditation files, especially in terms of the use of agrochemical products according to good agricultural practices so that they do not present unacceptable risks for humans and the environment, particularly the aquatic environment. Currently, the risks for aquatic organisms are being kept at an acceptable level by fixing buffers for each commercial formulation. These buffers are strips of variable width (2 to 20 m) established between the end of a treated plot and any stretch of water. At the request of the SPF in relation to public health, food chain security and environmental safety, the Department has been studying other methods to reduce spraying drift in order to:

- establish and validate new tools for assessing pesticide formulations;

- ensure that farmers are aware of the relevant precautionary measures;

- ensure that the precautionary measures are actually applied in the field;

- integrate the new measures into existing inspection procedures (e.g., *via* inspection of sprayers). *Collaboration: DvL (CLO), PCF-KOG (Gorsem).*

3. ERA Bioenergy Strategy – Shortterm measures to develop the ERA for Bioenergy RTD

Y. Schenkel (*), R. Crehay (**) (*) CRAW (**) CE-DGRECH

In order to establish a European Research Area (ERA) in bioenergy, the CRA-W organised an assessment of links between the different national research programmes on this topic and on how to optimise these synergies. The data gathered and the conclusions of this exercise were validated by



Belgian bioenergy researchers at a 'Belgian expert meeting' organized by CRA-W on 5 December 2003.

Collaboration: Joanneum Research (Austria), VTT (Finland), NOVEM (The Netherlands), CRES (Greecee)

4. Biomass Energy Guidebook

Y.Schenkel, J. Delcarte, D. Marchal (**), M. Temmerman (*) (*) CRA-W (**) RW – DGA, DGTRE

With the support of the Institute of Energy and Environment of French-speaking countries (IEPF), the Department coordinated the writing of the second edition of the Biomass Energy Guidebook. Oriented towards the utilisation of biomass to produce energy for industrial and domestic use, the book is not intended to cover all applications; it covers only validated procedures that have shown significant success. The authors describe the constraints in the utilisation of biomass. The Biomass Energy Guidebook provides its readers (public and private) with a source of much information which demonstrates the potential of using biomass resources, the feasibility of the technologies involved and the constraints of these technologies. The information should help users to make decisions about investment programmes or develop energy strategies which view biomass as a national energy source that is as modern and competitive as conventional fossil fuels. Collaboration: INRA, CIRAD-Forêt, ITEBE (France), Laborelec, UCL-TERM, TBW (Belgium), AIT (Thailand)

7. SERVICE ACTIVITIES

7.1.Tractor tests

(*) CRA-W – contrats divers

Tractor tests to assess power take-off (power, couple, consumption) were carried out in response to demand from users in the Department. In collaboration with a lubricants company, test rigs for power take-off were installed with tractors retailers (21 in Belgium, 61 in the Netherlands). The Department ensures the quality and maintenance of the test rigs installed in Belgium.

7.2. Sprayer inspection B. Huyghebaert (*), O. Mostade (*) (*) CRA-W

The inspection of sprayers has been compulsory in Belgium since 1995. The introduction of this action was motivated by the poor state of working equipment, the excessive fees paid by farmers for agrochemical treatments, and the negative impact on the environment of the irresponsible use of plant protection products (PPP). There was also a strong need to reorganise Belgian agriculture so that it remains competitive after CAP reform; the GATT negotiations also contributed to this decision.

The Agricultural Engineering Department is the officially recognized body for carrying out

the inspection of sprayers for the French and German-speaking parts of Belgium. This service is provided under the supervision of AFSCA. An average of 3,000 sprayers are inspected every year by two teams from the Agricultural Engineering Department.

Over the years there has been a considerable improvement in the state of sprayers, now reaching an 85% acceptance level on first inspection. Belgium's stock of sprayers is now among the best maintained in Europe. Indeed, the inspection of sprayers and the need to have them in a perfect working order has become part of everyday life in the sector, which illustrates a significant change of attitude. Farmers – the real specialists – are increasingly concerned about rationalizing the use of PPP. The fourth cycle of sprayer inspection begins in September 2004, while efforts to improve the inspection equipment and the treatment software continue.

7.3. Tests of fertilizer spreaders

O. Miserque (**) (**) CRA-W – contrats divers

The Department carries out fertilizer spreading tests in order to help farmers adjust their equipment, as necessary. More specific tests are also carried out for distributors and fertilizer manufacturers. The same protocol is used for testing spreading equipment for organic matter.

7.4. Inspection of snow-removal salt road spreaders

O. Miserque (**) (**) CRA-W – contrats divers

The MET (Ministry of Equipment and Transportation) proposed new contracts for builders favouring the utilisation of new snow-removal salt road spreaders with the addition of brine. About 300 spreaders have been subjected to tests. These have shown that, in general, they have not been properly adjusted and that users face many difficulties in achieving efficient spreading. The aspects inspected include the dosage and percentage of salt spread away from the road. In response to these tests, some manufacturers made changes to their equipment.

7.5. Costs indicator

O. Miserque (**)

(**) CRA-W – contrats divers

The updating and calculation of economic data relating to agricultural equipment is an ongoing process.

The calculation method is used to compare equipment and techniques. It has been used, for example , in relation to the cost of beet and chicory harvesting fields, and the cost of simplified seed techniques.

7.6. Regional Biomass Energy Agency (ERBE)

R. Vankerkove (**), J.F. Van Belle (*), Y. Schenkel (*) CRA-W (**) ERBE

ERBE is continuing with its development activities relating to biomass energy projects. In addition to a number of feasibility studies carried out for various enterprises, ERBE is also involved in about 10 projects focusing on developing bioenergy installations in the municipalities sector (Wood Energy Plan, Rural Development, Libramont, Philippeville, Enghien, Namur, etc.). In addition, ERBE is leading some projects at European level, within the framework of Phasing out (wood, biomethanation) and the Interreg III programme (Eurowood project). *Collaboration: BESEL (Spain), Rural Foundation of Wallonia, ICEDD*

7.7. ValBiom

D. Marchal (*), D. Vander Stricht (**), R. Crehay (*), F. Rabier (**), Y. Schenkel (*) RW – DGTRE, DGA (**) RW – DGA

(**) RW – DGA

ValBiom is currently working with CRA-W, FUSAGx, UCL.

Among the ongoing projects are the FARR-Wal, Wood-energy facilitator (private sector) and Diffuser programmes. FARR-Wal (Agriculture and Renewable Resources sectors in Wallonia) aims at diversifying farmers' activities (non-food utilisation of biomass and development of non-food crops), increasing the Walloon gross regional product (developing processing, distribution and non-food utilisation of agricultural products and biomass), and strengthening the economy of rural areas (setting up local processing and biomass utilisation ventures). This work involves various activities: monitoring technological development, developing communication plans, raising awareness in the agricultural and rural world of non-food utilisation methods of biomass (energy industrial biomass), coordinating methods, and setting up demonstration actions. With regard to biomass energy utilisation, an important activity is the setting up and organization of a working group on pellets.

ValBiom was chosen by the Walloon Region to act as wood-energy facilitator for the private sector. This assignment completes the work facilitators for the "renewable energy" sector set up by the Walloon Region. Within this framework, ValBiom aims particularly at helping those involved in the wood-energy sector in various ways (providing information on demand, conducting a study of the relevance of proposed projects, etc.). DiffuSER is a support service for the operators of the Energy Division (including the Energy Information Centre, facilitators) to popularise and disseminate information on renewable energies. Using various channels (training courses, conferences, documents for the general public, etc.), DiffuSER ensures that the information reaches private individuals

Collaboration: APERE, UCL, FUSAGx, ITEBE, FEDARENE





colza

7.8. Standardization ISO 17025

D. Vrebos – Bureau Assurance Qualité (CRA-W) Y. Schenkel, J. Delcarte, B. Huyghebaert (*), O. Miserque (**), O. Mostade (*) (*) CRA-W

(**) CRA-W – contrats divers

The establishment of the quality system is in its last stage, with the initial audit, led by Beltest, taking place in February 2004. The audit report was very positive and the auditors accepted all the propositions of corrective actions. The certificate of accreditation for three laboratories was given by the end of October 2004. The biomass laboratory: high calorific value in solid biofuels and wood charcoals, and proximate analysis of solid biofuels and wood charcoals. The sprayer laboratory: transversal distribution of spraying nozzles, and inspection of sensors and pressure manometers. The fertilizer laboratory: granulometry by sieving fertilizer particles, volumic mass of fertilizer particles after and/or without packing down, and angle of repose (30°-40°) and fluidity of fertilizer particles. The accreditation work and the international recognition obtained are the result of a remarkable effort by the scientific, technical and administrative staff of the Department, as well as by those running the CRA-W Quality Insurance Office.

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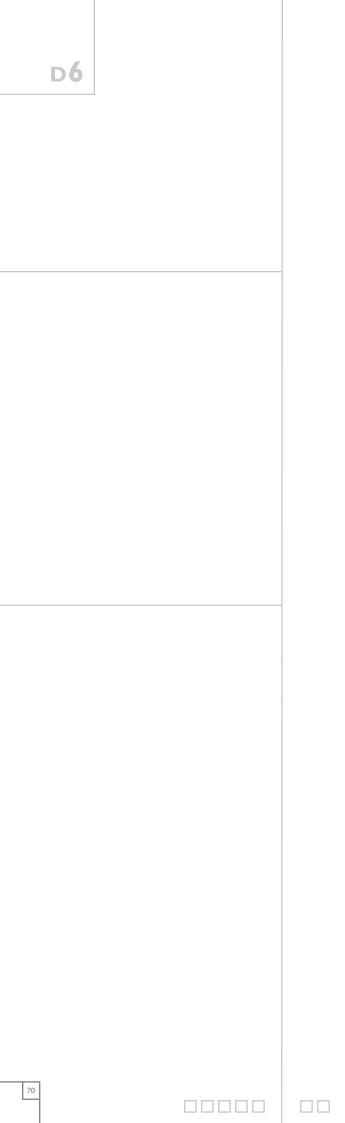
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Department

A N I M A L P R O D U C T I O N A N D N U T R I T I O N



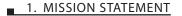
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Department ANIMAL PRODUCTION AND NUTRITION



. Research into nutritional parameters aimed at improving the quality and health impact of final products and reducing environmental pollution

. Research into the utilisation of farm produce and by-products as animal feed

. Research into herd management methods and animal welfare

2. SCIENTIFIC PERSONNEL

2 Permanent staff and 8 contract staff

2.1. Head of Department

N. Bartiaux-Thill, Inspecteur général scientifique

2.2. Scientists

C. Bauraind, Attachée scientifique (from 1/4/2004 until 31/5/2004) (RW, DGA)

L. Delbouille, Attachée scientifique (until 25/6/2004)

J. Fabry, Inspecteur général scientifique, Head of section (until 31/8/2003)

X. Frand, Attaché scientifique (until 31/1/2004) (RW, DGA)

E. Froidmont, Attaché scientifique

F. Pilette, Attaché scientifique (until 5/3/2004) (RW, DGA

P. Rondia, Attaché scientifique (RW, DGA)

Ph. Saive, Attaché scientifique

J. Wavreille, Attaché scientifique

3. NATIONAL AND REGIONAL REPRESENTATION

Fourrages Mieux, Centre agricole WR-DGA
 Filière Porcine Wallonne asbl (FPW, Official Adviser to the Pig Sector) – member of the Board of Management and Secretary/Treasurer – member of the Scientific and Technical Committees : Genetic, Animal Health and Feeding; Buildings and Facilities; Economics and Environment.

Filière Viande Bovine Wallonne (FVBW, Official Adviser to the Beef Sector) – member of the Board of Management (representing the scientific sector)
Fédération Interprofessionnelle Caprine et Ovine Wallonne (FICOW, Official Adviser to the Sheep and Goat Sector)) – active member and member of the Research Commission – Chair of the Dairy Commission

"Porc de Qualité Différenciée" Commission : Committee concerning improved quality products
Association Wallonne des Eleveurs d'Ovins et Caprins (AWEOC) Dairy Sheep breed commission

 Coordinator and Secretary to the Commission
 Coordinator and Secretary to the Commission
 Commission consultative scientifique pour les produits agro-alimentaires (Walloon Commission concerning the quality of agro-food products)
 deputy member and member of the Poultry and Pigs Scientific Committees

Cluster Nutrition (Interfaces: Universities

Industries, Nutrition and Health Professionals)
 member

 Association pour la Promotion des Protéagineux et Oléagineux (APPO, Belgian Association for Oilseeds and Protein crops) – member of the General Assembly • Animal Experimentation Ethical Committee: CRA-W, FUSAGx, FUNDP – Vice-President

• BASE scientific journal (Biotechnology, Agronomy, Society and Environment) – member of the Scientific Committee

• Regional Platform of Cow Mammary Gland Health (OSaM) – member

4. INTERNATIONAL REPRESENTATION

• European Association for Animal Production (EAAP) – Cattle Production, Pig Production and Animal Physiology commissions

• Association Française de Zootechnie (AFZ, French Animal Production Association) – member of the General Assembly

 Association Française de la Production Fourragère (AFPF, French Forage Production Association)
 – member

 European Association for Grain Legume Research (AEP) – active member (as an organisation/enterprise) European Society for Agricultural and Food Ethics (EurSafe) - member

5. RESEARCH TOPICS

Livestock farming in the 21st century is expected to produce healthy food, preserve the environment and meet producers' economic and social requirements. These criteria of concrete benefits for farming, environmental protection and product quality form the basis for our research work, the results of which are perceptible throughout society. For the Department's research staff, serving agriculture and society means being closely involved in the different agricultural sectors and maintaining contacts with producers and all the players in the agri-food industry. It also involves a variety of partnerships, of a scientific, economic or social nature. It should be pointed out that animal production in the Walloon Region and in Belgium as a whole is a key sector of the agricultural economy, alone accounting for around 61% of final agricultural production.

In order to remain competitive today, the sector is obliged to diversify in terms of activities, production techniques and scope for utilisation. Account has to be taken of a number of issues, such as product quality and health benefits, caring for the environment and animal welfare considerations, in a context of the revision of CAP and opening up of markets.

In accordance with the many and varied expectations of the sector and of society, the research activities of the Department are divided into five main topics. The aims of each of these are described below.

1. Herd management methods

• Trying out new, non-traditional herd management methods, often with considerable added value, permits diversification.

 Using appropriate technology to optimise herd management with the aid of decision support tools (e.g. milk urea as a dairy herd management indicator) to make our livestock enterprises more competitive and to make life easier for farmers

2. Utilisation of local forage resources as animal feed

Cattle feed is a major component (> 60 %) of the production cost.

Making use of forage produced on the farm and optimising its utilisation permits:

- a reduction in feed costs

- better traceability

- shrewd, sustainable management of the "plant – animal" system

3. Nutriment digestion with a view to sustainability

Environmental pollution (particularly by nitrogen) can be reduced:

 - by enhancing nutriment digestion (using enzymes)
 - by making good use of AAs in a free form, even in ruminants

4. Quality of products, safety of production methods and human health

- Producing food which is naturally rich in certain components that are beneficial to health as well as of high nutritional value.

- Adapting and developing raw materials in response to the agro-industry's expectations.

5. Animal welfare and ethics of production methods

Developing farming practices to promote animal welfare and greater convenience for farmers.
Stimulating dialogue between science and society and looking at prevailing production systems from an ethical point of view.

6. RESEARCH REPORTS

6.1. Herd management methods

Outdoor pig breeding Wavreille J., Pilette F., Bauraind C., Bartiaux-Thill N.

At the end of the WR–DGA project entitled "Outdoor pig breeding: an alternative for differentiated production", 23 Walloon farms are now keeping pigs out of doors. Outdoor production is mainly oriented to rearing, reflecting the primary reasons for the development of this alternative system, with 805 sows or 3% of all sows in the region being reared outdoors. One thousand one hundred and fifty-five fattening places were counted and fattening is carried out under the aegis of the Porc Qualité Ardenne cooperative, which is now marketing a new product at the supermarket, 'Outdoor Pork', with the approval of EQWALIS (a collective Walloon brand).

The outdoor method requires little in the way of initial investment and is equally suitable for a family farm or a larger operation, the only really limiting factor being the availability of land.

The installation cost of one sow hut amounts to \notin 700 as against \notin 3,500 indoors. The cost price per weaned piglet comes to \notin 36 in the first year of amortisation for 18 piglets weaned annually,



falling to € 21 for 22 piglets weaned after 15 years' amortisation of the insemination facility. The investment for one porker hut is € 143, compared to € 250 indoors. The cost price of a 125 kg live weight porker comes to € 140 in the first year of amortisation.

Duna and France Hybride sows account for over 80% of the total sow population on the farms. These breeds offer the best breeding performance, at 9.0 and 8.7 piglets weaned per litter respectively. The Department's results for a comparison of outdoors versus indoors confirm the expected values in the second year, namely one fewer piglet weaned per outdoor litter. As regards the genetic types studied, the Belgian Landrace underperforms the Scapaag sow at 7.8 and 8.8 piglets weaned per litter respectively. The number of piglets weaned annually per sow varies little, however, at 18.4 for the Belgian Landrace and 19.1 for the Scapaag. The Duna thus remains the favourite and is generally recommended to would be breeders. In earlier trials the Duna weaned 22 piglets per annum.

Outdoor post-weaning performance is comparable to indoor results. A hut similar to the fattening hut can be used.

Fattening performance on the farm can be quite appreciable, with a mean daily weight gain of over 800 g/d and a feed conversion rate of under 3. Addition of extended linseed to pig feed enhanced the nutritional quality of meat from pigs fattened outdoors. However, the formulation adds an extra ${f \in}$ 2.95 per tonne of feed. Soybean meal may be replaced by a pea-rape mix at the finishing stage, with an improved mean daily weight gain and feed conversion rate, especially since the formulation makes for a saving of € 2.5 per tonne. Generally speaking, outdoor pig rearing offers an alternative for the development of pork production in the Walloon Region. Outdoor pig breeding explores a quality niche and could be considered as a sustainable production method. For farmers, it offers diversification without major investment. Various labelled lines have appeared on the market, such as "Le Porc du Pays des Collines,""Le Porc des Prairies d'Ardenne,""Le Porc Plein Air" and "Porc Bio".

Assessing the need for iron injections for piglets born and reared outdoors

J. Wavreille, F. Pilette, L. Mandelaire*, N. Bartiaux-Thill Pig-rearing technicians more familiar with the methods used in indoor production generally recommend the same prophylactic measures to outdoor pig breeders, such as iron injections for 3-day old piglets. Some research was undertaken to establish the benefits or otherwise of injecting piglets born outdoors with iron dextran and to assess the effect of iron injections at three days or twelve days on the piglets' production performance and haemoglobin levels. None of the piglets showed signs of anaemia during the experiment and haemoglobin levels were all above the required minimum of 6 g/dl. At age 21 days, there was no significant difference in average haemoglobin levels between the piglets not receiving iron (16.1 g/dl) and their counterparts which received iron injections. In the latter case, haemoglobin levels were significantly lower in piglets treated at 12 days old compared to those

treated at 3 days, being 15.0 g/dl and 16.7 g/dl respectively. No significant differences in haemoglobin levels were revealed at 28 days. It was also found that iron injection, at either 3 or 12 days old, does not enable piglets to achieve a higher mean daily weight gain than those not receiving iron. No significant differences between treatments were in fact revealed for mean daily weight gain between 3 and 28 days old. Results were similar in the ensuing 45 day post-weaning period, with no significant differences in mean daily weight gain between the three modes of treatment. Our results confirm that the suckling and postweaning performance of piglets born and reared outdoors is not affected by not injecting them with iron dextran (Brown et al. 1996, Kleinbeck and McGlone 1999, Delbor et al. 2000 and Gueguen et al. 2002).

*Institut Supérieur Provincial d'Agronomie de Ciney

Effects of outdoor versus indoor sow keeping on the milk fatty acid pattern J. Wavreille, F. Pilette, F. Dehareng*, N. Bartiaux-Thill



Although their mother's milk is the piglets' primary food, there has been little research into the fatty acid pattern of sow's milk fat and still less into the effects of the rearing conditions. Eighteen sows fed on the same standard feed were kept half indoors on straw litter and the other half outdoors on grass. The sows were milked by hand on the weaning day. Analysis of the fatty acid pattern shows sows' milk to have a very low proportion of short - C4, C6, C8, C10 - and medium - C12, C14, C18 - fatty acids compared to



cow's milk. Sow's milk is rich in unsaturated fatty acids in both indoor and outdoor keeping, at 59.6 % and 61.2 % respectively. Cis versus Trans conformations are very well represented with the total proportion of C16:1 cis, C18:1 cis9, C18:2 c9c12 and C18:3 c9c12c15 amounting to over 55 %. The proportion of linolenic acid is significantly higher in outdoors milk (2.53 %) compared to indoor milk (2.08 %) due to eating grass when kept on pasture. The $\omega 6 / \omega 3$ ratio is significantly lower in the case of outdoors milk. Likewise, outdoors milk is significantly richer in conjugated linoleic acid at 0.13 % as against 0.09 % indoors.

Compared to cow's milk, sow's milk is characterised by more unsaturated fatty acids that are proportionally longer and tend to be of *cis* conformation. Our results are further evidence for the positive effects on milk quality of putting sows out to pasture.

* CRA-W, Quality of Agricultural Products Department

Dairy sheep rearing in the Walloon Region

P. Rondia, Ch. Delmotte*, N. Bartiaux-Thill Milk sheep production is attracting growing interest from producers and transformers in the Walloon Region.

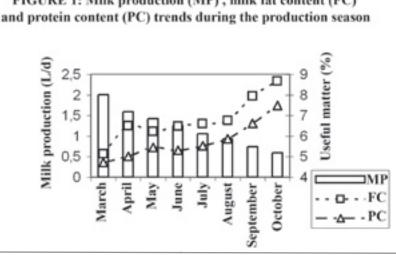


FIGURE 1: Milk production (MP), milk fat content (FC)



Rearing dairy ewes could offer a profitable avenue for diversification of animal production in the Walloon Region.

The main aim of this project was to set up a database focusing on the specific features and technical aspects of dairy sheep farming. Implementation involved not only measuring ewe production performance (milk production) and breeding performance (reproduction) but also describing the specific rearing techniques involved. This study was conducted in real conditions on

two sheep farms in order to produce a true image of the diversity of situations occurring in the Walloon Region. Each of these farms pursues a different aim: one focuses on producing milk and lambs for meat, while the other concentrates exclusively on milk production.

To establish the production performance of the ewes in terms of both quantity (milk production) and quality (useful matter as a combination of fat and protein contents; somatic cell count), official milk recording had to be put in place on the farms taking part in the study. Compared to multiparae, primiparae have a longer suckling period and a shorter lactation period (Table 1).

Parameters	Primiparae	Multiparae
Suckling period (days)	33	18
Lactation period (days)	142	208
Milk production (litres)	140	259
Daily production (L/d)	0,96	1,24
Fat content (%)	6,53	6,67
Protein content (%)	5,76	5,60

Production of primiparae and multiparae

The milk production trend is illustrated in Figure 1. Daily milk output dwindles as the production season advances, falling from 2 litres per ewe in March to 0.6 litres by October. At the same time, the protein and fat contents increase perceptibly, reaching over 7% and 9% respectively by the end of the season.

Bacteriological analyses show that tank milk from the two farms meets the standards in force. Mammary infections in smaller ruminants differ from those in cattle chiefly in their ethiology and a lower average incidence of clinical cases. The annual clinical case rate in sheep farming is no more than 5% of all animals, a notably lower level than in dairy cattle. However, the somatic cell count (SCC) in a healthy ewe is perceptibly higher than in the cow, with counts between 500,000 and 1,000,000 cells/ml being generally considered normal. We found that cell counts rise with rank and stage of lactation. Approximately 70% of the sheep have an SCC under 500 thousand cells and these account for 10% of the total SCC, whereas 20% of the sheep population make up over 80% of the total SCC.

(1) DGA, Development Department

6.2. Use of local forage resources as animal feed

The CRA-W has been studying the use of the lupin as animal feed since 2000, with the aim of achieving greater autonomy in plant proteins and thus making Europe less dependent on the American markets, ensuring the full traceability of our animal products and guaranteed GMO-free cattle feed. The research is directed at assessing the nutritional value of this legume for various of species (dairy cows, young bulls, pigs and poultry) and gauging the effectiveness of various treatments (enzymatic, mechanical, physico-thermal) in order to optimise its uptake by the animal.

- Use of lupin to completely replace soybean meal in the feed of high producing dairy cow
 - E. Froidmont, N. Bartiaux-Thill

Six Holstein cows were given 3 iso-N diets according to a Latin square experimental scheme. The soybean meal in the control diet (4 kg/d) was replaced on a nitrogen basis by 6.1 kg/d of lupin (*Lupinus albus*, var. Lublanc - lupin ration) and a mixture of 3.1 kg/d of lupin and 4.1 kg/d of pea (lupin + pea ration). The other components of the ration remained unchanged altered in a very similar fashion to that described in our 2000-2002 report. This trial demonstrates that protein from coarsely ground lupin is of equal value to soybean protein for the high-producing dairy cow.

Effect of total replacement of soybean by lupin seed or pea on nitrogen retention and digestive parameters in the doublemuscled Belgian Blue bull culard *E. Froidmont, L. Delbouille, X. Frand, N. Bartiaux-Thill*

Four double-muscled Belgian Blue bulls fitted with a cannula in the rumen, duodenum and ileum were given three iso-DM diets according to a 3*3 Latin square experimental design with one extra animal. The only difference between diets was the main protein source, namely 16% soybean, lupin seed or peas, coarsely ground. The key results show that the part of N digested that the animal retained is similar on the soyabean and lupin diets (38%) and lower on the pea diet (34%). The lower nitrogen value of the pea is due to the fact that it provides 60% less digestible dietary protein than the lupin or soybean. The coarsely grinding of lupin seeds enabled to optimize the digestible protein supply in the small intestine. The dietary and microbial components of these proteins were similar in both diets, indicating that the lupin can replace soybean on a nitrogen basis in this sector.

	Pro	Protein source			
	Soybean	Lupin	Lupin + Pea	ESM	P
Production parameters					
Production, L/d	34,2 ^a	b 35,7	a 34,7	0,15	0,001
F. c. milk production, L/d	31,8	31,8	31,4	0,18	0,616
Fat content, %	3,52 ^a	b 3,21	ab 3,34	0,03	0,001
Protein content, %	3,18	3,16	3,18	0,01	0,602
Fat, g/d	1186	1121	1127	11,89	0,077
Protein, g/d	a 1081	b 1124	ab 1100	4,96	0,004
Urea, mg/L	ab 403	а 416	372 b	5,07	0,004
fficiency of ingested protein, %	25,74	25,05	25,57	0,12	0,062
Veight at beginning/end of period, kg	750 [×] /735 [×]	742 / 743	752 [×] /742 [×]	0,85	0,001

h1- 0

Effect of the protein source on milk production parameters, feed efficiency and cow weight ^{ab} Within a row, means lacking a common subscript differ

^{*ab*} Within a row, means lacking a common subscript differ (P<0.05)

The main results (Table 2) show that fat corrected milk production was not affected by the protein source. However, the milk fat content fell on with the lupin diet, possibly indicating an excess of some dietary fatty acids, notably C18:1 which is present in a high concentration in the white lupin. Only the animals on the lupin diet maintained their live weight during the trial. The efficiency of the ingested protein was not affected by the protein source. The milk fatty acid profile was Value of mixed maize and lupin silage to high-producing dairy cows

E. Froidmont, L. Delbouille, N. Bartiaux-Thill

Maize silage is recognized as a good energy source for high-producing dairy cows. Conversely, the lupin is a protein-rich plant which, like all legumes, is hard to ensile on its own. The aim of the study is to mix maize and lupin in the same silage to produce a feed with a more balanced nutritional value in relation to the animal's requirements and thus to limit concentrate addition. The method involved planting a late variety of lupin (Lupinus albus, var. Energy) alongside an early maize variety (var. Pernel) so that both could be harvested the same day. The mixed silage was richer in proteins than conventional maize silage (11.8 vs 6.9%) and had a balanced OEB (0.8 vs - 30.0 g/kg DM). The animal trial involved feeding two basic diets comprising 56% maize silage or mixed maize / lupin silage to two homogeneous groups of nine cows. The diet provided the same quantity of net energy and digestible protein and enabled a milk production of 26 L/d. Those cows with higher production potential were supplemented with a production concentrate (200 g/0.5 L extra). Feeding mixed silage allows the total concentrate to be reduced by 500 g/cow/d while significantly increasing fat corrected milk production (27.5 vs 25.8 L/d) and without affecting the milk fat and protein contents. If this method proves costeffective it may offer a means of giving farms greater protein autonomy.



Lupin

Effects of replacing soybean by lupin seed, with or without α-galactosidase supplement, on porker production performance

E. Froidmont, J. Wavreille, N. Bartiaux-Thill

Six groups of 8 pigs were fed 3 rations throughout the growing-fattening stage according to an complete randomized block design. The diet were iso-N and iso-energy and all contained over 70% wheat and either 15% (soybean) or 20% lupin (*Lupinus albus*) with (Lupin + gal diet) or without (Lupin soybean diet) α galactosidase (Novozymes, S. A.). The results show that the animals fed the Lupin + gal diet turned in similar production performances to those fed on soybean, whereas the animals on the Lupin diet performed poorly (Table 3). Carcass quality and the meat fatty acid profile were not affected by the diet. The fatty acid profile of the backfat in the animals on the Lupin diet contained a higher proportion of C18:1 and less C18:2 and had a lower omega-6/omega-3 ratio. This research confirms that in the presence of α -galactosidase, lupin can replace the greater part of the soybean without impairing carcass quality, while actually improving the fatty acid pattern of the backfat.

Determining the most suitable lupin variety for pig feed, from Belgium's most productive varieties E. Froidmont, J. Wavreille, Ph. Saive

Three iso-N and iso-energy diets were compared in three groups of four pigs fitted with a cannula at the end of the small intestine. The diets contained 22% Lupin together with an α -galactosidase supplement and were distinguished solely by the variety of lupin (Lupinus albus cv'Lublanc', Lupinus albus cv amiga, Lupinus angustifolius cv Boltensia). It was noted that apparent N digestibility was greater on the 'Boltensia' diet. Fibre fermentation in the large intestine was also greater with that diet, reflecting differences in the polysaccharide composition of lupin varieties. Despite the greater N digestibility, the 'Boltensia' diet was characterised by greater urinary N excretion than was the case with diets based on white lupin. Nitrogen retention was thus better with the Lublanc variety, and poorer with Amiga and Boltensia (21.2, 18.0 and 18.4 g/d respectively). Similarly, the N ingested (P < 0.001) and digested (P < 0.001) was better utilised on the Lublanc diet compared to Boltensia. The results indicate that when supplemented with galactosidase, the Lublanc variety is a more suitable pig feed than the Boltensia variety.

Lupin as an alternative to soybean meal for poultry feed : dose rate and efficiency of an enzyme supplement E. Froidmont, Y. Beckers', N. Bartiaux-Thill, J-B. Aurov²

324 chickens were fed three iso-energy and iso-first limiting amino acid diets (Met, Lys, Thr and Trp) containing either 35% soybean, 30% lupin and 18% soybean or 58% lupin as the main protein sources, with or without an enzyme supplement (pectinase and hemicellulase) according to a complete randomized block design. All the diets included β -glucanase, cellulase and xylanase. The mean daily weight gain, feed conversion rate, N retention, apparent nutriment digestibility and metabolisable energy were significantly lower on the 58% lupin diets. The gizzard weight and intestine length (relative to live weight) and the viscosity of the digesta were greater (P < 0.05) in the birds on those diets. No effects of the enzyme supplement on the measured parameters were noted in this trial. Except for lower C16:0 and C16:1 levels with lupin-based diets, no differences were found in the thigh fatty acid content, fat or protein. These results indicate that the enzyme supplement in this trial does not effectively enhance lupin utilisation in poultry feed and, for the time being, lupin can only partially replace soybean in this sector. FUSAGx, Animal Science Unit ² ENESAD, Dijon, France

6.3. Nutriment digestion with a view to sustainability

Precision nitrogen feeding for the double-muscled Belgian Blue bull P. Rondia, E. Froidmont, Y. Beckers' et N. Bartiaux-Thill

The double-muscled Belgian Blue bull is one of the jewels of Walloon stock breeding. While often chosen for its big growth potential and high proportion of carcass muscle, its particular features also mean it has specific nutritional requirements, notably in terms of proteins and digestible amino acids (AAs), that are not always liable to be satisfied by conventional rations based on forage as the main raw material. The lack of one single essential AA is sufficient to inhibit protein accretion and thus prevent the animal's genetic potential from being fully exploited. Moreover, such a lack prevents greater utilisation of the non-limiting AAs, which are catabolised and thus increase urinary nitrogen excretion. Precision nitrogen feeding, providing the correct proportions of the AAs which the animal needs, ought therefore to have two major effects: increase growth performance and reduce nitrogen rejections.

As part of a DGA-WR research project CRA-W studied the AAs provided by a conventional, maize silage based ration and discovered a significant lack of histidine, methionine and lysine for growing animals (300 kg). (Table 4).

EAA (g/d)	His	Met	Arg	Lys	
Supply of digestible EAA (g/d)	12,5	10,5	37,0	38,8	
Digestible EAA requirements (g/d)	34,9	22,5	71,9	75,0	
Supply / requirements (%)	35,9	46,7	51,5	51,8	

Classification of EAA by limiting extent

_		Diet			
	Soybean	Lupin	Lupin + gal	ESM	P diet
Starting weight, kg	32,4	32,0	32,0	0,18	0,462
Final weight, kg	108,4	106,8	106,9	0,41	0,280
Fattening time, days	a 104,3	b 122,7	a 110,4	2,06	0,005
Mean daily weight gain, kg/d	a 0,742	b 0,613	0,684 ^a	0,01	0,001
Feed consumption, kg/d	a 2,230	b 2,035	ab 2,130	0,02	0,040
Feed conversion rate	a 3,080	b 3,355	a 3,140	0,01	0,004

Production performance according to diet

Based on these results, an additive enriched with these AAs was then formulated to increase growth performances during the growing and fattening phase. The special feature of our research was that the AAs were provided in free form as a considerable part of the AAs (35% in this study) can leave the rumen intact in the Belgian Blue. This casts doubt on the profitability of the AA protective coating, contrary to what is sometimes said in the case of the high-producting dairy cow. The additive also contains an appetizer to promote rapid ingestion, thus maximising the quantity of digestible AAs in the small intestine. Results indicate that the additive has the effect of increasing the mean daily weight gain by 250 g/d and the utilisation of ingested N by 12% during the growth phase, with no effect at the finishing stage.

The cost price of the histidine-rich additive is currently too high to be economically profitable. Our research shows the importance, in breeding and ecological terms, of a balanced ration for the Belgian Blue bull based on digestible AAs. Alternatives to free AAs can also be considered as a means of meeting the Belgian Blue's AA requirements, such as using co-products with a very useful AA pattern.

¹FUSAGx. Animal Science Unit

6.4. Product quality, safe production methods and human health

Human health and improving the fat composition of animal products



In Western societies an unbalanced dietary consumption is observed, in particular an excess of saturated fatty acids is consumed and a too high n-6/n-3 polyunsaturated fatty acids ratio is noticed. Numerous research activities have been devoted to increasing the levels of n-3 polyunsaturated fatty acids, having beneficial effects on human health, in widely consumed products of animal origin whose lipid composition is easily modified.

Various feeding trials have been conducted with extruded linseed with a view to improving the dietetic quality of the fat in cattle (milk), pork, sheep (meat and milk) and poultry (broiler and foie gras) products.

Lamb and ewe's milk P. Rondia, Ch. Delmotte¹, F. Dehareng², Y.Larondelle³, V.Decruyenaere⁴, J.Famerée¹, N. Bartiaux-Thill

Lambs, were the subject of two trials to investigate the effects of extruded linseed supplementation in ewe and lamb feed on production performance and the fatty acid composition of the meat from lambs reared either indoors or at pasture.

				table 0		Milk fatty acids pattern a
FA (% total FA)	Hou	ised		Grazing		type of supplementation ab Within a row, means lo common subscript differ
	т	L	т	L	н	
C18:0	15,6	16,8	15,2 ^a	14,6 ^a	18,4 ^b	In the "grazing" trial,
C18:1 trans	5,97	7,53	2,71 ^a	5,63 ^b	3,60 ^{ab}	supplementation do
C18:2 <i>cis</i> n - 6	7,71	6,71	5,81 ^a	6,58 ^a	4,37 b	have a markedly po
C18:3 n - 3	1,35 ^a	3,07 ^b	1,31 ^a	2,84 b	2,66 b	effect on the dieteti
CLA	0,95 ^a	1,17 b	0,82 ^a	0,94 ^{ab}	1,25 b	of the milk fatty acids. linolenic acid (C18:3 of
n - 6/n - 3	5,89 ^a	2,32 ^b	5,75 ^a	2,75 ^b	2,28 b	content of milk from
Σsfa	47,1	46,7	47,1 ^a	45,5 ^a	49,3 ^b	ewes remains relativ
Σμυγά	42,4	42,0	43,3 ^a	42,9 ^a	40,8 ^b	despite the consider
Σρυγα	10,6	11,3	9, 5 ^a	11,5 ^b	9,9 ^{ab}	quantities provided b
	1	1		1	· ·	grass and linseed. Co

table 6

Fatty acid pattern of meat from housed and grazing lambs with (L) or without (T) linseed supplementation or finished at pasture (H)

ab Within a row, means lacking a common subscript differ (P<0.05)

In the case of housed lambs, linseed supplementation (15% of their diet) significantly increases the C18:3 omega-3 and CLA levels while lowering the n-6/n-3 ratio (Table 5). The same observations

> can be made with the grazing lambs finished indoors (T and L). Likewise, the high proportion of C18:3 omega-3 in lambs finished on grass (H) can be attributed to significant PUFA intake (chiefly C18:3 omega-3) from the grass. Grazing also substantially increases the CLA content.

The milk feeding of the lamb also affects the meat fat composition.

The closer the date of slaughter to weaning, the more marked is this effect. This may account for the beneficial effect of feeding suckling ewes a linseed supplement on the fatty acid pattern of meat from housed lambs.

Ewes for milk production, were the subject of two trials, one indoors (winter) and the other at pasture (spring).

The "indoor" trials shows that adding 5% extruded linseed to the concentrate perceptibly improves the dietetic quality of the ewe's milk fat, increasing the C18:3 omega-3, CLA, MUFA and PUFA and reducing the SFA and the omega-6/omega-3 ratio (Table 6).

	Indoor t	rial	Grazing	trial
% total FA	Control	Linseed	Control	Linseed
SFA	78,88 ^a	70,46 ^b	70,18 ^a	67,05 b
MUFA	17,89 ^a	23,56 b	24,00 ^a	26,34 ^b
PUFA	3,22 ^a	5,98 b	5,82	6,61
C18:0	6,65 ^a	10, 22 ^b	8,38	9,81
C18:1 11 - trans	1,40 ^a	3,23 b	4,53 ^a	6,19 ^b
C18:3 cis	0,61 ^a	1,51 b	0,76	0,87
CLA	0,59 ^a	1,17 ^b	2,05	2,34
Ratio				
n-6/n -3	2,96 ^a	1,81 ^b	1,99	1,82

accordina to lacking a ·(P<0.05) , linseed

oes not ositive ic quality The alphaomega-3) m grazing ively low, erable by the Conjugated

linoleic acid (CLA) levels in the milk are very high in the spring trial, confirming the beneficial effect of grazing on endogenous production of these fatty acids.

A comparison of the milk fatty acids pattern in the control lots in the spring and winter trials shows that grazing generally has a positive effect on the dietetic quality of the fats.

(1) DGA, Development Department

(2) CRA-W, Quality of Agricultural Products Department (3) UCL, Unit of Nutrition Biochemistry

(4) CRA-W, Agricultural Systems Unit

Broilers and foie gras

P. Rondia, Ch. Delmotte¹, K. Raes², D. Maene³, J.Famerée¹, N. Bartiaux-Thill

The first study looked at the effects of the inclusion time of extruded linseed supplementation (3.25% of the diet) before slaughter on intramuscular fat composition in broilers. The second study focused on the effects of extruded linseed supplementation (2 and 4% of the diet) during the force-feeding period on the fatty acid composition of foie gras and intramuscular fat in ducks. The broiler trial comprised 900 subjects and three different feeding programs during the experimental period (between 46 and 73 days of age). Firstly, the control diet throughout the period (R0); secondly, the control diet from age 46 to 59 days followed by the linseed diet from 59 to 73 days (R14); and finally the linseed diet for the whole period (R27). A significant increase in the proportion of C18:3 n-3 was observed with linseed supplementation, the biggest increase accompanying the longest feeding period (27 days as against 14 days before slaughter) (Table 7).



Fatty acids (g/100g FA)	RO	R14	R27
C18:3 n - 3	1,74 ^a	2,93 ^b	3,80 ^C
Σ SFA	34,38	33,97	33,57
Σ MUFA	31,74	32,36	32,97
Σ PUFA	33,88	33,66	33,48
n-6/n -3	18,54 ^a	10,28 ^b	7,63 ^C

Fatty acid composition of chicken (thigh) intramuscular fat according to diet

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

The duck trial comprised 30 hybrid ducks during the force-feeding period (13 days). The test subjects were divided into three groups according to diet: maize grain on its own (control diet -T-); maize grain with 2% extruded linseed (L2%) and maize grain with 4% extruded linseed (L4%).

FA (g/100g)	т	L2%	L4%
Foie gras			
C18:3 omega 3	0,090 ^a	0,228 ^b	0,354 ^C
omega- 3 AGPI	0,158 ^a	0,396 ^b	0,558 ^C
omega- 6/omega - 3	23,4 ^a	6,7 ^b	4,5 ^b
SFA	37,8	37,6	39,3
MUFA	58,4	58,6	56,5
PUFA	3,05	3,10	3,08
Thigh			
C18:3 omega 3	0,654 ^a	1,788 ^b	2,548 ^b
omega - 3 PUFA	1,214 ^a	2,400 ^b	3,234 ^C
omega - 6/omega - 3	18,5 ^a	7,2 ^b	4,9 ^b
SFA	31,7	30,5	31,0
MUFA	48,1	49,0	47,7
PUFA	17,3	18,3	19,0
Breast			
C18:3 omega 3	0,526 ^a	1,448 ^b	1 ,912 ^C
omega - 3 AGPI	1,252 ^a	2,586 ^b	3,086 ^C
omega - 6/omega - 3	23,6 ^a	9,4 ^b	7,0 ^b
SFA	34,7	33,4	33,0
MUFA	42,2	40,3	39,6
PUFA	19,4 ^a	22,4 ^b	23,5 ^b

ble 8

Fatty acid pattern of foie gras and intramuscular fat in ducks

Linseed supplementation improves the fatty acid pattern by increasing the omega 3 fatty acids in the liver and intramuscular fat and lowering the n-6/n-3 fatty acid ratio (Table 8). Despite the higher alpha-linolenic acid level in the diets, the proportion of that fatty acid remains relatively low in the liver of force-fed ducks. Diet has a more significant effect on n-3 fatty acid levels in intramuscular fat. However, some differences in the fatty acid pattern emerge according to type of muscle (thigh or breast).

A sensory test by a panel of experts in fact shows that the diet with the lower proportion of linseed has a positive effect on the appearance of foie gras.

¹DGA, Development Department

²CLO, Department of Animal Production ³UCL, Genetics Unit Effect of vitamin E supplementation on ruminal biohydrogenation mechanisms and on the lower milk fat content Y. Larondelle (UCL), J. Pottier (UCL), E. Froidmont (collaborateur CRA-W)

The results of this experiment, conducted by UCL(Catholic University, Louvain la Neuve), suggest that vitamin E supplementation (12,000 Ul/cow/ day) in a diet containing extruded linseed (2 kg/d) and linseed oil (200 g/d) prevents the lowering of the milk fat content that often occurs with fat-rich feeding. Vitamin E supplementation also increases the biohydrogenation intermediate fatty acid concentration in the milk and the level of rumenic acid, the main CLA secreted. The beneficial action of vitamin E in limiting the transformation of trans-11 fatty acids into *trans* 10 also means that it has to be supplied at the same time as dietary fat, as the vitamin E loses its efficacy once the reaction has taken place.

Improving the fatty acid pattern of pork – Effect of the period and amounts of extruded linseed added (1) J. Wavreille, N. Bartiaux-Thill

Adding an extruded linseed mix to pig feed throughout the fattening period or only during finishing maintains the production performance, carcass quality and meat quality characteristics. It also naturally enriches the meat with alpha-linolenic acid and EPA, one of its long chain derivatives which is particularly important to lipid metabolism in humans. The $(\infty-6)/(\infty-3)$ ratio is significantly improved. It is more effective in the subcutaneous adipose tissue of the back than in Longissimus dorsi. Adding 10% extruded linseed mixture to pig feed leads to a ω -6/ ω -3 ratio less than 5 in the adipose tissue, even though the mixture was only fed to the animals at the finishing stage. In the muscle, the response is close to 5 if the supplement is provided throughout fattening, at 5.7 instead of 7.8.

Fee	d	Effects
L	LL10f	
0,42	0,46	S**, AxS*
10,8	4,0	A***, AxS*
10,4	4,0	A***, S***, AxS**
1,30	1,35	A***, S***
0,65	0,65	S**, AxS***
	L 0,42 10,8 10,4 1,30	0,42 0,46 10,8 4,0 10,4 4,0 1,30 1,35

able 9

Fatty acid composition of the back subcutaneous adipose tissue lipids

L = standard label feed, L10 = addition of 10 % extruded linseed mixture, f = finishing only, A = feed, S = sex, * = P<0.05, ** = P<0.01, a= C18:2 omega-6/ C18:3 omega-3, b = PUFA omega-6/PUFA omega-3

Fe	Effects	
L	LL10f	
0,48	0,46	S**
20,7	7,8	A***
11,3	5,5	Ä***
	L 0,48 20,7	0,48 0,46 20,7 7,8

ableau 10

Fatty acid composition of Longissimus dorsi lipids L = standard label feed, L10 = addition of 10 % extruded linseed mixture, f = finishing only, A = feed, S = sex, * = P<0.05, ** = P<0.01, *** = P<0.001, a = C18:2 omega-6// C18:3 omega-3, b = PUFA omega-6/PUFA omega-3

The addition of 5% results in an omega-6/omega-3 ratio under 5 in the adipose tissue if the feed is provided throughout the fattening period. In this case the ratio reaches 8.7 in the *Longissimus dorsi*. If provided during finishing only, this feed limits the expected ratios in the adipose tissue and *Longissimus dorsi* to 6.3 and 11.4 respectively. This research confirms that the nutritional quality of pork can be enhanced by adding extruded linseed to pig feed. The technological properties of the adipose tissue and the meat can also be maintained, as can the production performance. *' with the cooperation of Porc Qualité Ardenne sc*

6.5. Animal welfare and ethics of production methods

The future of animal products in our food in connection with the perceived welfare of farm animals

N. Bartiaux-Thill, Cl. Lamine¹, P. Stassart¹, Y. Beckers², D. Stilmant³, A. Théwis² and J. Wavreille

This project is part of the "Feed the Discussion (Alimenter le dialogue)" programme run by the King Baudouin Foundation and is being carried out in partnership with Gembloux Agricultural University and the University of Liege, Department of Environmental Sciences and Management. The aim is to reconcile animal welfare (animal rights) and consumer wellbeing (food rights) with farmers' welfare. Animal welfare is a topical issue in society that has, we feel, been treated in too compartmentalised a fashion to date. Fruitful debate should be stimulated by bringing together the various players with their varied knowledge and skills (farmers, consumers, economic players, scientists and so on). The public scientific institutions have a role to play here, especially in terms of passing on knowledge and thus also mediating.

The first phase of the project (January-April 2004) enabled us, through individual interviews with a number of players in this field, to establish exploratory dialogue and thus create confidence at an individual level before moving on to collective initiatives. It was also an opportunity to document and explore the arguments and stances of the various players with respect to animal welfare and the different methods of consultation. This work was undertaken and feedback supplied by an interdisciplinary expertise group of fifteen people drawn from the scientific community (zootechnicians, ethologists, agronomists, sociologists and philosophers).



The intention is for the group to develop gradually within the framework of the project (June 2004 – May 2006), *via* the pooling and comparing of individual areas of expertise, towards a joint building up of collective expertise. To this end, the dialogue begun by this group of scientists will be widened and enriched by the creation of forums bringing in producers and consumers. Finally, the results generated by the group of experts and the different forums will be fed back and discussed with the consultative bodies in place: the sectoral councils (under the aegis of APAQ-W), the Animal Welfare Council (Federal Ministry of Health) and of course CRIOC (the Consumer Organisations' Research and Information Centre).

Also, the creation of an Internet forum for 'virtual' exchanges of information and views between the different project partners and the sectors concerned should facilitate the expression and clearing away of barriers to comprehension and misunderstandings.

A intermediary report entitled "Strengthening the link between consumers, farmers and animals" is available on the King Baudouin Foundation site: http://www.kbs-frb.be/files/db/FR/Alimenter_le_ dialogue_Projet_ULg_Rapport1.pdf.

1ULg, Department of Environmental Sciences and Management 2FUSAGx, Animal Science Unit

3CRA-W, Agricultural Systems Unit

7. SERVICE ACTIVITIES

7.1. Cattle

 Expertise in controlling environmental factors in stock farming (production factors and use of farmyard manure in cooperation with the Agricultural Systems Unit), herd feed management and analysis of nutritional parameters for improving milk quality (fatty acids pattern, lipolysis, scientific cooperation with Catholic University of Louvainla-Neuve).

- Member of the OSaM platform (Dairy cow mammary gland health monitoring)

- Expertise in compiling the positive list of foods recognized by the FVBW (Official Adviser to the Bovine Sector)

- Member of the "Nitrogen efficiency of the Belgian Blue bull: potential for utilising negative OEB diets during the growing/fattening period" project support committee (MRW, DGA, D31-1072).

 Collaboration with the Quality of Agricultural Products Department in evaluating milk contamination by chlorohexidine, an antibacterial agent used as an udder disinfectant in dairy cattle, following pre-milking application.

7.2. Pigs

- Expertise in outdoors pig rearing (breeding and fattening).

- Support and guidance for the pig farming sector, including calculating pig rations, farm management and pig feed training.

- Expertise in connection with PSE meat at the abattoir.

- Scientific collaboration on carrying out pig farming experiments on behalf of Gembloux Agricultural University (FUSAGx):

 Utilisation of bovine colostrum in pig breeding, before and after weaning, as an alternative to antibiotic food additives'. FUSAGx Animal Science Unit and Animal and Microbial Biology Unit in cooperation with the UCL (Catholic University of Louvain-la-Neuve) Experimental Surgery Unit and the CER (Rural Economy Center) Colostrum Bank. 'Better health and a higher meat selenium content. Effects of organic selenium supplemen-

tation on pork'. FUSAGx Animal Science Unit in cooperation with the Strée Agricultural Technologies Centre, Kemira, Scar, the University of Bristol, the ULB Pharmaceutical Chemistry Laboratory and the FUSAGx Food Industry Technology Unit. • 'Effects of pig age and ration fibre content on *in vitro* fermentation of pre-digested beet pulp'.

FUSAGx Animal Science Unit.

Participation, as an expert member for pork products, in designing the 'Veal-cows-pigschicks... What do we eat?' exhibition. http://www.pass.be/fr/infos/espace/belvedere/ veaux-vaches-cochons-couv_es.shtml, Frameries Science Adventure Park, 28/02/2003
Member of the support committee for the MRW-DGA project on Ardennes Quality Pork "Traceabil-

ity, new products and PGI farm pork" - Member of the support committee for the MRW-DGA FPW (Official Adviser to the Pig Sector) project "Framework agreement for the Sector

- Council"
- Member of the reading committee for:

• the "Nitrogen production value concession procedure assessment report. Proposals for changes". NITRAWAL 2004, Lambert R.

• "Pork from A to Z". FPW 2004, Lambert L.

7.3. Digestibility of nutrients

1. Faecal digestibility of nutrients (pigs, ruminants, poultry)

2. Use of cannulated animals for the purposes of:
measuring the apparent digestibility of nutriments in the small intestine of pigs and ruminants (DM, OM, N, fats, raw energy, fibres, amino acids)
studying nutrient fermentation in the large intestine of pigs according to type of feed

 estimating digestible microbial protein flows in ruminants using purines as microbial markers
 estimating digestible protein flows of food and

endogenous origin in ruminants • determining microbial proteosynthesis yield in

the rumen according to feed • determining rumen fermentation parameters

(pH, N-NH3, VFA)

3. Measurement of nitrogen retention in animals and efficiency of utilisation of dietary protein

7.4. Catheterising cash stock

7.5. Analytical determination

blood metabolites (glucose, urea, creatinine, etc.), energy (bomb calorimetry), Cr₂O₃ (indigestible marker).

7.6. Supervision of 6 dissertations

by Belgian (FUSAGx, ISI-Huy, St-Quentin Ciney) and foreign (ENESAD, Dijon, France) students.



Publications

<u>Scientific</u> Publications (with a reading committee)

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Froidmont E., Bartiaux-Thill N., Delbouille L., Frand X. (2003). Influence de la substitution totale de tourteau de soja par des graines de lupin ou de pois sur la rétention azotée et les paramètres digestifs chez le taurillon Blanc Bleu Belge culard. *Journées 3R*, 10 : 395. Paris, France, December 2003.

Froidmont E., Bartiaux-Thill N. (2004). Utilization of lupin (*Lupinus albus*, var. Arès) seeds in animal diets: synthetic report. 5th European Conference on Grain Legumes & 2nd International Conference on Legume Genomics and Genetics, 41-42, Dijon, France, June 2004.

Froidmont E., Bartiaux-Thill N. (2004). Suitability of lupine and pea seeds as a substitute for soybean meal in high-producing dairy cow feed. *Anim. Res.* 6 (in press).

Froidmont E., Bartiaux-Thill N. (2003). Utilisation du lupin et du pois en substitution partielle du tourteau de soja dans l'alimentation des vaches laitières hautes productrices. *Fourages* 174: 285-292.

Froidmont E., Beckers Y., Dehareng F., Théwis A., Bartiaux-Thill N. (2004). Lupin seed as a substitute to soybean meal in broiler chicken feeding: incorporation level and enzyme preparation effects on performances, digestibility and meat composition. *55th annual meeting of the EAAP* – Bled. Slovenia. 138.

Froidmont E., Delbouille L., Romnée JM, Bartiaux-Thill N. (2004). Valorisation d'un ensilage mixte de maïs et de lupin par des vaches laitières hautes productrices. *Journées 3R*, 11 : 278. Paris, France, December 2004.

Froidmont E., Schoeling O., Deliège F., Wathelet B., Wavreille J., Bartiaux-Thill N. (2003). Influence de la substitution du tourteau de soja par des graines de lupin, avec ou sans complément d'a-galactosidase, sur la digestibilité des régimes et la rétention azotée du por cen croissance. *Journées Recherches Porcines*, 35 : 105-112. Paris, France, February 2003.

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Rondia P., Larondelle Y., Delmotte Ch., Fabry J., Bartiaux-Thill N. (2003). Influence du régime alimentaire sur la composition des matières grasses du lait de brebis : pâturage vs complémentation en bergerie avec ou sans apport de graines de lin extrudées. CEREL, *Journées Filière Lait*, July 2003.

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Supervisor: E. Froidmont

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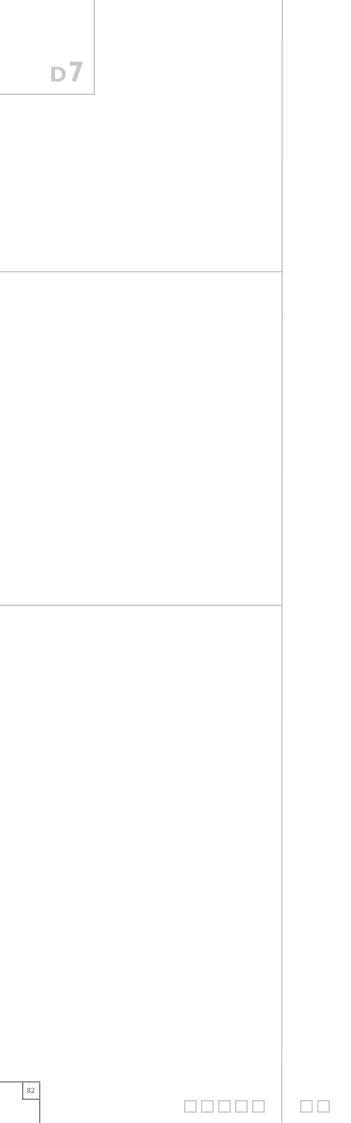
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Supervisor: J. Wavreille

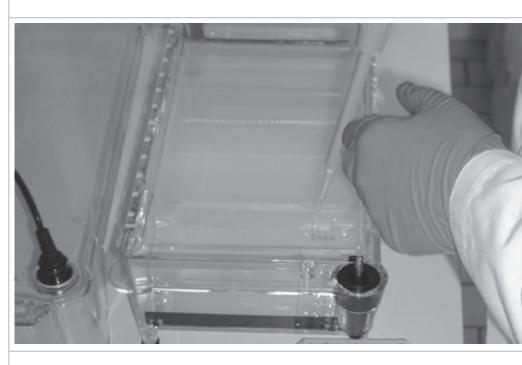
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Department

QUALITY OF AGRICULTURAL PRODUCTS



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1. MISSION STATEMENT

. Study of the chemical composition, the nutritional and hygienic quality of plant products and their technological value for the food and non-food industries.

. Analytical methods for milk and dairy products. Enhancement of milk constituents. Intrinsic, microbiological and technological quality of meat.

. Application of non-destructive physical analytical methods for global management of the quality of primary and processed products. Software development and network management.

2. SCIENTIFIC PERSONNEL

9 permanent staff and 11 contract staff

2.1. Head of Department

P. Dardenne, Inspecteur général scientifique

2.2. Scientists

V. Baeten, Attaché scientifique

- G. Berben, Inspecteur général scientifique, Head of Section
- A.M. Clarinval, Attachée

F. Debode, Attaché scientifique **

F. Dehareng, Attaché scientifique (GG)

J.A. Fernandez Pierna, Attaché scientifique (from 1/10/2004 FNRS)

O. Fumière, Attaché scientifique

B. Gaurois, Attachée scientifique – CRA-W Quality Assurance coordinator

E Jancson

- E. Janssen, attaché (SSTC) J. Laloux, Attaché scientifique
- B. Lecler, Attaché
- J. Lenartz, Attaché scientifique **
- A. Michotte Renier, Attaché scientifique **
- V. Ninane, Attachée scientifique
- G. Sinnaeve, Attaché scientifique
- J.M. Romnee, Attaché scientifique
- F. Rwagasore, Attaché scientifique (UE)
- A. Van Reusel, chef de section (until 31/12/2003)
- Ph. Vermeulen, Attaché scientifique (UE)

Sources of funding (for research teams or contracts): GG: guidance group; OSTC: Belgian Federal Science Policy; EU, European Union ; ** subsidised research by MRW-DGA-IG3-Direction de la recherche, *** subsidised research by SPF, Ministry of Public Health.

3. NATIONAL REPRESENTATION

• AFSCA : Agence fédérale pour la sécurité de la chaîne alimentaire, Member of the Scientific Committee.

 BASE scientific journal editorial committee.
 Advissory Commission of Agrofood products (RW)

Biosafety Council scientific committees.

• IDF – International Dairy Federation – Belgian National Committee.

• Ministry of Small Enterprises, Traders and Agriculture/Ministry of Public health, DG5 – Comité de coordination du lait – Groupe produits laitiers. • RéQuaSud asbl, Réseau Qualité Sud, Presidence. • National reference laboratory for dairy product microbiology.

4. INTERNATIONAL REPRESENTATION

BIPEA (Bureau interprofessionnel d'Etudes Analytiques). Member of the Executive Committee.
CEN (European Committee for Standardization). Expert on the ad hoc group "Quantitative detection of GMOs" within working group CEN/TC 275. Expert on working group CEN/TC 338 "Cereals and cereal products".

ICNIRS (International Committee of Near Infrared Spectroscopy). Member and Chairman-elect.
ICODRL (International Circle of Dairy Research Leaders). Secretariat: NIZO, Ede (Netherlands). Member.

• IDF (International Dairy Federation). Belgian delegate on milk analysis Commission E. Participation in the following working groups: E104: lactic bacteria and starters, E203: quality assurance, E301: fats, E303: infrared and other indirect automated methods, E713: firmness of butter.

• International Advisory Board for Forage for Foss-Tecator AB. Member.

• LCHA (Central Food Hygiene Laboratory)- AFSSA (French Food Safety Agency), Paris.

European project and network participation:

- ENGL: European Network of GMO Laboratories, JRC-Ispra (Italy). Member. http://engl.jrc.it/

- Dairy products working group - Prof. H. Glaeser. Participant.

 - KeLDA: Kernel Lot Distribution Assessment project. Participant http://biotech.jrc.it/sampling_ KeLDA.htm

- MEDEO: Development and assessment methods for the detection of adulteration of olive oil with hazelnut oil. Participant.

http://www.cica.es/aliens/igmedeo/

- STRATFEED: Strategies and methods to detect and quantify mammalian tissues in feedingstuffs. Coordinator. http://www.stratfeed.cra.wallonie.be - DG-SANCO project to design an interlaboratory test for the detection of meat and bone meal in animal feed. Joint organiser.

- SECUPROD: Modular courses on production quality – Cheese and wine making. Participant. http://www.secuprod.com/

- SIMBAG-FEED: Screening and identification methods for official control of banned antibiotics and growth promoters in feedingstuffs. Guest participant.

- TYPIC: Study of differentiated quality food products (wine and dried ham) designed to identify consumer preferences and establish objective criteria for evaluation of such products. Participant. http://www.typic.org

- TRACE: Delivering Integrated Traceability Systems that will enhance Consumer confidence in Authenticity of Food: http://trace.eu.org

5. RESEARCH TOPICS

The activities of the Department Quality of Agricultural Products are mainly concerned with the quality of raw materials and processed products for feed and food uses. The diversification of analytical methods for determination of both chemical and microbiological quality parameters is one of the Department's main focuses. New, faster and more effective methods for detecting certain microorganisms and antibiotics have been developed or are still undergoing research. Methods for detecting and quantifying mycotoxins have been developed by a Mycotoxins Unit at the CRA-W. Meanwhile, routine determination of composition parameters such as proteins, fats, sugars, etc. is carried out. Beside composition, elements (heavy metals and metal trace elements) are determined by ICP. These analyses, called reference analyses, are used in applications developed in near infrared spectrometry. The Department also deals with the specific requirements of particular sectors. The technological quality of cereals is one current topic, while in the dairy sector efforts are being directed, through Scientific Guidance for Interprofessional Organisations, towards harmonisation of milk analyses at both national and international level.

Some of our research topics have **environmental** implications. For example, the Department is involved in **precision farming** in co-operation with the Crop Production Department (D2). The aim is to take the laboratory out into the field in order, by means of direct measurements made with the aid of geographical positioning systems (GPS), first of all to determine and ultimately to adapt inputs so as to reduce the excessive use of fertilizers and plant protection products. In this connection the Department hosted an international symposium (2nd International Conference on Embedded Near Infrared Spectroscopy, Gembloux, 18-19 Nov. 2004) which was attended by more than 120 international participants.

Food safety is another of the Department's research topics. This is essentially approached by developing or refining efficient, innovative methods. The Department has for example gained expertise and European recognition in **detecting** and quantifying genetically modified organisms (GMOs). The Department has also established itself as a leading European laboratory in the area of detecting and quantifying meat and bone meal in animal feed.

Our work on the **authentication** and **traceability** of products has led to increased participation in some European programmes (FP5 and FP6) and it is another cross-cutting topic for the various sections of the Department. It covers a wide variety of products and uses most of the techniques available to the Department, such as analytical chemistry, molecular biology, spectroscopy and chimiometry.

6. RESEARCH REPORTS

6.1. Composition and technological and physico-chemical properties of cereals

G. Sinnaeve, J. Lenartz, J.M. Romnee, A.M. Clarinval, P. Dardenne

The Department's Cereal Technology Laboratory has for several years been performing analyses to determine cereal quality, working principally with wheat and spelt.

In addition to the conventional analyses relating to wheat acceptance standards (moisture, Zeleny sedimentation index, Hagberg falling number, etc.), methods for describing the rheological properties of doughs (Chopin-Dubois alveograph, Brabender farinograph, etc.) and the Belgian standard breadmaking test are also carried out on behalf of the main players in the cereal sector, both upstream (farmers, breeders, retailers/stockers, etc.) and downstream (millers, bakers, the starch and gluten industries and the client food processing industries).

In its capacity as a reference laboratory, the Cereal Technology Laboratory organises ring trials for the analytical laboratories of Requasud, a non-profitmaking organisation. It also shares its cereal quality analysis experience (forming control samples, problem-solving, etc.) with the various partners of this network.

As part of its research work the Laboratory participates, in close collaboration with the private sector, in the development and evaluation of new methods for defining, precisely and reproducibly, the technological properties of the various matrices obtained from the kernels such as whole meal and white flour for breadmaking. The Mixolab, likely to be marketed by Chopin-Dubois, which provides an assessment of the rheological properties of doughs, and the Rapid Visco Analyser (RVA) viscometer produced by Newport for investigation of the gelling properties of starches and flours in connection with amylolytic activity are particular focuses of study. Like other analytical tools, the Mixolab assays the flour proteins and their gluten network but it also assays the starch fraction of doughs and associated amylolytic activity based on reduced sampling of both whole meal and white flour and thus offers potential for integration all along the wheat chain, from the breeder to the end-user.

Besides the conventional criteria already mentioned, these new methods play a part in the description of varieties for the National Catalogue, supporting the tests carried out by the Crop Production Department (D2) within the framework of its "plant accessions" tests and the new wheat and spelt variety breeding work carried on by the Biological Control and Plant Genetic Resources Department (D3).

In cooperation with the Temperate Regions Crop Husbandry Unit and the Food Processing Industries' Technology Unit of FUSAGX, the Laboratory is also involved in the project entitled "Description of factors affecting the composition and structure of starch and consequences for the development of native wheat" funded by the Walloon Region Directorate of Agriculture (project D31-1088). The main aim of the project is to describe the genetic (variety), crop husbandry (quantity and splitting of nitrogen manuring, fungicidal protection, sowing dates, etc.) and environmental factors likely to affect, on the one hand, the wheat starch structure (granule size, amylose content, gelling properties, degree of amylopectine polymerisation, and so forth) and, on the other, the enzymatic (amylasic) activities in the grain that are likely to cause this starch to deteriorate. The Laboratory's share of the work has focussed on the interactions that take place between enzymes and the starch component and their specific role with respect to the gelling properties of whole meal. To this end an experimental design using an AgNO, solution as an amylolytic activity inhibitor has been developed to identify the intrinsic rheological properties of starches via RVA rapid analysis. Now routine, this method has already been used to screen varieties grown in Belgium according to the properties of their starch fraction.

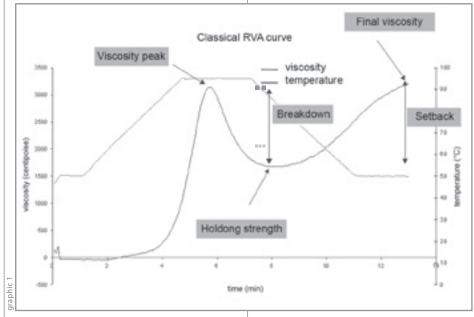
Results for the first two years show that the starch characteristics of Belgian varieties vary considerably. Moreover, growing conditions also appear to affect starch properties to a significant extent. It now appears possible in the medium term for farmers to be provided with information on precise growing techniques designed to produce wheat with starch fraction characteristics to match a range of industrial applications.

6.2. Authentication and compliance of agricultural products

Qualitative and quantitative detection of genetically modified organisms (GMOs) or their derivatives in food matrices *E Janssen**, F. Debode*, G. Berben*

During the past two years the Department has continued to work on GMO detection, focussing particularly on describing the quality of the DNA extract used to quantify GMO content and, within the framework of a cooperative link-up with FUNDP to develop a "plant species" biochip and a quantitative *biochip*. The problem of conversion factors for percentage by weight and percentage by number of copies per haploid genome has also been tackled, taking maize MON810 as a model. Finally, work has been done on plant fat extraction, showing that DNA can remain in oil. The residual DNA depends on the oil refining process. As a member of ENGL (European Network of GMO Laboratories), the Department made a sizeable contribution to the KeLDA project coordinated by the Ispra Joint Research Centre (JRC-IHCP, Ispra, Italy). This project is designed to assess transgenic contaminant distribution in kernel lots based on soya beans. The ultimate aim is to establish sampling methods for large-volume cargoes that offer proven scientific reliability. The Department took part in three ring trials to

validate "event-specific" quantification methods organised by the JRC-IHCP (lspra, Italy) acting as the Community reference laboratory in the latter two tests. These were validation tests for maize Bt11, NK603 (validation comprising DNA extraction) and TC1507.



Viscosity profile established by means of the Rapid Visco Analyser (RVA) viscosimeter

The Department has been approached with a view to cooperate with CLO (Centrum voor Landbouwkundig Onderzoek, Ghent) and ISP (Institut scientifique de santé publique, Brussels) in connection with establishing the national reference laboratory under the aegis of the ISP. Lastly, it may be mentioned that within this area of expertise, several members of the Department



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have been invited to organise training or to give talks to technicians and executives in developing countries, Belgian students and people working in the food processing chain.



Grinding of "KeLDA" samples by CRA-W at IRMM

Detection of processed animal proteins in compound animal feed

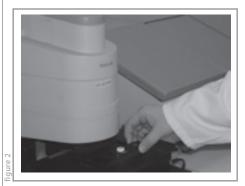
V. Baeten, O. Fumière, Ph. Vermeulen, A. Michotte Renier, J.A. Fernandez Pierna, F. Dehareng, G. Berben, P. Dardenne

Within the framework of the European STRATFEED project, coordinated by the Department, and a national infrared imaging project, a large body of new results obtained during the last two years is expected to lead to tighter control of compound animal feedingstuffs. First of all, initiatives have been taken to set up and maintain a European network of research teams involved in developing analytical methods and working towards building up consumer confidence in animal products. An international conference on the topic of food chain safety in the context of prion diseases was held in Namur in June 2004, under the aegis of the STRATFEED project, and was attended by over 120 researchers. At the same time, edition no. 8(4) of the BASE journal was devoted to the latest developments in this field. A separate initiative involved the publishing of a book by the European Commission describing all the achievements of the STRATFEED project in developing the conventional microscopy method and the latest alternative methods. The Department also contributed to various working groups organised by the European Commission with the aim of optimising official controls and developing an effective strategy for the traceability of the various categories of animal by-products.

In response to requests from DG-SANCO and DG-Research at the European Commission, the CRA-W has played a role in a number of initiatives designed to tighten the official control performed by laboratories in order to ensure the safety of animal feed. A European ring test coordinated by

JRC-IRMM (Geel, Belgium) to measure the ability of new member states to implement the European directive on the detection of animal meal was organised in cooperation with the AFSCA laboratory at Tervuren. Another objective of the ring test was to assess new versions of the analytical design used in conventional microscopy and to test the performance of some new immunological kits. Two workshops were also held at Gembloux to enable new member states to benefit from the achievements of the STRATFEED project in connection with animal meal detection by conventional microscopy and a validation study was organised in conjunction with JRC-IRMM and RIKILT (Wageningen, Netherlands). The latter study led to amendment of the European directive to include the improvements resulting from the method developed within the framework of the STRATFEED project.

Scientific studies were also carried out in order to develop new methods and strategies for detecting animal meal in compound feedingstuffs. During the 2003-2004 period the Department has built up expertise in all the analytical methods used to detect animal meal, namely conventional microscopy, methods based on molecular biology and immunology and infrared spectroscopy methods. The advantage of a single laboratory covering all the techniques is that it enables innovative methods to be proposed, such as infrared microscopy and imaging, as well as strategies for combining microscopic and molecular biology methods. On the strength of the research undertaken the Department is often asked to make its expertise available to the international scientific community through lectures and courses. The Department has also contributed to various high-calibre scientific publications (Gizzi et al., 2003; Gizzi et al., 2004; Fernandez Pierna, 2004).



Detection of animal proteins by infrared microscopy

Description of the specific origin of animal fats

G. Berben, O. Fumière, F. Rwagasore, V. Baeten

Alongside the issue of processed animal proteins turning up in animal feed, the related problem of the specific origin of animal fats likely to be used in animal feed was also tackled from an analytical point of view in cooperation with JRC-IRMM (Geel, Belgium). In this connection the Department was responsible for applying spectrometric methods and PCR to a series of mono- or multispecific fat samples. The study demonstrated the potential of near infrared spectrometry for identifying the animal species from which a pure fat originated, but in the case of mixed fats from different animal species, its discriminatory power is not sufficiently fine for practical use. For the real-time PCR tests the bovine target developed as part of STRATFEED was used to detect the presence of fat of bovine origin. It was found that of all the techniques used, PCR was the one to detect the lowest levels (2%) of tallow added to lard. However, PCR is limited in this application by the indirect nature of the test, as some DNA has to remain in the fat. Therefore, as soon as the residual insoluble contaminant level falls below a certain level (<0.15%) it is no longer possible to detect a bovine target by PCR. One notable case is highly refined fat described as premier jus, though this does not pose a TSE risk. The results were written up as a scientific publication submitted in 2004.

Development of analytical methods for the typicality of differentiated food products V. Baeten, F., Rwagasore, J.A. Fernandez Pierna, G. Sinnaeve, P. Dardenne

The Department has continued to maintain its expertise in the development of analytical methods based on infrared spectrometry for the authentication of food products. This expertise is put to good use in connection with the Department's involvement in two projects within the European Community Fifth Framework Programme. The first of these is the European Commission's MEDEO project (2001-2003) for the detection of adulteration of olive oil with hazelnut oil (http://www.cica. es/aliens/igmedeo/). The second is TYPIC (2003-2005) (http://www.typic.org), a European Community project designed to study differentiated quality food products (wine and dry-cured ham) in order to define consumer preferences and lay down objective criteria for the evaluation of such products. Consumer behaviour and consumer perception of differentiated quality products are analysed using objective parameters defining the typicality of the products. The TYPIC project is also concerned with developing analytical methods to describe and guarantee the specific gualities of such products and their traceability.

Traceability and training modules in connection with the quality of cheese products. J.Laloux, F. Rwagasore

As part of an EU Leonardo da Vinci project called SECUPROD (http://www.secuprod.com/) a training tool has been created to improve the skills of cheese makers with respect to food safety (hazard analysis critical control points) and traceability. Being relevant to practical cases, it can also be useful for trainers. The aim was to contribute to food hazard control by motivating SMEs while at the same time ensuring knowledge transfer as part of global quality. This project had an economic dimension insofar as SMEs that practise

Department QUALITY OF AGRICULTURAL PRODUCTS

quality are more competitive and economically more performant. Project evaluation was carried out by the partner SME (cheese maker Fromagerie de Chimay) throughout the tool design phase and end-user assessments were also carried out in the partner countries before distribution. The information will be distributed on CD and *via* the Web, thus enabling users to access other sites in order to update the economic and regulatory contexts.

Traceability information and communication systems

Ph Vermeulen, V. Baeten, R. Oger

Over the past 20 years or so, each laboratory within the Department has built up a stock of data and results on each technique in respect of clearly defined matrices. The grouping together of the laboratories in 1999 brought the benefits of synergy.

Today, the multidisciplinary nature of the work and the involvement of a number of outside partners in the research, coupled with the volume of analytical data and documentary information handled, requires information and communication systems to be developed. One such example, with the support of the CRA-W Biometrics Section which has acquired considerable experience in developing various information systems, is a Web-based complete system developed for the European STRATFEED project (FP5) which is concerned with the detection of mammalian tissues in feedingstuffs (http://stratfeed.cra.wallonie.be). This system includes a Website (extranet, intranet), specialised databases, sample data management and exploration tools and a decision support tool for tissue recognition based on conventional microscopy images.

The STRATFEED Website has opened new doors to other projects involving both sample and analytical data management and information and project management. Within the framework of the new European Commission TRACE project (FP6), for example, a system combining scientific data utilisation tools and tools for communication between scientists is under development. Designed for the various players in the food chain, this system aims to promote quality and traceability systems for label products (http://trace.eu.org).

6.3. Microbiology, chemical composition and residue analysis in primary and processed products

Microbiology of fermented milk V. Ninane, F. Dehareng, G. Berben

The Department's lactic fermentation know-how has been available to the public for a long time now. Over the past two years the Department has provided information, assistance and advice in connection with fermented milk and fromage frais production to private individuals and dairy producers, to technicians at the Ciney College of Agriculture in charge of the regional project to provide technical and technological assistance to dairy product manufacturers and processors in the Walloon Region and to one of the academic staff at the Haute Ecole Charleroi Europe. The scope of this tool transcends traditional products and the borders of the Walloon Region: the Department has in fact provided guidance to a French goat breeder in connection with an innovative application for kefir grains.

Dealing with enquiries from the public involves some applied research on specific technological aspects. Home storage conditions for kefir grains and the conservation period for fermented milk prepared and kept in standard domestic conditions have thus been established.

Kefir has also been the subject of some experimentation concerning a health aspect, namely the effect of fermentation on the milk fat and in particular on the transformation of some of the components into substances beneficial to health (omega 3 and CLA).

Finally, the Department set out to identify the intricate, diversified microbial consortium of kefir grains. Traditional microorganism identification has two main drawbacks when applied to a complex fermenting agent, in particular a lactobacillus population: firstly, the necessary step of growing and isolating the different species, with the risk of "overlooking" some of them, and secondly, the unreliability of the results. The Laboratory has accordingly adapted a molecular method to lactobacillus identification and during the past two years has sought to confirm the species identified by this molecular method by conventional determination of their phenotypical characteristics. The applicability of the method to microbial enumeration has also been verified. In view of the performance of the method, it has been adapted for identification of another type of bacteria present in the grain, namely lactic acid cocci. The description of the grain yeast population, which is less random than in the case of bacteria, has in contrast been undertaken in a traditional way, and Saccharomyces cerevisiae was isolated from the kefir grains distributed by the Department. This latter research forms part of a doctoral thesis.

Lipids have been a focus of growing interest in many areas for some years now. Food sciences, particularly as related to nutrition, dietetics, food technology, health and traceability require increasingly detailed knowledge of lipid composition.

Gas chromatography has for many years been an acknowledged analytical method for lipids. Much research has been undertaken by the Department in the past, in particular into the fatty acid profile of butter fat. The former Dairy Station thus developed the bases for a new product which went on to become a big success: butter that spreads straight from the fridge.

These chromatographic methods have lately found favour at the Department again as a means

of tackling problems such as separation and quantification of omega 3 and 6, *cis* and *trans* fatty acid isomers, some specific isomers (CLA: conjugated linoleic acids) and triglyceride separation.

Several tests have been conducted in cooperation with the Animal Production and Nutrition Department (D6). With respect to cows we have shown that, compared with soya cake or pea supplementation, lupin seed supplementation reduced the milk C16:0 concentration and increased the C18:0 content. Other work has been concerned with the effects of lupin supplementation on chicken meat. The results showed a lower C16:0 content on the lupin-based diet compared to soya-based feeding. Inter-species (sow, cow and ewe) comparison of milk fatty acid profiles revealed that sow's milk is much less rich in saturated fatty acids and also has a much higher C18:1 and C18:2 content than is the case for the two ruminants.

The fatty acid profiles of various hams have been determined as part of the European TYPIC project, using analytical methods to establish the typicality of a variety of products. This project is still on going.

In the area of fermented milk we have set up a test to investigate the influence of different kefir strains on fatty acid composition.

In partnership with the Ministry of the Walloon Region's Development and Extension Division we have monitored the fatty acid composition of Walloon farm butter and have demonstrated the effectiveness of feeding strategies currently implemented at certain farms in order to increase omega 3 and CLA levels.

Detection of antibiotics in agricultural products J.M. Romnee, F. Dehareng, A. Ciza

Antibiotics are still the most commonly used active ingredients in livestock farming. Their intensive use, for both prevention and cure, has led to the emergence of bacterial resistance that undermines the effectiveness of some treatments in human medicine. Checking for the presence of these substances in food and agricultural products (residues in milk and meat) thus remains a current issue.

1. Antibiotics in milk

The Analytical Chemistry Laboratory has continued to assess the test for detecting antibiotics in milk (Delvotest MCS, now adopted in Belgian legislation). A report has been produced on the assessment of the scanner reading system developed by DSM. This research highlighted variations in the sensitivity of the test that were not evident from visual reading of the control test. The technique is in fact based on analysing the image obtained after incubation. Each microplate

image obtained after incubation. Each microplate well is assigned a value calculated by a program that incorporates the three basal colours and that value is then compared with a threshold defining

Lipid analysis by gas chromatography F. Dehareng, V. Baeten, F. Rwagasore., V. Ninane.



the positive samples subject to penalties. The determination of this value has been studied by the Department in order to standardise reading at the various laboratories performing routine analyses. A detailed analysys of the results produced by the Laboratory and by the Battice Milk Committee demonstrated the futility of attempting to apply analytical chemistry concepts to the microbiological tests used in screening. Even industrially prepared tests are not totally homogeneous. The variability observed cannot always be explained.

Study of a new detection test (Copan Milk Test, Copan SA – Italy) has started. This test has similar characteristics to the Delvotest MCS (a microbiological test using the same microorganism, *Bacillus stearothermophilus* var. calidolactis, growth of which is revealed by a pH indicator). The work involves comparing the sensitivity of the two tests in identical conditions of use and assessing the homogeneousness (within the same microplate, the same lot and between lots). Other parameters, such as the effects of the somatic cell and germ count, pasteurisation and composition, will also be looked at in this study.

2.Antibiotics and growth promoters in food

Within the framework of the European Simbag-Feed project the Department has implemented a method at the Analytical Chemistry Laboratory for carbadox and olaquindox detection. These two growth promoters, banned by the European Union in 1999, were used until then with piglets. The method used is based on chromatography after extraction and extract purification. The limits of detection are 0.83 mg/kg (carbadox) and 0.08 mg/kg (olaquindox) respectively. Using the UV spectrum, obtained with the aid of a diode array detector, enabled some false-positive results to be discarded (figure 3), notably in the case of the "veal" analysed. The Laboratory has also been appointed to carry out the validation study for the HPLC method developed for the project.

Mycotoxin detection in cereals

Mycotoxins are secondary metabolites produced by certain fungi. Among the best-known of these, trichotecenes (nivalenol, deoxynivalenol, etc.) are produced in particular by the agents that cause *Fusarium* disease (*Fusarium* sp.). Due to changes in European legislation, these substances will in future be included in the quality criteria for cereal acceptance. The permitted limit in the case of deoxynivalenol (DON) will be around 1,000 µg/kg in cereals.

The Analytical Chemistry Laboratory has developed a method for separating nivalenol, DON, 15-acetyl-DON and 3-acetyl-DON. These four substances can be recovered in a single extraction using a mixture of water and acetonitrile. The detection limits for DON and nivalenol are 160 and 200g/kg respectively. By using the diode array detector we were able to demonstrate interference in the extract purification process.

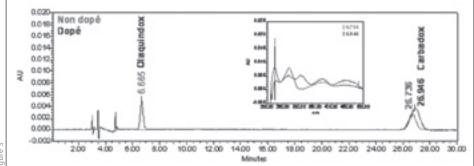
An analysis of 38 flours from field inoculation tests allowed the results to be compared by ELISA and chromatography. The results show a good correlation between methods. This is a joint study with the Biological Control and Plant Genetic Resources Department (D3).

The Laboratory has also assessed the rapid detection test for DON for direct use at cereal delivery.

Developments in inductively coupled plasma atomic emission spectrometry J.M. Romnee, A.M. Clarinval

Changes to the legislation concerning wood preservatives and the replacement of arsenic salts by boron salts require the development of analytical methods for tracking boron after disposal of the wood. Sample preparation (microwave digestion) and analysis (ICP-AES) methods have been developed. The fractions resulting from wood pyrolysis (smoke, charcoal, oily residues) have been analysed to locate the boron. This is a joint project with the Agricultural Engineering Department (D5).

Determination of other major (Na, K, Ca, etc.) or minor (Se, Cu, Fe, Mg, etc.) elements has been undertaken on behalf of other CRA-W departments



L Chromatogram of artificially spiked and unspiked food. UV spectrum of peaks with a retention time of 26,736 minutes and 76.946 minutes

(carbadox) (Non dopé = Not fortified, Dopé = Fortified).

and external customers (FUSAGx, Valbiom, etc.). This work involving different types of matrices (food, soils, plants, meat, mineral and organic oils, etc.) required the development of specific analytical parameters.

6.4. Application of spectrometry to agricultural and food product quality management

G. Sinnaeve, J.M. Romnee, V. Baeten, J. A. Fernandez Pierna, B. Lecler, P. Dardenne

Near and mid infrared spectrometry (NIR and PIR)

1. Management of databases and networks of instruments

The spectral databases built up over the years and our accumulated expertise in instrument standardisation form the basis of the NIR chain and cereal and forage chains within the ReQuaSud network. This instrument networking experience has put the Department at the centre of the instrument network for use in research (INRA network - six laboratories throughout France) or by private companies (Provimi network comprising forty-five laboratories around the world). These databases, originally compiled using a single type of spectrometer, are undergoing some modifications for adaptation to the newer types of spectrometer on the market. Potential users of NIR technology can contact the Department for calibration transfer to their own instruments and will thus rapidly reap the benefits of all the variability introduced over the years (growing years, varieties, arowing sites, etc.).

2. Study of new devices

A handful of manufacturers dominated the NIR spectrometer market until a few years ago. Over the past two to three years much more choice has become available, with spectrometers using a different measuring principle (dispersive, Fourier transform, post-dispersive diode array, etc.), with different spectral ranges and using different software. The Department is currently studying methods for transferring databases from one instrument to another to enable calibrations to be used irrespective of spectrometer type.

3. Study of new software

NIRS calibration was conventionally based on linear modelling techniques which the Department has been involved in assessing. While such techniques are widespread and commonly used, the development of calibrations based on artificial neurone networks (ANN) is booming. This method does not only perceptibly improve the accuracy and ruggedness of the models but also facilitates transferability from one instrument to another. As part of H. Prévot's doctoral thesis (2004) in cooperation with the Statistics and Data Processing Unit of FUSAGx, comparisons were made of 14 dependent variables associated with five spectral collections. The results written up in the thesis show that ANN methods are often competitive with more conventional multivariate calibration methods (MLR or PLS). As none of the methods consistently outperforms the others, the "trial and error" method still applies.

The RINA concept (Foss-Infrasoft International) offers a new approach to NIR spectrometer network operation and management. Predictive models are stored on a server (in this case, the CRA-W server) and spectra are sent in by authorised users *via* an Internet link. The values estimated by the models are then returned to them. This system considerably facilitates the network coordinator's work as well as ensuring that each customer uses the latest model.

Support Vector Machine: this is a new chemometric method for building classification or quantification models. This approach has now been applied to spectral data classification in near infrared imaging and a number of papers and publications have resulted.

4. Development of new applications

A number of applications are being developed for both primary and processed products. Some of these are described below.

As diode array spectrometers have no moving parts they are more rugged and are therefore coming to be increasingly used for on-board applications. The object of our research is to mount NIR spectrometers directly on the harvesting machinery (combine harvesters and forage harvesters) for continuous assessment of crop quality, specifically the protein content. We are liaising with breeders in order to develop calibration methods for determining moisture and protein content during harvesting. This considerably alleviates the work of collecting, preparation and analysis of thousands of samples. From these measurements in conjunction with the GPS coordinates of the harvest machine, field "protein mapping" can then be carried out, nitrogen manure applied on that basis and the use of inputs thus cut down. NIRS is also used to determine the nitrogen content of plant tissue for the purpose of crop monitoring during growth, optimising nitrogen manuring and thus helping to reduce input levels (for instance, in cereals and potatoes).

Work towards O. Pigeon's doctoral thesis (Pesticide Research Department – D4) involved NIRS analysis of single cereal seeds treated with anthraquinone, tefluthrin or imidacloprid. Calibration following HPLC and GC determination enabled a homogeneity index to be produced for treated lots. This work will now be extended to beet seed treatment.

The Department was approached by some NGOs involved in clothing recycling with a request to develop a sorting method by type of textile. Type sorting is essential for successful recycling of textiles no longer suitable for selling through second-hand clothes outlets. Discrimination models have been designed to distinguish between six different groups according to the dominant fabric (cotton, wool, acrylic, viscose, polyester and polyamide). A correct allocation rate of 96.8% was achieved by the two block partial least squares (PLS2) method.

CARI, a beekeeping research centre, had a collection of 600 honey samples collected in Belgium. A total of 116 samples were selected according to chemical data. The NIR spectra for these samples were measured to create a database taking account of the inherent variability of honey (single flower, mixed flowers, nectar or honeydew). The results show that the moisture can be determined and honey can be classified according to glucose and fructose content. Based on the results for melezitose, classification into three groups by low, medium or high levels can be carried out. High levels of this trisaccharide pose problems with honey extraction.

With a view to reducing CO2 emissions, biodiesel produced by transesterification of vegetable oil can be added to diesel without having to retune the engines. Biodiesel is regarded as a carbonneutral energy source. Limits of 2% in 2005 and 5.75% in 2012 will apply in the European Union. In a cooperative study with the Agricultural Engineering Department (D5), biodiesel/diesel mixtures (from 0.0 to 6.0 %) were created from two samples of biodiesel and four samples of diesel. The aim of this research was to assess the performance of NIRS in detecting and guantifying biodiesel in diesel. The study showed the two products to have guite different spectral signatures. It was also found that the diesel spectra are fairly similar with respect to one another, as are those of the biodiesels. The precision of calibration is such that NIRS can be used as a means of checking the biodiesel content of diesel fuel. This preliminary study will now be continued in order to develop rugged universal databases.

7. SERVICE ACTIVITIES

1. Scientific assistance to Belgian I.O. (Interprofessional Organisations)

The Department has provided scientific support to I.O. laboratories responsible for determining the quality and composition of milk for payment to suppliers since 1994. This work was harmonised at national level in 2001. The Departments of Quality of Agricultural Products (CRA-W) and Kwaliteit van dierlijke Producten en Transformatietechnologie (CLO-Melle) were appointed by Royal Decree dated 3rd March 1994 to perform this role, according to a joint protocol approved by the National Milk Coordinating Committee which covers, in particular:

 weekly supply of known-content reference samples, - bimonthly supply of a range of samples for calibration of the Interprofessional Organisations' FTIR equipment.

- comparative analyses on a monthly basis.

2. GMO detection

The volume of routine analyses to detect GMOs or their derivatives is growing steadily and has reached a total of 500 samples per year in the past two years. A quality assurance system is being set up with a view to applying for accreditation in 2005. Among events of note, we must point out here that the Department revealed the first case in Belgium of an unauthorised GMO derivative (specifically, maize GA21) in a food product. The Laboratory is partly funded under a WR-DGA agreement.

3. Fermenting agents for small-scale dairy product manufacture

Since the late nineteen-thirties the Department has maintained a stock of lactic ferments for cheese, yoghurt and kefir making. These starters are sold to individuals and craft-scale enterprises along with appropriate guidance to ensure quality products.

4. Near Infrared Spectroscopy (NIRS)

The Department offers a consultancy service in this field, as well as acting as a reference laboratory for the members of the ReQuaSud network. Regular requests for the Department's NIRS expertise are received from private companies (breeders, food manufacturers, food processors, etc.) for contract analysis, spectrometer standardisation, calibration transfer or feasibility studies.

International conference on the item of "Food and feed safety in the context of prion diseases" organised in June 2004 at Namur







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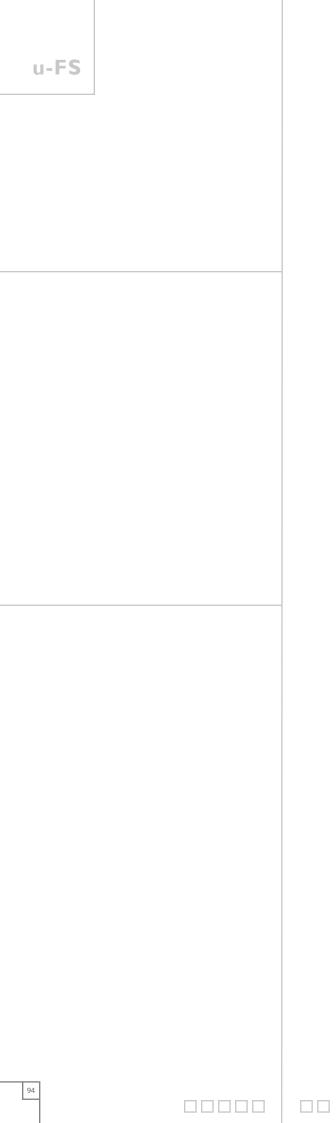
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Department QUALITY OF AGRICULTURAL PRODUCTS





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. Conduct research on the potato production chain

. Analyse farming systems in terms of economic, environmental and social sustainability . Conduct research on integrated livestock

farming systems based on using locally grown fodder crops

2. SCIENTIFIC PERSONNEL

3 permanent staff and 14 contract staff

2.1. Head of Section

D. Stilmant, Inspecteur général scientifique

2.2. Scientists

R. Agneessens, Attaché J.F. Collard, Attaché (FIRST project and DGRNE – RW project) V. Decruyenaere, Attachée scientifique F. Delire, Attaché scientifique (until 31/03/04) B. Dupuis, Attaché scientifique * (from 15/01/03) D. Haine, Attachée scientifique * J.P. Harvent, Attaché S. Hennart, Attaché scientifique D. Jamar, Attaché scientifique (SSTC) D. Knoden, Attaché scientifique (from 01/02/04) D. Michelante, Attaché scientifique J.L. Rolot, Attaché scientifique H. Seutin, Attaché Y. Seutin, Attaché A. Soete, Attachée scientifique (from 12/01/04) F. Thilmany, Attachée scientifique

* Subsidized researches

3. NATIONAL REPRESENTATION

• MRW-DGA (formerly MCMA – DG 6) – Agricultural Pilot Centers (Non-Profit Organization): for Better Fodder Crop Management (FM) – for Potato (CPP) – for Organic Farming (CEB)

• MRW-DGA (Product Quality Direction) – Working group on establishing a National Catalogue of recommended species and varieties of agricultural plants

• MRW-DGA (Product Quality Direction) – Working group on 'Potato seeds'

• Walloon council for the potato agro-food chain • Technical group for the 'Terra Nostra' label

- recrimical group for the field Nostra laber
- APAQW Working group on 'Label Politic' • Ethical Committee (FUNDP, FUSAGx, CRA-W)
- Fluorescence X methodology working group

 REQUASUD Network; Inter-professional Office for Analytical Studies (BIPEA) – PAMESEB Non Profit Organisation

• BPQA (Belgian Potato Quality Award): Working group on promoting high quality potato production. • MRW-DGA (Product Quality Direction) – Working group on 'Seeds and starting material for crop production under organic farming'

4. INTERNATIONAL REPRESENTATION

• AFPF – French Association for Fodder Crop Production.

- British Grassland Society.
- EAPR European Association for Potato Research.
- Livestock Farming System Working Group.
 OEPP European and Mediterranean Organisation for plant Protection; Sanitary Certification of potato seeds working group.

• EUNET-ICP European network for development of an integrated control strategy of potato late blight.

5. RESEARCH TOPICS

The first theme is centred on three topics. The first is the study of potato diseases (such as virus Y, late blight, Erwinia) for the development of Decision Support Systems (DSS) for sustainable farming systems. The second focuses on producing, for the potato seed production sector and for fruitbearing culture, starting material of high quality (1) by improving the techniques for diagnosing the presence of viruses in this material, (2) by improving the techniques needed for producing starting material (minitubers and vitro-tubers) of high sanitary quality for potato crops and (3) by maintaining varietal collections that are as diverse as possible. The third topic is the development of unbiased techniques (such as Near Infrared Reflectance Spectroscopy [NIRS], image analysis) for characterising potatoes utilisation value (culinary and technological behaviour).

The 'sustainable livestock farming systems' theme also focuses on three topics; they overlap considerably and are distinguishable mainly in terms of scale. This scale goes from: (1) farming systems, including the producer-to-consumer chain, where we are looking for coherent approaches to address economic, environmental and social constraints and to respond to land-use issues; (2) to the analysis of 'soil-plant-animal" relations, especially under grazing; (3) to the study of sward composition and feeding value with the development of the appropriate analytical techniques, especially NIRS.

6. RESEARCH REPORTS

6.1. Potato production

- Potato viruses: epidemiology, detection and decision support systems
 - J.-L. Rolot

In potato seed multiplication, potato viruses, transmitted mainly by aphids, are among the most dangerous infections. With the economic risk that downgrading or even rejection of products by the official control service carries, producers are being pushed towards increasingly intense protection of multiplication fields, often without the necessary understanding of virus transmission and its causes or of protection systems that are less dependent on pesticide use.

Against this background, our work focuses on: - Analysing the influence of the quality of starting material (tubers) on infection by potato epsilon virus (PYV) at harvest;

- Developing a system to predict flying aphid populations;

- Developing a control strategy;

- Developing reliable methods for detecting potato leaf-roll virus (PLRV) to assist in applying official sanitary measures.

Our results stress the importance of the internal infection sources that usually constitute the starting point of PYV dissemination in the field. This dissemination is strongly linked to the dynamics and species composition of aphid populations in our region. These parameters allow us to calculate an infection pressure index that is a good indicator of the final quality of potato seed lots. Our research also highlights the influence of climate, mainly with regard to the temperatures between December and March, on the intensity of aphid activity in the spring. Our observations also show that efforts to reduce the spread of PYV should be based on the use of mineral oils rather than insecticides. Our findings have been incorporated into the development of an early warning system for producers, based on monitoring aphid incidence through the use of suction traps and the calculation of the resultant weekly PYV infection pressure.

Potato seeds starting material: germplasm collection, *in vitro* and *ex vitro* production techniques J.L. Rolot and H. Seutin

The *in vitro* techniques for potato micropropagation were widely accepted in the 1990s as basic techniques for supplying the potato seed production sector with material of high sanitary quality. Current sanitary requirements (to prevent the introduction of quarantine diseases onto farms through imported potato seed from contaminated areas, such as *Clavibacter michiganensis* spp. *sepedonicus*, Ralstonia solanacearum) are pushing producers to develop their own pre-basic and basic potato seed with starting material of *in vitro* origin.

Against this background, the overall aim of this project is to seek and develop techniques for germplasm conservation and starting material production in order to provide useful support to the production sector.

More than 300 varieties have been preserved, as vitro-plants, on Murashige and Skoog medium under a photoperiod of 16h/8h and 20°C, in our laboratory . All this material has been pathogen tested and constitutes a starting point for producing healthy starting material for distribution to the potato seed production sector. Techniques for long-term conservation by cryopreservation need to be developed to reduce the expense of maintenance.

A technique for *in vitro* tuberization, leading to the production of vitrotubers or microtubers, is also being explored. This technique, which carries a guarantee of maximum sanitary health because the whole production process is conducted in the laboratory, is very simple and inexpensive as it does not require the use of infrastructures such as greenhouses. However, the reduced size of the vitrotubers remains a problem to their practical use in multiplication fields.



Microtuber production through temporary immersion.

The objective is therefore to develop new techniques for producing vitro-tubers with a larger average size. Particular focus has been on production in larger bio-reactors, using the temporary immersion technique. Complementing this work is the testing of hormones added to the media and promoting tuber formation.

Mini-tuberisation in soil-less systems (hydropony) in the glasshouse is being studied in order to improve the economic efficiency of this system (number of mini-tubers with a calibre greater than 10 mm per surface unit, quantity and type of starting material needed). This involves studying tuber formation induction with regard to (1) specific hormone use (jamonic acid, flurprimydol, coumarin and kinetin), (2) the management of nutritive solution composition in terms of nitrogen concentration and (3) the management of plant density.

Potato late blight (Phytophthora infestans) D. Michelante and D. Haine

Over the past 2 years we have been studying the incidence of new *Phytophthora infestans* (Mont.) de Bary populations and of varietal resistance on the development of the disease in order to improve the Guntz and Divoux DSS. This model was developed in the 1960s for the Bintje variety. During the 1980s and 1990s new populations of this pathogen were accidentally introduced into Europe, so the meteorological criteria used in the model had to be adapted to the epidemiological characteristics of these news populations, also taking into account the added dimension of varietal resistance.

The monitoring of potato late blight incidence over several years showed a strong occurrence of type A2 strains not observed in the old populations. Metalaxyl resistance is also very common. On the other hand, the virulence profiles (against the host resistant [R] genes) of the collected strains are complex and highly diversified. This enables field populations to ensure sexual reproduction and thus generate important genetic mixing, with the production of genotypes likely to be well adapted to local agro-ecological conditions. In the aggressivity trials, the study of incubation

In the aggressivity trials, the study of incubation time showed that many strains are able to perform their cycle in a shorter period than that predicted by the Guntz and Divoux model. With regard to infection frequency, laboratory trials have demonstrated maximum infection at relatively low (10°C) and high (25°C) temperatures. In addition, the potato leaf moistening delay necessary to allow the infection to take hold could be as short as 1 hour.

In terms of varietal resistance, field trials need to characterise the type of resistance (partial, total, or a combination), to evaluate their importance and durability and to test the possible modulations of plant protection that such resistance can promote. The initial results showed that a high level of resistance (more than 6 on a 10-point scale) is linked to the presence of at least one major gene. The erosion of this type of resistance is rapid; it can occur after only a few years of cultivation in the same place. For such a variety, there can be a significant reduction in plant protection, but this will lead to stronger selection pressure and to the risk of a rapid occurrence of virulent potato late blight strains. A partial level of resistance is only of value when disease pressure is weak. Our results, obtained under strong disease pressure, do not allow us to draw conclusions about the possible modulations that such resistance implies for plant protection strategies.

In the context of the INTERREG III 'Walloon area - Flanders - France' project VETAB (Drawing on the trans-national experience in organic farming: concerted action for diffusion, experimentation and extension, in a large area of production facing common problems in the development of organic farming), our section is characterising products and strategies to replace or reduce the use of copper to control potato late blight. Out of 24 products tested in the laboratory, five gave good results in terms of protection against infection: Myco-sin (clay minerals and plant extracts), Ulmasud (clay minerals), Proval PK2 (kalium phosphonate), Penta Cu55 (low copper dosage fungicide) and Glutex Cu 90 (low copper dosage fungicide). The protective action of two other products was less efficient and more variable: Kubig (low copper dosage fungicide) and IRF (sea plant extracts). When the efficiency of these products was evaluated in field trials, none gave sufficient protection. Their sensitivity to leaching, as observed in laboratory assays, could explain this low efficiency in the field.

The protective practices based on the application of cupric fungicides at a rate of 800 g/ha/pulverization did not prevent the total destruction of foliage by late blight under our trial conditions (strong pressure due to artificial inoculation and the presence of unprotected rows). However, the more efficient treatments did postpone foliage destruction by about 15 days, enabling an acceptable yield to be obtained. The spraying programme applied according to instructions provided by the DSS was statistically better than the systematic 7-day programme. *in association with the GABNOR and the SRPV (Nord-Pas de Calais), the CARAH (Hainaut) and the PCBT (Beitem).

Potato soft rot diseases due to pectinolytic *Erwinia*: definition of a method for evaluating susceptibility *B. Dupuis and D. Michelante*

This project began in response to a demand from potato seed growers who are increasingly having to face this problem, resulting in losses in the field, during storage or during transport.



Figures 2 and 3: Some symptoms of Erwinia: black leg (Eca) and tuber soft rot.



The objective of this project is to develop a susceptibility indicator for potato seed lots (batches) to soft rot, based on latent contamination level measurement and on identifying the responsible pathogen. The most contaminated lots should be moved away. The validation of such an indicator will be done through correlation with the maceration level observed after artificial incubation of the tubers. Later, it will be validated under field conditions through comparison with the damage observed during the cropping season or during storage. Such a tool will allow producers (1) to identify the risk factors in production systems that could influence soft rot development and (2) to evaluate new systems of potato seed protection.

During the first phase, a new serological method was tested (Enrichment ELISA or E-ELISA) in order to quantify the bacterial inoculum on tubers artificially contaminated with *Erwinia carotovora subsp. Atroseptica* (Eca) strains. This approach required the development of several methods to be able (1) to perform a rapid quantification using spectrophotometry of the bacterial concentration within a suspension, (2) to quantitatively enrich bacterial suspension and (3) to verify the specificity and sensitivity of commercialised ELISA tests.

Potato quality audit in the Walloon Area A. Soete, H. Schyns, R. Agneessens, P. Dardenne¹, Chr. Anceau², M-J Goffaux², P. Lebrun³, E. Somerhausen³, O. Pigeon⁴, J-P Goffart⁵

This project has two main objectives. The first is to conduct an inventory of pollutants (heavy metals, pesticide residues, nitrates and glyco-alcaloïds) present in potatoes, intended for consumption, in the Walloon area. The second is to develop new techniques for analysing potato quality that are both rapid and reliable. Techniques will be

implemented and harmonised in local laboratories where potato growers evaluate the quality of their production in order to optimise their value. Our section is involved mainly in coordinating and developing the second objective. We are examining the difficulties of using NIRS to characterize potato quality when applied on whole unpeeled tubers. To address these difficulties, we will look at approaches involving tubers cut in two. In contrast, image analysis allows one to determine frying colour and after-cooking blackening indexes. Until now, these indexes were determined by visual comparison with reference cards (i.e., according to the judgment of colours by the human eye, and thus rather subjective). However, these reference cards are necessary for determining internal defects that are too diverse, in terms of coloration, to allow easy identification by the image analyser. Such defects are therefore assessed by comparison with a reference card, developed by the section, showing 18 pictures of potatoes damaged at increasing levels. To develop this card, the percentage of defects compared with total surface area of the tuber was calculated using image analysis.

The defects were then divided into five categories of increasing gravity, which correspond to a coefficient allowing the calculation of the internal defects index. This card is now validated and disseminated.

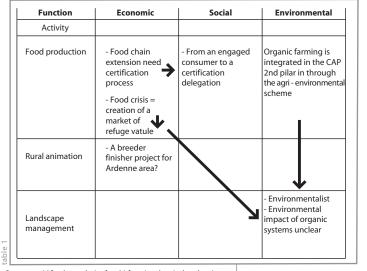
- ¹ Department for the quality of agricultural products ² REQUASUD npo
- ³ FIWAP npo
- ⁴ Department of phyto-pharmacy
- ⁵ Plant production Department
- Fiant production Department

6.2. Study of farming systems in terms of economic, environmental and social sustainability

Methodology to take into account farming systems' multi-functional dimension in relation to the multiple land-use in the surrounding area⁽¹⁾

D. Jamar, D. Stilmant, P. Stassart*, S. Vanderborght*, B. Morhain**, J. Stoll***

The general public's expectations of the agricultural sector are becoming greater and greater. If the sector's main role is food production, this cannot be achieved without taking into account other dimensions such as animal welfare or the environment (water quality, biodiversity, etc.). Also, food chain security is of major importance for consumers and the general public. Improving these aspects of production could increase its value. Apart from food production, farmers are taking on new activities, linked mainly to public services, such as rural tourism, landscape management, organic waste management and use, and energy production. These new functions imply multi-functional development of the land, particularly in the study zone, which is characterised by a patchwork of rural areas and dense population centres with important population movement between areas. This land-use dimension justifies a transboundary approach which works at the right spatial scale for such problems as water resources management. The area considered in our study includes the Province of Luxembourg in Belgium, Luxembourg, and the Departments of the Moselle and Meurthe and Moselle in France.



Common grid for the analusis of multi-functional agricultural projects (Institut de l'Elevage)-Application on organic cattle meat production systems

Within this area, the main aim of this project (INTERREG III 'Walloon area – Lorraine – Luxembourg') is to define and test a methodology and tools to help local partners set up joint, multi-functional agricultural projects. The specific objectives are: (1) to evaluate and exchange existing experiences (natural parks, Farm Territorial Contract, agri-environmental schemes, etc) and disseminate the results of these evaluations to the three areas; and (2) to help local partners, including farmers, in ongoing efforts to identify the priorities to be addressed, in relation to local land-use priorities, and to develop together the operational tools for implementing, monitoring and evaluating multifunctional project.

The first step consists of identifying 10 completed or ongoing projects combining agriculture with other land-use functions such as projects focusing on the development of meat production systems of varying quality, taking into account, implicitly or explicitly, their effect on the environment or on the development of farming systems incorporating recycling organic waste as a public service. Having performed an analysis on the same grid (table 1) of these projects, a first set of tools for diagnosing their evolution in line with their initial objectives will be proposed and validated. * University of Liège – SEED Unit, ** Institut de L'élevage, *** Fédération des Herd-Books Luxembourgeois.

Reconcile the economic and environmental performances of dairy farms based on grassland

S. Hennart, D. Stilmant, V. Decruyenaere, L. Fabry*, E. Froidmont**, JP Destain***, Ch. Vandenberghe****, R. Lambert***** and J.P. Harvent

The main objective of this project is to complete and validate the list of indicators to take into account, together with the definition of their critical thresholds, in order to ensure the proper application of the Walloon government decree of 10 October 2002 for sustainable nitrogen management in agriculture. Another target is to identify way of

reconciling the economic and environmental performances of dairy farms. The main aspects that will be developed are (1) the identification, validation and diffusion of agronomic practices that enable farmers to reduce the nitrogen balance in dairy farming systems based on grasslands and (2) the validation and definition of the critical limits for different indicators of environmental pressure at the plot level, such as

the number of grazing days (BLU*day/ha). In collaboration with the Technico-Economic Service of the AWE, 26 specialized dairy farms (OTE 411) were selected in the Herve Country, Ardenne and High Ardenne areas. This selection was based. primarily, on the good economic performance of these farms. The second selection criteria was nitrogen balance. For half of the selected farms this parameter is lower than the average minus one standard deviations and they are expected to exert limited environmental pressure; the nitrogen balance on other farms is greater than the average plus one standard deviations, showing important environmental pressure. The practices in terms, particularly, of grassland management that are used in these two groups of farms are recorded at the plot scale.

The evaluation of economic and social sustainability, performed on the 26 farms using the IDEA method, showed that these farms are efficient from these perspectives, even if the way they achieve good levels of efficiency differs. This result was expected, given the known economic performance of the selected farms. In contrast, their ecological sustainability was guite limited. Indeed, all the farms are highly specialised and so do not carry many animal breeds (Holstein and red Holstein) or plant species (permanent grassland with or without maize in a mono-cropping). In order to analyse the relevance of the number of grazing days (BLU*day/ha) indicator and the value of this indicator in autumn (after 1 September) when the leaching risk is important, we use a drained area ('superficial' draining system) in the Jurassic area. This will allow us, after applying different stocking rates, to determine the nitrogen balance in the different plots and to quantify leaching water composition, leading to a better definition of the link between environmental pressure indicators and leaching risks. The nitrogen balance will be established for each plot, with a weekly evaluation of the quality and quantity of available and ingested grass, animal performance (weight profit of BBB heifer), quantity and nitrogen content of soil water, climate conditions, and amount of nitrate residue in the autumn. * Technico-Economical Services of the Breeding Walloon Association, **Livestock Nutrition and production

Association , **Livestock Nutrition and production Department, *** Plant production Department, **** GRENERA-FUSAGx, *****ECOP-UCL



 How does organic farming contribute to the development of a sustainable system of production and consumption ? Study of cattle meat production
 D.Jamar, P Stassart*, Y. Seutin, J. Aertsens**, M. Mormont*, G. Van Huylenbroeck**, V. Decruyenaere and D. Stilmant

In this project, system sustainability is considered as the "capacity, for agro-food actors' networks, to take into account, in the long term, social, economic and ecological modifications in their activities". Sustainability is not a desirable state that must be defined, validated and reached, but a capacity to adapt. The context is the one of a collective construction, and in the long term, of articulated and innovative systems for the production, management and consumption of organic meat. This sector, after a significant increase in demand for organic products and in their availability in supermarkets, and the successive crises in the agri-food sector in the 1990s, is now in a consolidation phase. Although these systems were developed according to the Belgian Blue Beef production protocol, they are now raising questions about this protocol.

Cattle meat and organic farming sectors have been selected to assess the sustainability of their development. Indeed, some tensions, expressed through the strategies used by various operators, to circumvent the rules associated with organic farming (with regard to feed, health, welfare, etc.) and global issues, such as environmental norms, question this sustainability.

This project aims to:

1) Test, in collaboration with the actors in the chain, some new management schemes, new production techniques, and processing, distribution and consumption schemes, in terms of sustainability.

2) Define and test an action-research methodology using a multidisciplinary approach. This methodology will make it possible to assess the constraints of the sector with the actors involved and to develop new applied and scientific knowledge by changing the existing knowledge and the relationships among the actors.

In this multidisciplinary approach (sociology, economy and agronomy), the CRA-W contribution lies in the organic farming systems analysis, particularly in the analysis of the relationship between farmer, grassland, fodder crops and livestock. The production system is considered as a whole, alongside the agro-food chain. The social environment and the environmental factors are seen as being influenced by these systems.

Over two growing seasons and on 13 organic farms, chosen for the diversity of their farm structure and their approach to the organic farming rules. Breeder knowledge, young bull and heifer performance, and grassland and crop production were recorded with a special focus on animal sanitary status (parasitic pressure) and behavior, plant biodiversity, sward structure and pressure on the environment.

* University of Liège – SEED unit, ** Universiteit Gent

Optimising heifer growth to promote a first calving at 24 months V. Decruvengere

To remain competitive and increase their profits, farmers have to reduce their production costs. In suckling cattle systems, heifer management for early calving, ensuring an average daily gain (ADG) from birth to first insemination of 0.75 kg/day, can help to reach this target. This practice can reduce the unproductive life of breeding animals by 6 to 12 months and, theoretically, with the same calving number, reduce the stocking rate at farm level. Although this practice is technically feasible, it is fairly uncommon and farms practising early calving tend to be moreintensive (more concentrate feed and more fertilizers for forage production). The objective of this 3-years study is to show that it is possible to manage heifers for early calving with optimal use of grass and forages produced on the farm. To achieve this objective, two topics are being explored: (1) what will the impact of winter diets on heifer performances be during the subsequent grazing period ? and (2) what will the grazing performances of 6-month-old heifers, born in autumn, be during their first grazing season.

With regard to the first topic, a significant influence of both the basal diet (forage = FOR or concentrate = CON) and the level of nitrogen supplementation (115N or 100N) was observed on winter performance, with no interaction between these factors. Concentrate-fed heifers or heifers fed with high levels of nitrogen were heavier at turnout (+20 to 30 kg). Nevertheless, from an economic point of view, forage-based diets remained more attractive (-€0.31/animal/day)

But did the winter diet affect grazing performance? Heifers previously fed with forage diets maintained their level of performance and at the end of the grazing season there was no weight difference between the basal diets (FOR or CON). On average, a weight of 420 kg was reached for all heifers between 15 and 18 months.

With regard to the use of grazed grass by 6-monthold heifers born in the autumn, grazing performances were, on average over the 3 years of the trial, 0.576 kg/day, with a high between-year variability: 0.649, 0.311 and 0.765 kg/day, respectively, in 2001, 2002 and 2003. Such performances were achieved with good quality grass (940 VEM, 89 g of digestible protein in the intestine (DVE in Dutch system), 16.5% of crude protein) supplemented with 0.5 to 1 kg of breeding concentrate. In 2001 and 2003 grazing resulted in satisfactory carcass development (in term of increase of thorax diameter). During the subsequent stalling period, ADG was significantly higher for the concentrate diet, with 0.885 kg/day against 0.630 kg/day and 0.746 kg/day, respectively, for the grass-, silage- and hay-based diets. As in the first experiment, forage diets remained economically interesting. All heifers could be inseminated between 15 and 18 months old but concentrate-fed heifers weighed 420 kg 1.5 months before the others.

Sheep production to diversify cattle farming systems V. Decruyenaere

Grazing is an useful basal diet for suckling cows as it does not need any harvest and yet results in good animal performance. So it is the cheapest way to feed cattle from spring till autumn (about €0.40 and €0.49/animal/day, respectively, for heifers and young bulls). However, to reach a good performance level (0.750 kg/day and 0.850 kg/day, respectively, for heifers and young bulls), it is necessary to ensure a good quantity of good quality grass throughout the season. Both these parameters change with the season and according to the pasture management or plant development stage. In a rotational grazing system, therefore, grass availability and quality decrease continuously after cattle have been introduced to a new paddock. To maintain a good level of performance, grazing time on the paddock could be reduced. However, important residual grass quantity could be prejudicial to the pasture (in terms of plant cover degradation, installation of undesirable species or species of less nutritional value, etc.). The grazing behaviour of sheep is different from and complementary to cattle behaviour. Sheep can reach a lower sward surface height and, because of their small bite size, they are more able to select their diet. The use of this 'sheep-cattle' complementarity, also recognised in the management of gastro-intestinal parasite problems, could lead to good animal performance in both cases and high quality sward persistence. This is being tested in this project. Such diversification could also promote sheep-meat production, an interesting possibility as Belgium is not self-sufficient in this sector and as sheep production remains associated with a positive assessment by consumers. Such considerations sustain sheep meat prices. The sheep race selected to test this approach is a 'grazing sheep', the 'Swifter' (photo 1). It is a crossbreed between a Texel ram and a Flemish dairy ewe : its products being allocated to lamb meat production. Their principal characteristics are: a high prolificity; confirmed by the 1.87 lambs born per ewe, for our 40 first lambing ewes; a long reproduction period; good dairy production; and the production of lambs of good conformation. We observed no problems after lambing; Swifter ewes are very maternal.



Swifter suckling ewe

From their birth till turn out, lamb performances were on average, and with hay as the only supplement, 272 g/day (ADG), reflecting their mothers' good milk production.

Three approaches to grazing are being tested: separate grazing of Belgian Blue White heifers and sheep; mixed grazing on six paddocks; and leaderfollower grazing, also on six paddocks. For separate grazing, heifers grazed either in continuous grazing or in rotational grazing on three paddocks, while sheep were only in continuous grazing. In spring there were few differences between the grazing systems with regard to heifer performance (ADG May-June = 0.715 kg/day). Only the heifers in continuous grazing showed poorer performances (0.613 kg/day). In summer, the heifers under a simplified rotational grazing, with an ADG of 0.451 kg/day, had a lower performance than the other group (0.719 kg/day), probably because of higher parasitic pressure.

The spring performance of ewes with lambs was higher than 200 g/day for all grazing systems. The performance of the group in the 'leader-follower' grazing system was, at 208 g/day, lower than that of the other groups (239 g/day).

Lambs took maximum advantage of their mothers' milk and of the spring grass quality. Their performance level was, until weaning (07/08), 372 g/day, irrespective of the grazing system.

The first results indicate that parasitic infestation of sheep or heifers is influenced by the grazing system. Heifers grazing with sheep had a lower serum pepsinogen level. For the leader-follower grazing system, this could be explained by the fact that heifers always grazed grass with a low infestation load; indeed, they left paddocks when the grass height was 7-8 cm and parasites were restricted to the lowest sward stratum, close from the soil surface. In this system, sheep grazed paddocks till there was a low residual sward height and its serum pespinogen level was higher than in the other systems. Under mixed grazing, it was expected that ewes would clear the paddocks of cattle parasites, and thus there was a lower infestation level in these paddocks. The effect of cattle stocking rate - lower in the leader-follower and mixed grazing systems - could also explain these results

6.3. Grassland and other fodder crops phytotechny in south-east Belgium

Leguminous species, an alternative for farming systems seeking a low level of nitrogen input

(D. Stilmant, D. Knoden*, B. Convier**et B. Mernier**)

In organic farming, to which Walloon wants to convert 10% of its UAS before 2010, crop nitrogen fertilisation management at different plant development stages remains one of the main constraints to good yields and the development of this type of production.

The main aim of this project is to determine alternatives that include leguminous species in the rotation while taking into account the technical factors required to obtain high quality yields. The issues being investigated during this 2-years period are: - the advantage of using high diversity grassland mixture instead of the traditional 'perennial ryegrass (PRG) – timothy (T) – red clover (RC)' association;

- the potential of a cereal under-sown with a white clover sward to extend rotation length within organic farming systems;

– legume drying management, a key factor in reducing losses in quantity and quality.

With regard to the study of a species-rich combination for meadow, after 9 years of records and analysis, the combinations of more than five legume and five Graminae species have not shown, under our soil and climatic conditions, any clear advantage from either a qualitative or quantitative perspective in comparison with the classical PRG-clover association. Species-rich sward often showed an advantage during the sowing year but, because of the lower persistence of the clover varieties used, their performance fell more rapidly. These observations are of value with or without the use of a cover crop during the sowing year. It is also worth noting the rapid decline in the floristic diversity in these mixtures; species adapted to a cutting regime, such as cocksfoot, perennial rye-grass and red clover are more persistent. These observations, in addition to the high cost of such mixtures, lead us to warn against using this type of mixture in organic farming under our soil and climatic conditions.

The aim of the 'winter cereal planting within a white clover sward' trial, initiated in the 2003-2004 planting season, is to lengthen the rotation scheme of '3 years of temporary grassland - 3 years of cropping' usually applied in organic farming to ' 3 years of temporary grassland - 6 years of cropping' with an under-sowing of white clover cover in the spring cereal in the fourth year of cropping. This practice will be followed by two winter cereals that will be sown, without deep tillage, in the white clover cover. In the first year, various soil preparation techniques, before the winter cereal (spelt) was planted in the white clover sward, were tested. They ranged from deep tillage to direct sowing using shallower tillage techniques. The objective was to slow down white clover development before the spelt was planted. The first results show a decreasing yield gradient from the deep tillage, stimulating organic matter mineralisation and nitrogen release, to direct sowing, where spelt suffered from a strong white clover concurrence. A shallow tillage allowed intermediary yields and a good white clover cover to be obtained. This configuration could surpass the deep tillage one, which over the forthcoming years will no longer benefit from the white clover. The third research topic focuses on finding alternatives that will reduce the quantitative and quali-

natives that will reduce the quantitative and qualitative losses observed during legume-rich sward drying. Legume leaflets break off easily during forage drying. The associations investigated are PRG-white clover (WC), PRG-RC, T-RC and cocksfoot (C)-lucerne (L). The alternatives investigated in 2003 were, during the first cut for hay production, the impact of mower type, with or without conditioner, and, during the second cut for silage making, the impact of tedder rotation speed (270 *versus* 540 rotations per minute (r/min) at the power plug). Legumes accounted for, on average, 65 and 80% of the dry matter (DM), respectively, during the first and second cuts.



Evaluating legume-rich mixed swards

The losses observed, on a DM basis, were 41 and 27%, respectively, during hay and silage making. There was no effect of the mower type, but an increase in tedder rotation speed increased the protein losses by more than 11%, without significant impact on the final DM content. As expected, the main losses were observed at the level of legume leaflets. This was confirmed by developing a NIRS calibration allowing us to quantify the proportion of legume leaflets in a Graminae-legume sward. The type of Graminae-legume mixture also had a significant impact. However, this factor combined various parameters such as the ratio of legumes and initial yield differences which were independent of the management type.

*Best Fodder npo **Trial Centre for organic agriculture and horticulture

Grass varieties adaptation to grazing: persistence and palatability

(Y. Seutin, D. Stilmant, P. Luxen*, D. Knoden, P. Dardenne**, D. Leconte***)

This trial, also supported by Best Fodder npo, aims to determine the biochemical and morphological characteristics required to achieve, through a genetic selection process, improved sward ingestibility and persistence. The final objective is to be able to advise farmers on the choice of varieties to re-seed grazed grasslands.

To achieve this objective we need to develop the analytical tools that will quickly determine the morphological and biochemical characteristics of interest from a great number of samples, especially through the use of the NIRS technique. The good performances of this technique (table 2) will allow us to predict the species composition of a sward (Graminae, legumes [white clover, red clover, lucerne] and other species) as well as its morphological composition (leaf versus stem ratio) in terms of both Graminae and legume species. With regard to the guality of the NIRS tool developed, the 'Standard Deviation of the population / Standard Error in Cross Validation' ratio being higher than 3 for all these calibrations highlights their good performances (table 2). Such NIRS calibrations also exist for determining sward biochemical characteristics.

	N	Mean	SD	R ²	SD/SE cv
Legume leaf	868	20,2	12,77	0,98	5,7
Legume stem	869	50,9	15,93	0,97	5,7
Grass	869	15,7	12,30	0,97	5,5
Rest	872	12,7	8,49	0,96	4,3

Evaluating legume-rich mixed swards

The last step is to determine the potential links between the variety palatability index and the various morphological and biochemical characteristics. The first results show the significance of organic matter digestibility in explaining the observed palatability index variations. * Best Fodder npo, **Agricultural Product Quality Department, ***INRA-Domaine Expérimental Fourrager du Vieux Pin

Compost: impact on nitrogen fluxes in a grazed grassland

(D. Stilmant, V. Decruyenaere)

In line with the implementation of the Nitrate Directive in the Walloon area, with its limitations on organic fertilizer spreading, it is important to be able to spread farm manure on the greatest part of the farm surface. In grassland-based livestock farming systems, therefore, a solution to the spreading of solid manure on grazed grasslands needs to be found. Composted manure seems to be an interesting alternative. Some 10 t of compost/ha/vr meets the requirements of grazed grasslands in terms of P,K,Ca,Mg and oligo-nutrients, without any intake modification. But, what will the impact of such a fertilization practice be on the nitrogen flux within this agro-ecosystem ? In order to explore this problem, a nitrogen balance trial was conducted over 6 years (1995-2000) in two systems grazed by young cattle at an initial stocking rate of 1506 kg liveweight/ha. Simplified rotational grazing systems on three paddocks, one paddock being mowed once in spring, were used. One of these grazing systems received only composted manure at the rate of 16.7 t/ha, on average, on the grazed paddocks and at the rate of 35.5 t/ha on the mowed and grazed paddock. The second grazing system received only mineral fertilisers to ensure similar sward productivity in terms of quantity and quality. Nitrogen dressings were 0, 13.5, 27, 33, 47 and 75 kg/ha, respectively, from 1995 to 2000, and P and K dressings were 50 and 60 units/ha, respectively, on exclusively grazed paddocks and 100 and 120 units, respectively, on mowed and grazed paddocks. This experiment was duplicated. In order to perform the N balance at paddock level, we took into account the available N fraction of the compost: 15 and 25% of the total N amount as a direct effect, respectively, for mature and new compost, 10 and 8.6% of the total N amount, annually, as a long-term effect, during the following 7 years. The other inputs taken into account were the N fixation by white clover and animal supplementation under grazing. Outputs considered were animal exportation, silage exportation and gaseous losses.

Over the 6 years of the trial, organic fertilisation (62.4 kg/ha) provided twice as much available N than mineral fertilisation (31.8 kg/ha). The other main N input, representing 41% of available N input, came from symbiotic fixation by the legumes. The total inputs were 135 and 98 kg/ha/year, respectively, in the 'compost' and 'all mineral' fertilisation schemes. The outputs are, due to silage exportations, linked to the exploitation method. Only gaseous losses, always lower than 10%, were dependant of fertilisation method. On this basis we determined the available N balances influenced by both the fertilisation and exploitation methods :62.9 and 79 kg/ha within grazed paddocks and 5.1 and 36.8 kg/ha in mowed and grazed paddocks, respectively, for mineral and organic fertilisation schemes. *In fine* N use efficiency was lower in the 'all organic' scheme((46% as opposed to 65%). This lower efficiency has to be put in perspective as N balance performed at the farm level will favour compost use coupled with lower N fertiliser imports! However, to reach such a target while minimising the environmental impact of grazed grassland, the compost application rate had to be as low as 10 t/ha. This means that the compost available in a system with an animal stocking rate of 1.8 BLU/ha, corresponds at the equilibrium to the release of +/- 55 kg N/ha.

Compost: the basis of the fertilisation in organic farming systems (D. Stilmant, D. michelante et D. Jamar)

The Walloon area is seeking to convert 10% of its UAS to organic farming before 2010. This system promotes the use of composted manure as the basis of crop fertilisation. What performances could be achieved taking into account the manure availability in the system? How does one adjust the additional fertilisation needs (N-P-K)? In order to answer to these questions, we have been running a long-term trial since 1998. It aims to perform nutrient (N-P-K) balances in an organic meat production system with special production (potato and spelt).

The system analysed has 45% of its surface occupied by a 7-year rotation: 3 years of temporary grassland, 1 year of spelt (crop of economic value), 1 year of triticale, 1 year of potato (crop of economic value) and 1 year with a triticale/pea mixture. Compost availability is linked to an animal stocking rate of 1.7 BLU/ha, giving 7.6 t of compost/ha/yr. This compost is spread at a rate of 15 t/ha for 3 years on the permanent grasslands, at a rate of 15 t/ha in the first and last years of temporary grasslands and on the triticale, and at the rate of 30t/ha on the potato crop. The compost composition is, on average, 6 kg nitrogen, 4.5 kg P2O5 and 6 kg K2O. The evolution of the quality and quantity of production is recorded alongside soil fertility parameters.

Over the 5 years of recording, good yields were

observed on the temporary grasslands; with 5t/ha produced in the year of sowing and 10 t/ha in the years of full exploitation, after a deduction of 20% of drying losses for mixtures rich in legume and for potato crops, with a mean vield of 30 t/ha. However, cereal crops suffered from a nitrogen deficit. This led to low yields of

this mid-mountain area.

Such performances led to mean nutrient exportations of 22 kg/ha/yr of phosphorus and 127 kg/ ha/yr of potash. These exportations were covered, for the phosphorus, by compost spreading, while a net deficit of 72 kg/ha/yr was recorded for the potash. In order to reach an equilibrium and maintain soil fertility, an annual spreading of potash is necessary (e.g., in the form of patenkali at the rate of 300 kg/ha/yr).

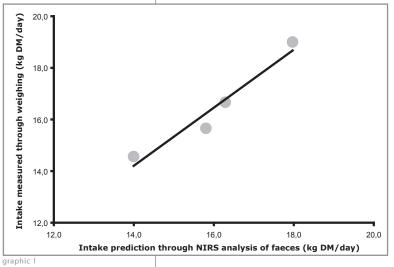
6.4. Development of analytical methodologies and tools

Determination of herbivore intake under grazing and of the composition of this intake by NIRS analysis of its faeces (V. Decruyenaere, R. Agneessens, Ph. Lecomte*, M. Boval**, M. De Visser***)

Under grazing, animal performance is influenced by the quality and quantity of grass available. The measurement of these parameters using conventional techniques is difficult and time consuming. Herbivore faeces, a residue of the digestion, can be considered as a good indicator of the animal intake. As shown in past years, NIRS applied to sheep or cattle faeces allows a good estimation to be made of the voluntary intake and digestibility of grazed grass, as well as its composition and, in particular, the legume fraction ingested, in PRG-WC mixed swards.

In our work on this approach, we have developed some calibrations to quantify the white clover proportion in sheep and cattle intake (in collaboration with the Animal Sciences Group, Wageningen UR, Lelystad, The Netherlands). The statistics associated with these calibrations highlight the potential of this method (n=177; mean = 46.12 %; $R^2 = 0.9$; SECv = 4.59 %).

Similarly, in collaboration with CIRAD, this tool has been used to assess dairy herd intake on Reunion Island. These predictions have been compared to real intake measurements in four herds. The results show a good correlation between the two parameters (figure 6), highlighting the potential of such a tool for assessments at the herd level.



2.5 to 3.2 t/ha, the latter obtained with cereal/pea mixtures. The normal cereal yield was 5-7 t/ha in

Link between the levels of intake predicted through NIRS analysis and through weighing, of four dairy herds on Reunion Island.

* Centre International in Agricultural Research for Development (CIRAD-EMVT), ** Institut National de la Recherche Agronomique (INRA) Antilles-Guyane, Station de Zootechnie, Guadeloupe, ***Animal Sciences Group, Wageningen UR - Lelystad

Co-composting: Study of the process and its environmental impact, and evaluation of the agronomic value of such organic fertilisers

(J.F. Collard, R. Agneessens, J. Devillers* et P. Dardenne**)

A significant part of the organic wastes of industrial and/or urban origin can be considered as harmless. This allows one to assess the value of composting and biomethanisation techniques. However, in their original state, organic wastes are not all compostable as such. Co-composting consists of gathering various organic materials, without an optimal constituent balance if taken alone in terms of composting, and then mixing them in proportions aimed at optimising bio transformation.

Although the process is familiar, questions persist. What about the rules of organic matter combination? What about the environmental impact (e.g., greenhouse gas emissions, filtering capacity against organic contaminants, impact on bioavailability of heavy metals) of the process? What about the effect of this process on the sanitary status, in term of pathogen concentration, of the end product? What is the agronomic value of such products?

The purpose of this project is to study these issues. All the information collected will be used to write a 'good practices in co-composting' code in order to improve compost quality and reduce the negative effects on the environment.

The development of NIRS allows us to determine parameters such as dry matter, organic nitrogen, total carbon and organic matter (figure 7). The main difficulty is the lack of homogeneity of fresh samples. At present, NIRS allows us to determine important parameters in 72 hours (including drying). quality, and to prevent any lixiviate production, the compost was piled up inside a shelter. These results revealed a decrease of 50% of the initial weight. Lost gases are composed of 75% water vapour, 15% carbon dioxide and methane and about 1% of various other constituents, including nitrogen.* Agricompost Society

** Agricultural products quality Department

7. SERVICES ACTIVITIES

Control of sanitary quality (double budding technique, of the indicator and of the variety to be tested), conservation, multiplication and diffusion of 135 fruit tree varieties and 11 stock types (guarantee of the identity of the variety), without any known virus (virus-free, VF) or without a list of viruses of economic importance (virus-tested, VT).
Identification of phyto-sanitary problems on potato, with advice on addressing them.
Analysis of variety conformity for potato tubers for the RW-DGA – Quality Direction, for enterprises, etc.
Analysis of potato quality from a technological and culinary point of view (e.g., the lots proposed for the Terra Nostra label).

- Provision of a 'potato late blight' and 'aphid flight' advisory service to reduce production costs while preserving the environment (for interested farmers and private individuals).

Determination of the presence of virus agents in all lots of potato seedlings produced in Belgium.
Production of starting material (micro-tubers, mini-tubers, acclimated vitro-plants, etc.) for potato production chains in Belgium or abroad.
International expertise for potato seed production (Democratic Republic of Congo, Montenegro, Kosovo, Madagascar, Senegal, Rwanda, Lebanon, etc).
Development, with other CRA-W Departments and other institutes or private partners, of analytical methods for organic substrates of agronomic interest

- In collaboration with the Quality of Agricultural Products Department and for the REQUASUDnpo, NIRS model maintenance and improvement through adding to databases the samples selected and analysed through reference methods.

- In collaboration with the Quality of

Agricultural Products Department and

through the non-

profit organisation

CEFAWAL, assist in

the establishment of quality control

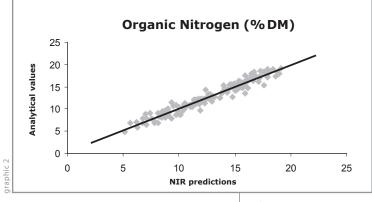
facture, common in

the Walloon area, in

order to maintain

these enterprises in the agro-food

(GMP) in small animal feed manu-



Correlation between labs and NIR measurement, on dry material, for organic nitrogen (%DM)

For the environmental impact of the co-composting process, the first results concern a mass balance performed during such a process. In order to quantify all the inputs, in terms of quantity and scheme.

Advisory service for ruminant nutrition.
Agricultural and Agronomic Engineering students training during their end of study work.

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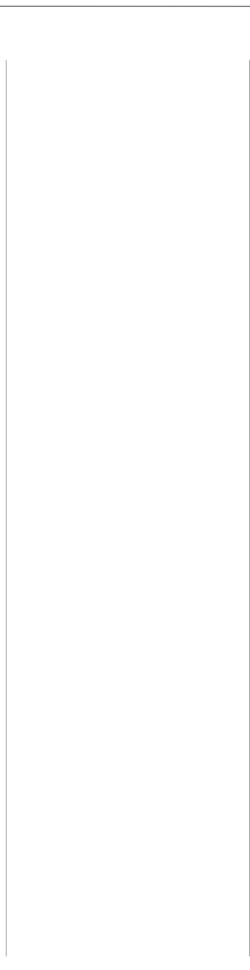
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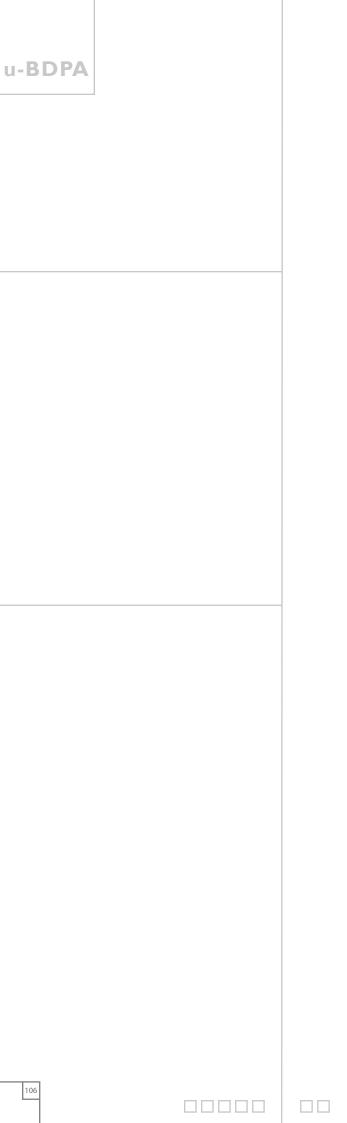
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u-BDPA

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Unit

BIOMETRY, DATA PROCESSING AND AGROMETEOROLOGY



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Unit BIOMETRY, DATA PROCESSING AND AGROMETEOROLOGY

1. MISSION STATEMENT

This section focuses on developing research and support activities in statistics and data processing as applied to agronomic research. It seeks to develop and promote the use of innovative data management methods and to create mathematical and statistical models representing agro-environmental systems with spatio-temporal components. The work is done not only in collaboration with other CRA-W departments but also with researchers in other Belgian or foreign institutions.

2. SCIENTIFIC PERSONNEL

2 permanent staff and 5 contract staff

2.1. Head of Section

R. Oger, Inspecteur général scientifique

2.2. Scientists

A. Antofie, Attachée scientifique (EU) D. Buffet, Attaché scientifique Y. Curnel, Attaché scientifique (BSP) A. Krafft, Attaché scientifique (EU) B. Leteinturier, Attachée scientifique (BSP) V. Planchon, Attachée scientifique (OPI)

3. NATIONAL REPRESENTATION

Steering committee of the TELSAT 4 program and of the Plan of Scientific Support for a Durable Development policy (PADD II - BSP).
Steering committee of the 'STEREO-CROP' project of the research programme on earth observation 'STEREO', supported by the Belgian Science Policy
Participation in a study group on sampling, organized by the Scientific Committee of the Federal Agency for the Safety of the Food Chain (AFSCA).

4. INTERNATIONAL REPRESENTATION

• EU, member of the Management Committee of the action COST 718 'Meteorological Applications for agriculture'.

• EU, member of the Management Committee of the action COST 725 'Establishing a European Phenological Data Platform for Climatological Applications'.

• European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR).

• CNES – Direction of the Strategy and Programmes, Team 'Observation of the Earth' (DSP-OT). Representing Belgium in the ORFEO study group 'Agriculture'.

• EC-JRC, Belgian representative in the group of experts Crop Growth Monitoring System (CGMS) of the programme Monitoring Agriculture by Remote Sensing (MARS Stat).

5. RESEARCH TOPICS

Research activities in the unit have, in recent years, adopted a multidisciplinary approach to the projects and a partnership approach based on complementary strengths and common objectives. These activities all relate to science and technology knowledge advances in data processing with regard to sustainable agricultural development, the environment and food consumption.

Four overlapping fields constitute the unit's research tasks:

1. Sustainable development of agricultural ecosystems

Current political measures aimed at promoting the sustainability of agriculture focus on maintaining a rural heritage by conserving the production potential of the environment and ensuring long-term agriculture viability. The implementation of this policy requires improved monitoring of agricultural activities and improved evaluation of the impact of agriculture on the environment, as well as promoting the need for competitive agricultural production. Particular emphasis is placed on identifying relevant indicators to help people in charge of natural resources management implementing the required agro-environmental measures. The research projects on this topic are closely related to the development of Web-based geographical information systems (GIS). They also seek to use information arising from remote sensing data to monitor and manage the environment and areas used for agricultural purposes. The integration of satellite imagery is likely to facilitate the spatialization of the information and the formulation of regional inventories.

2. Quality, traceability and food safety

With the globalization of information exchange, people are becoming better-informed consumers who demand to know about the origin and safety of the food they eat and about the environment in which it is produced. To meet this demand, the implementation of systems tracing products from the producers to the distributors of agro-food products has become essential. The need for traceability of agricultural products varies from one agricultural sector to another. At the production level, it relates to the need to be able to offer a choice of products. At the supervisory level, in charge of food safety, traceability prevents unsuitable products being marketed and consumed.

The objectives of the research on this topic are to define a methodology for acquiring, using and processing georeferenced data which enable geographical traceability indicators to be produced. One aspect of these activities relates to the problem of data exchange and building data processing infrastructures that guarantee the geographical traceability of agricultural products. The assessment of the mycotoxin production risk in winter wheat is an example of a project in this field. It aims to develop a strategy of effective sampling for the control of production lots before harvest.

3. Genetic resources and agricultural biodiversity

The documentation systems for genetic resources and, in particular, the centralized databases play an essential role in the conservation of genetic resources and in the promotion and evaluation of the use of these resources in the context of international management. The aim of the projects on this topic is to build specific databases covering phenotypic as well as genotypic characteristics and to develop user interfaces that make it possible to compare genetic material and exchange information between researchers and breeders. These objectives can be achieved by ensuring a greater standardization of the contents and by defining conceptual models for databases adapted to this type of information.

4. Conception of new methods and techniques for data management

The aims of creating and managing databases that cover the various research activities undertaken by CRA-W are to make available information on various topics related to agrometeorology and to enable product quality and environmental characteristics to be evaluated. These activities are closely related to the installation and evaluation of new information technologies such as Web services and the study of the constraints related to the inter-operability and exchange of the data. These activities also aim at developing and maintaining expertise in the development and use of new agricultural output forecasting models on a local and regional scale, as well as the use of information resulting from satellite imagery.

The aims of the scientific work related to this activity are to develop quality control methods specific to the management of large datasets. It is envisaged that operational techniques for detecting outliers or extreme values in spatial databases will be developed.

6. RESEARCH REPORTS

6.1 Sustainable development of agricultural ecosystems

Development of an agri-environmental monitoring system for the Chinese province of Heilongjiang Y. Curnel

This project is directly linked to two earlier projects in which CRA-W was involved and which sought to develop crop yield monitoring systems, the first one on the scale of Belgium (B-CGMS) and the second one on the scale of the Chinese province of Heilongjiang.

The cooperation with two other Belgian research institutes (VITO, FUL/ULg) and the Heilongjiang Province Institute for Meteorological Sciences (HPIMS) was motivated by the fact that for more than a decade agricultural policy had been oriented towards the sustainable management of natural resources. It is against this background that agri-environmental indicators, which enable,



for example, the impact of the agricultural or forest activities to be evaluated, have been developed.

The overall objective of this project is to develop and implement an integrated, multi-purpose land monitoring system, which combines all types of available inputs, including results from CGMS, which can help in planning and decision-making in agriculture, forestry, wetland and environmental monitoring. In line with the earlier collaboration, the emphasis remains on agriculture, crop monitoring and yield forecasting, but the applicability of the system has been extended to monitoring drought stress and land use. The spatial component of the studied phenomena is taken into account using remote sensing indicators, which are a core component of the project.

Although the project title refers only to China, part of the activities are conducted in Belgium. The methodological improvements, for example, were first tested on Belgian datasets, which are readily available, and then transferred to China.

Hyperspectral imagery for monitoring grassland and controlling agri-environmental measures. D. Buffet, Y. Curnel et B. Leteinturier

With CAP reform, Agri-Environmental Measures (AEM) are now compulsory and contained in all rural development plans in the EU. Due to the diversity of farming systems and their importance in the agricultural landscape, grassland and headland constitute an important part of the agrienvironmental measures.

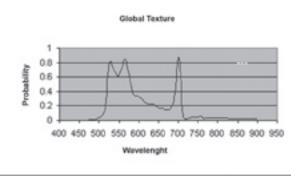
Within the framework of sustainable agriculture, monitoring permanent meadows is closely linked to knowledge of grassland management systems and their physico-chemical and environmental characteristics at regional level. This information is usually collected by systematic ground observation exercises and by laboratory analyses. Unfortunately, this approach takes a long time and can be expensive. A solution could be the use of remote sensing as a consistent and non-intrusive verification tool in the management and control of plot-based agricultural activities.

The objective of this study is to show that hyperspectral imagery meets some management needs by providing continuous spatial and temporal monitoring of parameters that characterize the canopy structure of every grassland plot, as well as its biochemical and biophysical properties.

Three airborne hyperspectral imagers were studied: the CASI sensor for VIS and NIR wavelengths, the SASI sensor for SWIR and the ATM sensor for the thermal infrared band.

Remote sensing data were acquired during three flight campaigns in September 2002, June 2003 and October 2003 from a representative study area of about 50 km² in south-eastern Belgium. Biophysical characteristics, reflectance data and grass samples were collected simultaneously in a large numbers and from various grasslands. These samples were then analysed in the laboratory to determine their chemical characteristics.

The first measurement exercise showed the potential of hyperspectral imagery for estimating quantity parameters (e.g., wet matter, biomass, grass height) and the quality of the grass canopy (e.g., protein, VEM, DVE) and thus to establish regional inventories. In addition, and based on textural analysis at the intra-parcel level, spectral response curves highlighted a potential discrimination and classification (Figure 1) between the various types of meadows (pasture, mowed meadows, etc.).



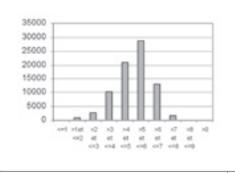
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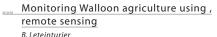
Probability level of the difference between grassland management classes: variance analysis using global texture parameter calculated for each waveband.

Discriminant analysis with cross validation showed that about 83% of correct grassland management classification can be obtained. This important observation is very promising in terms of the monitoring and control of agri-environmental measures. These initial results demonstrate the potential of hyperspectral imagery to perform a first screening of the plots being controlled. The SAGRIWATEL project was undertaken with the financial support from the Belgian Federal Science Policy Office and the Walloon Region. It brings together partners from various institutions (CRA-W, FUSAGx, WR, UCL and ULG) in an effort to strengthen this approach by putting a pre-operating system at the disposal of the Directorate of Agriculture of the Ministry of the Walloon Region . Three types of tools are required to set up an integrated system for monitoring the state of Walloon agriculture: (i) spatial information on annual agricultural land use, using IACS (Integrated Administration and Control System), (ii) CGMS (Crop Growth Monitoring System) yield forecasting, adapted to Belgian conditions, and (iii) satellite

> information, with different levels of spatial and temporal resolution (SPOT-VGT, SPOT5, LANDSAT5, IKONOS, etc.). These tools are used to produce a set of agro-environmental indicators (AEI) which are considered as promising tools for assessing, quantifying and monitoring the effects of agriculture, with the aim of ensuring sustainability and minimising environmental impact. From the huge list of AEI published by the European Com-

mission, our attention focuses on those where the accuracy of results is most likely to be improved using remote sensing. Among these indicators is the example below of the crop sequence agroenvironmental indicator (Isc). Crop sequences observed over a 7-year period were evaluated at plot level for the whole Walloon territory, on a scale varying between 0 to 10. The assessment was based on the effect of the previous crop, the next crop, keeping to recommended minimal return time for the different crops, and crop diversity. Figure 2 shows crop sequence indicator frequency distributions from 1997 to 2003 for the Loam (left) and Ardennes (right) agricultural regions.





The legislative context of agriculture in the Walloon Region is a complex one, with three main components: support, monitoring and control. The Walloon Rural Development Programme contains a set of measures aimed at controlling and supporting agricultural development in the Walloon Region.

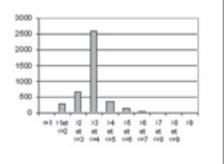
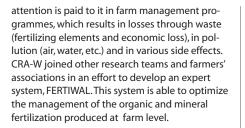


Figure 2. Crop sequence indicator frequency distributions from 1997 to 2003 for the Loam (left) and Ardenne (right) regions.

Optimization of the organic and mineral fertilization management in the Walloon agricultural systems A. Krafft

Manure production is an important source of fertilizer on a farm. Unfortunately, not enough





The objective of this project is to provide farmers and technicians with powerful and reliable software to optimize the economic returns from fertilization at both farm and plot level, in line with Walloon regulations.

The tool, now under development, takes account of the data inter-operability concept to enable data to be transferred using other agricultural software packages. This process is based on a structured and standardized scheme. It also includes a Geographical Information System (GIS) in order to increase the accessibility of fertilization management information.

Working at parcel level makes it possible to also include non-productive elements such as those imposed by agri-environmental regulations. The coordination of the project is handled by the Industrial and Agronomic Research Centre of the Free University of Brussels. The other partners are Agra-Ost and the Walloon Breeders Association.

6. 2 Quality, traceability and food safety

Geographical traceability in agriculture (GeoTraceAgri) A. Krafft

GeoTraceAgri is a project of the 5th FP (IST) carried out in collaboration with the Chamber of Commerce and Industry of Gers in France, CIRAD, the University of Liège, the Laval University in Quebec, Canada and the group CDER Informatique.

A prime objective of the GeoTraceAgri project was to devise a methodology for acquiring, using and processing georeferenced data which makes it possible to produce agro-environmental indicators at various spatial scales. This work led to different indicator classes allied with geographical traceability in agriculture being determined.

The various geographical scales considered are the plot, the basin and the region for which the product origin is certified (registered designation of origin - label guaranteeing the origin and quality of the product). A series of indicators of geographical traceability was identified by user groups selected by the project consortium. This allowed us to define a frame of reference for geographical traceability in several agricultural sectors (cereals, potatoes, fodder, fruit and vegetables).

The project resulted in the construction of a dataprocessing infrastructure which guarantees the geographical traceability of agricultural products using internet-oriented tools. The system was built on an internet platform using open-source software. It incorporates extended GIS facilities such as spatial data analysis, Web mapping, consultation of metadata catalogues and interconnection between geographical and semantic databases.

The GeoTraceAgri project ended in December 2004. As of 1 January 2005, all the agricultural products now have to be traced. Therefore, a new project (GTIS-CAP) was initiated within the framework of the 6th PCRD. It aims to develop a prototype of an integrated control and management system adapted to the new requirements of the reformed CAP.

Evaluation of risk contamination of winter wheat by mycotoxins R. Oger, P. Detrixhe, et D. Buffet

The Biometry Unit is involved in a research project, in collaboration with the Department of Biological Control and Plant Genetic Resources (CRA-W), that aims to develop an agrometeorological model for assessing the risk of head blight development and of mycotoxin contamination in winter wheat. The risk of head blight development and mycotoxin contamination depends on meteorological conditions during the flowering of wheat. It is generally assumed that frequent rainfall associated with a temperature above 16°C in the few days before and after flowering are favourable conditions for head blight development. Nevertheless, in terms of meteorological parameters, the duration of leaf wetness associated with dew deposit and rain is probably the parameter of greatest importance.

To address this risk at the field level, it is necessary to include complementary information about the cropping system (the previous crop, fungicide protection, level of resistance in the wheat variety, level of disease development in the area, etc.). The spatialization of rainfall events is a key element required in order to be able to estimate the risk at parcel level. It is estimated using meteorological data provided by the RMI meteorological radar in Libramont.

These parameters are used to build probabilistic models aimed at estimating the risk of head blight development and DON contamination. The development of this kind of model required a calibration and validation phase based on data collected in the field over the past 3 years.

One of the interesting aspects of this kind of model is that it is possible to characterize, before harvest, the geographical areas according to their level of risk. Thus, it would be possible to restrict the quantitative analyses of DON to samples from parcels in geographical areas showing a high level of risk and for which previous cropping systems would be conducive to head blight development.

6.3 Genetic resources and agricultural biodiversity

High-quality Disease-Resistant Apples for Sustainable agriculture A. Antofie, M. Lateur

The objective of this project is to build a European database for apple (*Malus* x domestica) cultivars to enable breeders to use existing genetic resources collections in a better way and to help them to select more easily and more quickly parents for new cultivars which have required characteristics (e.g., the external appearance of the fruits, their digestive quality or their disease resistance). The AppleBreed database was developed within the framework of the HiDRAS research project (High-quality Disease Resistant of Apples for Sustainable Agriculture), implemented with financial support from the European Commission. The database collects the observations and results of genomics analyses of several families (parents and progenies) of apple cultivars.

A specific feature of the AppleBreed database is managing a multi-annual culture and enabling the trait from the parents to the progenies to be monitored; in other words, to ensure the traceability of the genotype and to link the trait of the cultivars to the QTL (Quantitative Trait Loci). The structure was developed in several phases: the conceptual data model level (giving a global view of the database), the logical data model level (describing the logical structure and the metadata) and finally the physical data model (describing all tables from the database structure with data types and theirs relationships). A website with a graphical interface is used to visualise output results from the database (http://hidras.cra. wallonie.be).

In the 2003-2004 period, the AppleBreed database structure was finalised. Two guidelines have been produced. They concern the structure and the data model used in the development of the database (defined data standards adopted by all partners) and the standardization of the encoding files (release of reference data-input templates).

Studying *Malus sylvestris* biodiversity A. Antofie, M. Lateur

The BelBiodiv*Malus* database was developed under a research project called 'Studying apple biodiversity' conducted with financial support from the Belgian Federal Science Policy Office (EV/42/28C). BelBiodiv*Malus* contains the phenotypic observations and results obtained from genomic analyses. These observations were made on several old apple varieties and on *Malus sylvestris*, found throughout Belgium, studied *in situ* and *ex situ*.

The BelBiodiv*Malus* database structure was created in the 2003–2004 period, together with encoding forms used within the framework of the project.

To define the structure of BelBiodiv*Malus*, a specific data model has been developed. This data model allows phenotypic and genomic data to be managed at the same time.





Unit BIOMETRY, DATA PROCESSING AND AGROMETEOROLOGY

This work enabled us to develop a graphical interface to show and explore data and to see the distribution of *Malus sylvestris* in Belgium, using geographical maps accessible *via* the Internet (http://applebiodiv.cra.wallonie.be).

6.4 Conception of new techniques and methods

Research of outlier detection methods for structured data with spatial constraints V. Planchon, R. Oger

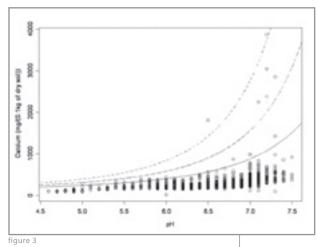
Over the past decade, various areas of research such as precision farming and geographical information systems (GIS) have grown very rapidly. In the wake of the development of these techniques, laboratories are facing an increased demand for all kinds of analyses (chemical composition of soils, nitrate or heavy metal content, wheat quality, etc.) and are required to manage large volumes of data where errors can occur with respect to units, orders of magnitude, etc. In view of such problems there is a need to detect outliers in related databases.

The chemical composition of soils is a very interesting part of the information held in spatially referenced databases with soil analysis results. Therefore, it is important during statistical analyses to take account of various problems generally associated with spatial constraints, including neighbourhood information and the presence of various soil associations in sampling areas.

The distribution of elements studied in soil analysis is very asymmetrical, with a very marked tail to the right. The presence of a large number of very high or extreme values at the right of the distribution makes it difficult to identify the parameters required to perform tests to detect aberrant values. Another problem is that of the possible mixing of several asymmetrical distributions within the same municipality, due to the presence of various soil associations.

Part of the work consists of studying relationships between physico-chemical characteristics (e.g., pHKCI) and various components, such as calcium. Suspect values can be detected graphically by superimposing the conditional quantile function, estimated from the Pareto distribution, on the scatter plot of calcium in relation to pHKCI. Observations located below the estimated quantile curve are considered to be suspect, as shown in Figure 3.

The overall aim of this study is to propose an operating procedure to detect aberrant values that can be applied to large sets of spatially referenced data. The method will ensure statistically and spatially consistent acceptance or rejection of the data, linked to neighbouring municipalities and to the presence of several soil associations in these communes. The perspectives of applications relate to management and follow-up of database quality and to spatialization within geographic information systems (GIS).



Ca versus pHKCl scatter plot with estimated quantiles superimposed with three levels of probability: (a) p=0.99 (solid line), (b) p=0.999(broken line), (c) p=0.9999 (broken-dotted line).

7. SERVICE ACTIVITIES

Biometry and data processing are considered as support activities essential to implementing research projects and strategies of general interest. Biometry is closely related to the improvement of the quality of agronomic experiments and the validation of data by contributing to their better use. Data processing takes into consideration the management of scientific information and ensuring wider access to it from centralized databases. Operational aims covered by these activities include:

1.Biometry

• Organization of 'quality' systems in various CRA-W laboratories (validation of methods, control charts, measure of uncertainties, statistical analysis of inter-laboratories assays, evaluation of lot homogeneity, writing procedures).

Planning and analyses of agronomic experiments.
Preparation of sampling plans for the control of agricultural productions.

• Elaboration of a monthly agro-meteorological bulletin giving yield forecasts for the five most important crops at national and district level • Internal training in CRA-W.

2. Data processing

• Management of the computer infrastructure, networks, servers and software packages indispensable to scientific and administrative data processing at CRA-W.

Management of intranet and internet servers.
Study of problems concerning the configuration of networks, systems and services, as well as the implementation of tools to ensure computer safety.

• Management of the meteorological database of the Ernage meteorological weather station (Gembloux) and publication of a bioclimatological bulletin.

 Implementation of a system for the collection and management of information within the framework of the Walloon Potato Chain (FIWAP). • Building knowledge bases on the development of Geographic Information Systems (GIS) at CRA-W.

• Development of software for the creation and management of control charts.



Publications

Scientific publications (with a reading committee)

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1-2 February 2003

7th International Stock Farming and Agriculture Fair, Tournai

7 February 2003

National debate on "Disease control in organic arboriculture", Biological Control and Genetic Resources Department, Gembloux

19 February 2003

Fertilisers and health protection for cereals (White Paper), Gembloux

4 March 2003

Commissioning at the Agricultural Engineering Department: first start-up of the experimental solid biomass combustion plant, Gembloux

12 March 2003

Geographical traceability, a new opportunity for developing agricultural products (*), Gembloux

<u> 31 March - 6 April 2003</u>

Spring Science Fair in cooperation with Gembloux Agricultural University, Gembloux

8 May 2003

Presentation of forage trials, Agricultural Systems Unit, Libramont

7-8 June 2003

Farm open days: presentation of outdoor pig rearing at a pig farm, Animal Production and Nutrition Department

15 June 2003

Strawberry Day, Biotechnology Department, Gembloux

24 June 2003

Tour of trials in progress, Crop Production Department, Gembloux

11-12 July 2003

Farm open days: presentation of the dairy sheep project at a sheep farm, Animal Production and Nutrition Department

<u>25-28 July 2003</u> Libramont Agricultural Fair, Libramont, Libramont

6-7 September 2003

Lesdain Rose and Nursery Festival, Biological Control and Genetic Resources Department

9 September 2003

White Paper, Pre-drilling information, Gembloux

13-14 September 2003

Kitchen Garden Festival / Moestuin dagen at Château d'Hex, Biological Control and Genetic Resources Department

17 September 2003

Tour of 'Cocksfoot' trials set up by the Agricultural Systems Unit in the DER showcase, Marloie

05 octobre 2003

Company Open Day[®], Agricultural Engineering Department, Gembloux and Agricultural Systems Unit, Libramont

5 October 2003

Château de Modave Apple Festival, Biological Control and Genetic Resources Department

8 October 2003

3rd Pork and Poultry Products Day, 'Towards a quality policy – at what price?' (*), Gembloux

18 October 2003

Old horticultural varieties market, Namur, in partnership with the Office of the Deputy Burgomaster for the Environment, Biological Control and Genetic Resources Department

22 October 2003

Developing an ethical approach at our agricultural institutions, Gembloux

1-2 November 2003

Pomexpo 2003 (9th exhibition) with the Regional Genetic Resources Centre, Villeneuve d'Ascq (France), Biological Control and Genetic Resources Department

6 November 2003

FUL seminar on 'Outdoor pig rearing'. Presentation of our pig production research work, Animal Production and Nutrition Department

8 November 2003

Torgny Pear Festival, Biological Control and Genetic Resources Department

26 November 2003

Milk, quality and guidance, cooperation without borders, symposium to mark the retirement of André Van Reusel, Head of Section, Quality of Agricultural Products Department

26 November 2003

Outdoor pig rearing, an opportunity, Animal Production and Nutrition Department, Gembloux

26 November 2003

National debate on "Application for extension of the number of active ingredients authorised for organic fruit growing in Belgium" (lime sulphur and neem), Biological Control and Genetic Resources Department, Gembloux

28-30 November 2003

Guest of honour at the Plant, Tree and Fruit Conference, Saint-Jean du Gard (France), Biological Control and Genetic Resources Department

10 December 2003

10th New alternatives in Forage Day: Pasture management in response to economic, environmental and social expectations on the threshold of a new century (*), Agricultural Systems Unit, Libramont

21 January 2004

9th Animal Production Symposium: The Impact of Eastward Enlargement on Animal Production in Western Europe (*), Gembloux

<u>9-15 February 2004</u>

AGRIBEX exhibition, Brussels

18 February 2004

Fertilisers and health protection for cereals (White Paper), Gembloux



3 March 2004

Which pasture for which development? Study conference for organic farmers, Agricultural Systems Unit, Libramont

12 March 2004

Training day on "Apple tree growing: new fruiting control techniques developed by the MAFCOT group," Biological Control and Genetic Resources Department, Gembloux

29 April 2004

Gembloux Spring Meeting, Europea Belgium, Gembloux

<u>6 June 2004</u>

Sheep conference organised by CISO and FICOW, presentation of dairy sheep breeding, Animal Production and Nutrition Department, Faulx-les-Tombes

16-18 June 2004

International Symposium on Food and Feed Safety in the context of Prion Diseases, European Stratfeed project, Quality of Agricultural Products Department, Namur

16-17 June 2004

"Tree growers' and nurserymen's conference" as part of the Nord-Pas de Calais celebration of 20 years of the conservation orchard (Regional Genetic Resources Centre, Villeneuve d'Ascq, France), Biological Control and Genetic Resources Department

22 June 2004

The problems of sustainable development: what are the implications for research?, Agricultural Systems Unit, Libramont

2 July 2004

Tour of the 'Cocksfoot' trials set up by the Agricultural Systems Unit in the DE showcase, Marloie

20 July 2004

Tour of the crop husbandry trials conducted by the Agricultural Systems Unit, Libramont

23-26 July 2004

Libramont Agricultural Fair, Libramont, Libramont

23 August 2004

Cross-border conference for organic tree growers, Biological Control and Genetic Resources Department, Gembloux

28 August 2003

Afternoon conference with a group of French tree growers from GABNOR, Biological Control and Genetic Resources Department, Gembloux

4-5 September 2004

Lesdain Rose and Nursery Festival, Biological Control and Genetic Resources Department

9 September 2004

White Paper, Pre-drilling information, Gembloux

11-12 September 2004

Kitchen Garden Festival / Moestuin dagen at Château d'Hex, Biological Control and Genetic Resources Department

14 September 2004

Half-day seminar on integrated pest management and frames of quality reference in potato growing, in cooperation with the Integrated Pest Management Study Group at the Agricultural University Pesticide Research Laboratory, Biological Control and Genetic Resources Department, Gembloux

26 September 2004

Open Day, Biological Control and Genetic Resources Department, Gembloux

1 October 2004

Tour of the Animal Production and Nutrition Department, Gembloux

1-2 October 2004

Biomass conference, Agricultural Engineering Department, Gembloux

2-3 October 2004

La Roseraie nursery open days, Biotechnology Department, Gembloux

3 October 2004

Château de Modave Apple Festival with the C.R.I.E., Modave, Biological Control and Genetic Resources Department

14 October 2004

Results of the 2004 cereal campaign and prospects opened up by new rheological methods, Quality of Agricultural Products Department, Gent

16-17 October 2004

Flore et Pomone, Biological Control and Genetic Resources Department, Orp-Jauche

20 October 2004

4th Pork and Poultry Products Day, "Towards harmonious relations between producers and neighbours? (*), Gembloux

23 October 2004

Old horticultural varieties market, Namur, in partnership with the Office of the Deputy Burgomaster for the Environment, Biological Control and Genetic Resources Department

18-19 November 2004

2nd International Conference on "Embedded Near Infrared Spectroscopy", Quality of Agricultural Products Department, Gembloux

05 octobre 2003

Journée Découverte Entreprises au Département Génie rural, Gembloux et à la Section Systèmes agricoles, Libramont

27 November 2004

Torgny Pear Festival, Biological Control and Genetic Resources Department

(*) available on request from the CRA W







Biotechnology



Departement Crop production



Departement Biological control and plant genetic resources

Departement Pesticides research



Departement Agricultural engineering



Departement Animal production and nutrition

Departement Quality of agricultural products



Unit Farming systems



Biometry, data processing and agrometeorology