



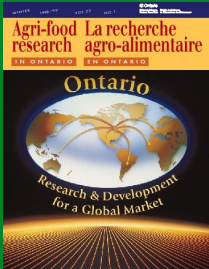
HOME

WHAT'S NEW

CALENDAR

PRODUCTS

NEWS RELEASES



**Winter
1998-99
Vol. 22 No. 1**

Agri-food research La recherche agro-alimentaire

IN ONTARIO EN ONTARIO

Formerly Highlights of agricultural and food research in Ontario

| [Cover & Credits](#) || [Forefront](#) || [Crop Technologies](#) || [Livestock Technologies](#) || [Food Technologies](#) |
| [Les Recherches En Bref](#) |

COVER AND CREDITS

- [Front Cover](#)
- [Back Cover](#)
- [Table of Contents and Credits](#)

| [Top of Page](#) |

FOREFRONT

- [Ontario: Research and Development for a Global Market](#)

| [Top of Page](#) |

CROP TECHNOLOGIES

- [Measuring Risk Education in Pesticide Use](#)
- [Standardizing the Beneficial Components in Herbal Medicines](#)
- [Preserving Cut Flowers](#)
- [- Keeping the Ethylene Out With 1-MCP](#)
- [Towards a Real Time, Intelligent Precision Sprayer](#)
- [Ontario Soybeans: An Ongoing Success Story](#)
- [Fighting Fusarium in Ontario Corn](#)
- [- Biotechnology Builds Disease Resistance](#)
- [Healthy Hydroponics: Getting a Headstart on Disease Control in Greenhouse Crops](#)

| [Top of Page](#) |

LIVESTOCK TECHNOLOGIES

-  [A Novel & Natural Source of Lysozyme](#)
-  [Mosaic Arthroplasty:
Equine Joint Repair is Away to the Races](#)
-  [Super Plants Mean Super Pigs](#)

| [Top of Page](#) |

FOOD TECHNOLOGIES

-  [Enriched Milk With That Oh-So-Important Omega 3](#)
-  [Modeling to Minimize Pre-Harvest Contamination in Beef](#)

| [Top of Page](#) |

LES RECHERCHES EN BREF

-  [Les Recherches En Bref](#)

| [Research, Education, Laboratories & Risk Management Home Page](#) |

| [Central Site](#) | [Feedback](#) | [Search](#) | [Site Map](#) | [Français](#) |
| [Home](#) | [What's New](#) | [Calendar](#) | [Products](#) | [News Releases](#) |



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HERE'S WHAT'S NEW INSIDE:

- **Healthy hydroponics: Getting a headstart on disease control in greenhouses.** *(above)*
- **A novel and natural source of lysozyme: the egg yields an important consumer and industrial protein.** *(below)*

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PHOTO: KERRY LITTLE, OMAFRA

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Agri-food research La recherche agro-alimentaire

IN ONTARIO

EN ONTARIO

WINTER 1998-'99
VOL. 22 NO.1

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CROP TECHNOLOGIES



MEASURING RISK REDUCTION IN PESTICIDE USE	2
STANDARDIZING THE BENEFICIAL COMPONENTS IN HERBAL MEDICINES	4
PRESERVING CUT FLOWERS – KEEPING THE ETHYLENE OUT WITH 1-MCP	6
TOWARDS A REAL TIME, INTELLIGENT PRECISION SPRAYER	8
ONTARIO SOYBEANS: AN ONGOING SUCCESS STORY	10
FIGHTING FUSARIUM IN ONTARIO CORN – BIOTECHNOLOGY BUILDS DISEASE RESISTANCE	12
HEALTHY HYDROPONICS: GETTING A HEADSTART ON DISEASE CONTROL IN GREENHOUSE CROPS	14

LIVESTOCK TECHNOLOGIES

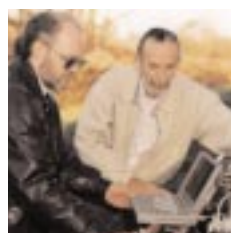
A NOVEL & NATURAL SOURCE OF LYSOZYME	16
MOSAIC ARTHROPLASTY: EQUINE JOINT REPAIR IS AWAY TO THE RACES	18
SUPER PLANTS MEAN SUPER PIGS	20



FOOD TECHNOLOGIES

ENRICHED MILK WITH THAT OH-SO-IMPORTANT OMEGA-3	22
MODELING TO MINIMIZE PRE-HARVEST CONTAMINATION IN BEEF	24

LES RECHERCHES EN BREF	26
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ONTARIO: Research and Development for a Global Market

Development of new technologies is revolutionizing the agri-food industry globally. From nutraceuticals and health diagnostics to new industrial uses of agricultural raw materials, the potential for economic growth is huge. R&D will continue to be the cornerstone of competitive and successful marketing of these products abroad.

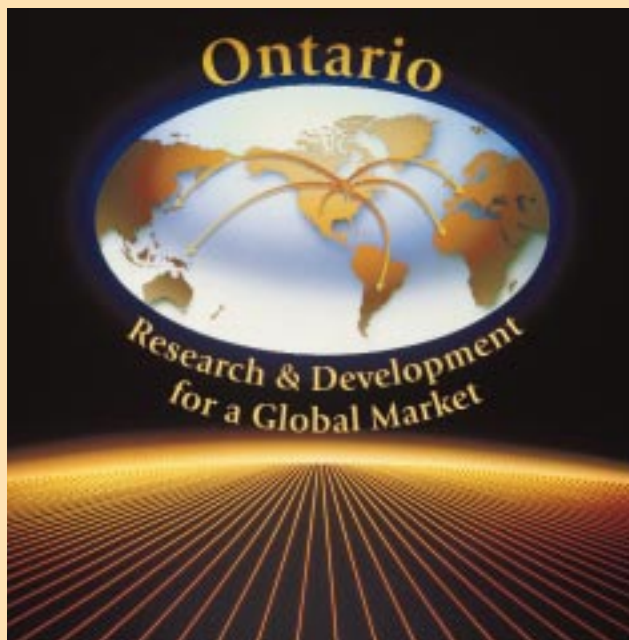
Ontario fosters a climate of collaboration between research and industry. More than 500 scientists at ten public and private world-class research institutes conduct a wide range of market-driven, basic and applied agri-food research. Provincial funding programs match industry and public dollars, ensuring industry research needs are met, and quick uptake of new technology.

In 1997, Ontario led all other provincial

governments in agri-food research investment, with \$41 million – as much as the governments of Quebec and Alberta combined. Ontario also led all other provinces in Canadian agri-food exports by shipping more than \$5.6 billion (1997) in products.

Exports have increased 167 per cent over the past decade.

With the greatest diversity of crops and livestock anywhere in the country, it's not surprising that Ontario's R&D capability is so broad. This issue profiles twelve top new technologies to watch – research in the works that will serve the needs of the



Ontario agri-food industry – as it strives to serve the global consumer. From flowers that last longer to favourable fatty acids in soybeans; mycotoxin-free corn to mosaic arthroplasty in horses and humans, it's truly R & D for a global market.

MEASURING RISK REDUCTION IN PESTICIDE USE

Reducing pesticide use by 50%, while maintaining agricultural productivity, is a major goal of the Food Systems 2002 program in Ontario. Most would agree that the objective of any pesticide reduction program should include at least an equivalent reduction in risk.

GORD SURGEONER

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Reducing pesticide use by 50%, while maintaining agricultural productivity, is a major goal of the Food Systems 2002 program in Ontario. A reduction in pesticide use should represent an equivalent reduction in risk – taking into account such factors as changes in area of land farmed, hectares of crops grown, changes in cropping patterns or crop yield – and the fact that different pesticides have different levels of associated risk. An assessment of how far we've come in risk reduction makes the goal of Food Systems 2002 meaningful for farm workers, consumers and the environment.

The Ontario government initiated the Food System 2002 program in 1987. The most recent year of data available at that time from Ontario's census of pesticide use was 1983, which has become the baseline for further comparisons. The government's five-year census of pesticide use has monitored kilograms of active ingredient, since 1973.

In our study, risk was defined as the inherent toxicity of a pesticide, and exposure to that chemical. But exposure by whom? The list could be very long, but we have considered three general risk categories – farmworker, consumer and ecological environment. We measured pesticide risk for each active ingredient using a scoring system, called Environmental Impact Quotient (EIQ), developed by the Integrated Pest Management (IPM) program at Cornell University.

From 1983 to 1993 pesticide use in Ontario declined 28.5%, as measured by kilograms of

active ingredient. Over the same period, there was a 10% reduction in total area of land farmed and a 16% increase in tonnes produced per hectare.

Comparing Field and Horticultural Crops

The reduction in pesticide use was primarily on corn and soybeans, which account for a large proportion of hectares farmed, but have a relatively low pesticide risk per hectare or tonne of crop produced. The risk per hectare was higher on tobacco, fruits and vegetables, even though the total amount of pesticide used was much lower.

New technologies have encouraged reduced pesticide use. These include new formulations requiring much lower doses – grams rather than kilograms – of active ingredient per hectare. Methods of pesticide application have improved, e.g. electrostatic sprayers, improved sprayer calibration, banding of pesticides, and IPM programs have been more widely adopted. Changes in cropping patterns, such as rotational cropping, have reduced the need for pesticides. Voluntary changes in pesticide use have resulted in significant declines of some pesticides, such as atrazine.

Pesticide Risk Declined for Farmworker, Consumers and Environment

Overall risk, as measured by the EIQ, declined 29% from 1983 to 1993. This is similar to the reduction in the amount of pesticides used. The largest declines occurred in risk to



farmworkers (41%), but there has also been a decline in risk to consumers (28%) and the environment (21%).

Risks to farm workers have declined substantially because of mandatory pesticide education programs, increased custom application, new formulations for safer mixing and more enclosed tractor cabs, which reduces exposure to drift.

At the consumer level, there is no evidence of residue contamination in major field crops that constitute the most hectares grown. Increased numbers of pick-your-own operations may have, however, increased the potential for exposure to pesticides.

At the ecological level, Environmental Farm Plan and Integrated Pest Management programs have increased awareness of risks associated with pesticide use and encouraged improved farming practices.

All of this leads to an important question. Should we target our resources to further improvements on large-hectare field crops, which have a relatively small risk per hectare? Or should we concentrate our resources on fruit and vegetable crops that have a higher risk per hectare, even though they account for less than 11% of the amount of pesticides used?

We have almost achieved a 50% reduction in risk to farmworkers. Perhaps now our efforts should concentrate on developing pesticides or production methods with greater risk reductions at the consumer and ecological levels.

However you look at it, just how we evaluate a 50% reduction in pesticide use has big implications for where best to invest our research and management dollars. ■

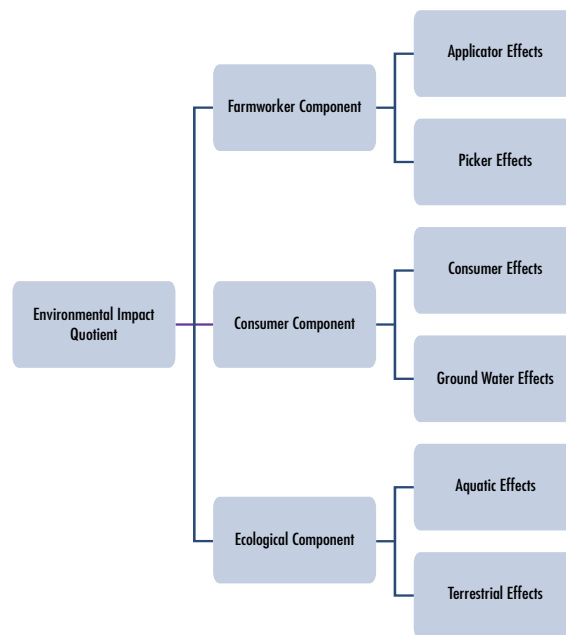


Fig. 1. Three major categories of the Environmental Input Quotient (EIQ).



$$EIQ = \left(\frac{C[DT*5] + (DT*P)}{3} + \frac{C*((S+P)/2)*SY + (L)}{3} + \frac{(F*R) + (D*((S+P)/2*3) + (Z*P**3) + (B*P*5))}{3} \right) / 3$$

DT = dermal toxicity D = bird toxicity
 C = chronic toxicity S = soil half life
 S = systemicity Z = bee toxicity
 F = fish toxicity B = beneficial arthropods toxicity
 L = leaching potential P = plant surface half life
 R = surface loss potential

Fig. 2. Formula for calculating the EIQ for individual pesticides. The blue box at top is the Farmworker component, the yellow box is the Consumer component, and the green box is the Ecological component.

Standardizing the Beneficial Components in Herbal



In 1997, Canadian consumers spent approximately \$340 million on herbal plant preparations. For many, these products have provided health benefits, with fewer side-effects than traditional pharmaceuticals. With consumers' increased interest in their health, and public acceptance of plants as alternatives or supplements to conventional medicine, North American markets for herbal products are increasing. But the question remains for consumers: how to be sure of the quality of the product they purchase.



Currently, most herbal preparations are non-standardized plant products. For future expansion of the industry in Ontario, the development of consistent, standardized products is essential. But there are several challenges in achieving this. For example, there can be year-to-year variability in crop quality, which affects levels of active ingredients in the product. Similarly, concentrations of medicinally active components can vary between locations and among individual plants. Insects, bacteria and fungi can contaminate plant material and product, and preparations can contain misidentified plant species. And, with non-standardized products, there is the potential for consumer fraud.

The popularity of herbal preparations, in the face of these challenges, clearly indicate the need for new methods of plant production.

To address this need, we are developing new technologies for micropropagating medicinal plants in our laboratory at the University of Guelph. Plant cells or tissues, such as hypocotyl

or stem tissue, are grown on nutrient-enriched culture medium with optimized levels of plant growth regulators. These cells or tissues possess a unique capacity to develop into embryos and ultimately whole plants, identical to the parent plant. We grow these regenerated plants in consistent, ideal nutritional and environmental conditions, free from bacterial, fungal or insect contamination. Individual plants are then screened for uniquely high levels of active medicinal compounds. Once we have identified chemically superior plants, we can again clonally mass-produce these plants using tissue culture techniques.

We have successfully micropropagated several medicinal plant species, including St.

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Medicines

John's Wort (*Hypericum perforatum*), Feverfew (*Tanacetum parthenium*), Echinacea (*Echinacea* sp.), Huang-qin (*Scutellaria baicalensis*) and garlic (*Allium sativum*).

The micropropagation technologies we've developed allow for the mass-multiplication of medicinal plants in a sterile, controlled environment.

It's also an efficient means of producing plants with consistent, standardized characteristics.

Our research represents the first step in the production of high quality herbal products for Ontario and international markets. ■



Fig. 1. Clonally mass-produced medicinal Feverfew (*Tanacetum parthenium*) plants in the greenhouse.



Fig. 2. Using micropropagation techniques, a single shoot forms at the apical meristem of Feverfew cuttings in tissue culture.



Fig. 3. Formation of multiple shoots on a 1 cm. stem cutting from St. John's wort (*Hypericum perforatum*), in response to optimized growth regulators in the culture medium.



Fig. 4. Two month old St. John's wort plantlets grown from tissue under sterile conditions.



Preserving Cut

KEEPING THE

Ethylene (C_2H_4) is a gas that occurs naturally in plants. It functions as a growth regulator, promoting the ripening of fruit, and is also produced when the plant is diseased, cut or damaged. Plants can be exposed to ethylene from external sources such as fruit ethylene, engine exhaust, improperly vented greenhouse heaters or CO_2 generators.

But ethylene – even at low levels – can spell bad news and lost profits for the floraculture industry, and reduced quality of flowers for retailers and consumers.

The effects include accelerated aging, leaf yellowing, and flower or leaf abscission. Cut flowers are particularly sensitive. But given the pervasiveness of the gas, it's impossible to avoid exposure. The floraculture industry has recognized the need for environmentally sound, inexpensive, easily applied methods to counteract the effects of both endogenous and exogenous ethylene.

Putting the Bite on Ethylene Binding

Traditionally, the industry has used silver thiosulphate (STS) to inhibit ethylene effects. But environmental concerns over potential heavy metal contamination have prompted research into alternate controls. A new gaseous inhibitor of ethylene binding, 1-Methylcyclopropene (1-MCP), is a volatile,

cyclic olefin that is proving to be a useful, relatively stable alternative. It acts by preventing ethylene from binding to specific sites in the plant, which would then initiate the plant's damaging response.

1-MCP is highly effective at low concentrations against both plant-produced and external ethylene. It is non-toxic to humans and plants and there is no disposal concern. 1-MCP is applied as a volatile, therefore treatment is done in a relatively sealed area. It requires a minimum exposure time that increases as temperature decreases and protects only existing growth.

With sponsorship from OMAFRA, the Canadian Greenhouse Conference and Agriculture and Agri-Food Canada's CanAdapt Program, we screened many Ontario-grown floriculture crops, including bedding, potting and cut flowers for ethylene sensitivity. Ethylene levels >10ppm are unlikely to occur in greenhouse, transport and retail situations, so we did

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flowers

ETHYLENE OUT WITH 1-MCP

not screen plants with sensitivities greater than that for 1-MCP response.

We have tested four species of cut flowers that showed ethylene sensitivity including snapdragons, alstroemeria, delphinium and gypsophila. At room temperature, 1-MCP performed well on all four species. We observed the most pronounced results with delphinium.

At room temperature in the absence of 1-MCP, no flowers remained on the ethylene-treated controls after three days. The control, without ethylene or 1-MCP had 50% of the number of flowers initially present. By contrast, the flowers remained attached and the buds continued to open with both 1-MCP treatments. The final flower counts were 170-200% of initial counts. Flower counts were slightly lower for the 1-MCP treatment with ethylene than without, possibly indicating that 1-MCP

did not provide total protection. Test results at 4°C were similar, but less pronounced.

Further research will concentrate on optimizing the 1-MCP treatment, with various combinations of 1-MCP concentration, temperature and exposure time. Registration for the use of 1-MCP on floriculture and food crops is being pursued in both Canada and the U.S..

We hope to expand our research to include greenhouse vegetables, and to determine more precisely where ethylene poses the greatest problem – in greenhouse, storage and transportation facilities, and retail outlets and homes.

Conservative estimates of losses to ethylene damage are \$17 million annually. With a 1995 floriculture industry value of \$332 million and market expansion into the United States, 1-MCP could offer significant savings. ■



Fig. 1. Cut flowers treated with 1-MCP (right) compared to control (left) after three days in room temperature at 5 ppm ethylene.

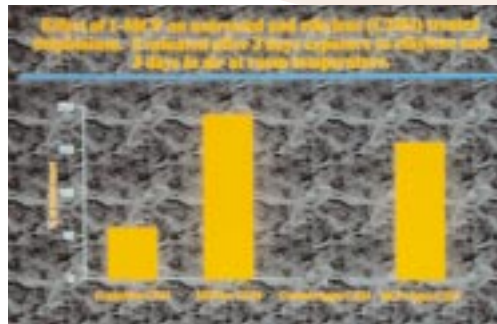


Fig. 2. Effect of 1-MCP on untreated and ethylene treated delphinium. Evaluated after three days exposure to ethylene and three days in air at room temperature.

TOWARDS A REAL TIME, INTELLIGENT



Rear view of sprayer showing metering pump apparatus with supervisory control computer and injection nozzles on spray boom.

Precision farming uses information technology to prescribe field inputs according to localized field conditions and crop needs. Ontario farmers rely heavily on chemical herbicides, using over 5 million kg in corn and soybean production each year. More efficient spray application techniques could reduce this amount, with no loss of productivity, and offer both economic and environmental benefits.

Traditional blanket spray application of herbicide at one recommended rate is only efficient when weed distribution is uniform over a large field. In many fields, particularly in no-till situations, weed populations are not uniform, but tend to cluster in known locations from year to year. This variability makes blanket application inefficient, since it wastes herbicide in areas of low weed concentration and may be insufficient in areas of high concentration.

A herbicide spray management system that accounts for variable weed distribution has three main components – weed mapping, prescription modeling and prescription delivery. Weed mapping involves production of a grid model of the field layout in a computer database. Weed populations are then overlaid on the grid, block by block, for decision analysis.

Prescription modeling analyses each block to determine if the level of weed concentration justifies herbicide treatment. This information and other decisions, such as delivery rate and type of chemical, are then transferred to the supervisory computer on the sprayer.

Prescription delivery depends on the ability of the sprayer's control system to establish accurate field positioning relative to the grid and on the fluid delivery system to target the correct grid block.

A Responsive System and Precise Delivery

Currently, we are developing an effective precision sprayer as part of this system. The sprayer incorporates a direct injection system (DIS) that can deliver a variable rate of active ingredient directly at the nozzle. The sprayer tank contains only a water carrier and requires no pre-mixing of chemical. During application, water is sprayed constantly. The herbicide concentrate is introduced from a separate tank into the water carrier only when needed, as indicated by the prescription map. The metering of only a small amount of fluid makes the system very responsive, allowing for more precise delivery of the herbicide. We can also tank mix a chemical to target an evenly distributed weed, such as grass, while injecting a second chemical for another one less evenly distributed.

The metering pump consists of an array of syringe pumps, bound together, sharing a common stepping motor drive. These pumps are simply reciprocating pistons within long cylinders, using check valves to permit flow in either direction. Each nozzle is injected from its own independent, positive displacement pump. With this, we can precisely control the injection rate, independent of the viscosity of the herbicide.

BY KEN BENNETT AND RALPH BROWN

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PRECISION SPRAYER

We field-tested a prototype application system during the 1998 growing season in collaboration with crop scientist, Dr. François Tardif and MSc student, Heather Goudy at the University of Guelph. They produced weed maps and prescriptions for corn and bean test fields in southwestern Ontario, using intensive, in-field weed counts and GPS (Global Positioning System – Halltech Environmental Inc. of Guelph, Ontario). We positioned the sprayer using an origin location stake and real-time velocity/position sensing wheel.

During our experiments, we varied metering rate only as a correction factor to speed changes in the tractor. Under certain circumstances, however, lower than recommended spray application rates can still achieve acceptable weed control. Herbicide effectiveness depends on a number of factors – species susceptibility, growth stage, environmental stress factors, and weather conditions at time of application. Including parameters such as these in the prescription model could further reduce metering rates.

Field trials will continue through the 1999 growing season. Further modifications to the sprayer will reduce its retrofit cost and increase its reliability and versatility. For example, most herbicides are effective against either weeds or grasses. The development of a dual injection precision sprayer that could deliver two types of herbicide would have application in most typical field situations.

GPS will be an integral part of the precision management system as it becomes more reliable and less expensive. A future version of the sprayer will include an on-board GPS receiver to locate position. The applicator will be able to start anywhere in the field and will always know what grid blocks still require treatment.

We have more work to do in the areas of remote sensing and prescription modeling.

While work in 1998 involved intensive field scouting, earlier work with aerial photography showed feasibility for field mapping. Analysis of the spectrum of digitized colours in an aerial photograph can produce weed distribution maps.

Prescription modeling is also in its infancy. Until recently, decision management and control systems have relied on precise mathematical models or logical relationships. The relationship of crop yields to field inputs are situations of such complexity, however, as to defy traditional modeling. The introduction of fuzzy logic and fuzzy modeling techniques can bring novel approaches to the determination of yield-inhibiting weed thresholds, chemical types and application rates.

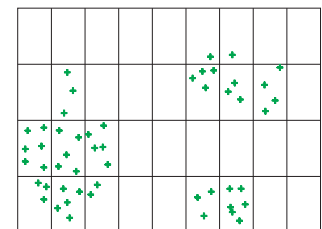
While a real-time, intelligent, precision herbicide sprayer is not yet a reality, our research will continue to focus on a management system that reduces chemical inputs while maintaining farm productivity and food quality in Ontario. ■

Acknowledgements

We would like to acknowledge the support of OMAFRA Food Systems 2002, the Ontario Corn Producer's Association and the Ontario Soybean Producer's Association.



Researchers Ken Bennett and Ralph Brown are using weed mapping and herbicide prescription modeling and delivery to reduce total herbicide needs.



Precision Grid Overlay on Weed Patches

OFF	OFF	OFF	OFF	OFF	+	+	OFF	OFF
OFF	+	+	OFF	OFF	+	+	+	OFF
+	+	+	OFF	OFF	OFF	OFF	OFF	OFF
+	+	+	OFF	OFF	+	+	OFF	OFF

Herbicide Prescription Map

Fig. 1. Weed mapping. The top square shows the positions of weed patches in a field. Overlaid is the coordinate grid map used to block out sections of the field to be sprayed. The bottom square shows the decisions that will be used by the computer to spray or not spray each respective block of the field.

BY GARY ABLETT

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ONTARIO SOYBEANS AN ONGOING SUCCESS STORY



Cultivar and Germplasm Improvements are the Key

PHOTO: ONTARIO SOYBEAN GROWERS MARKETING BOARD

The soybean industry in Ontario is a well known success story, from its humble beginnings fifty years ago, to a \$690 million crop grown on 2.1 million acres in 1998. Soybeans and its oil are used in a huge variety of foods, and an ever-increasing range of industrial products like lubricants, inks, cleansers, solvents and fuels.

As a result of public sector breeding programs, soybeans can now be grown across a wide range of crop heat unit areas and soil types. Varieties developed by the University of Guelph (Ridgetown and Guelph) and Agriculture and Agri-Food Canada (AAFC -Ottawa and Harrow) occupy about 45% of the soybean acreage grown in Ontario.

At Ridgetown College, the major focus of soybean breeding is cultivar development and germplasm enhancement. The goal of germplasm enhancement is to capture the improvement in established cultivars of commercial significance.

Yields, Lodging and Disease Resistance

Improved cultivars with superior yield potential, and good lodging and disease resistance have been developed by crossing productive genotypes from a range of diverse sources. The breeding system for cultivar development at Ridgetown primarily uses the 'modified single seed descent' method. Breeding lines are evaluated for yield just 2½ years after the initial cross, and breeder seed can be released five to six years after that.

The program uses locations representing a wide range of soil types and heat unit areas (2800-3400) to evaluate this material. Parent material includes the best available lines from the Ontario Soybean Variety Trials (both public and private), from the USDA Uniform tests and from U.S. company programs. Recent cultivar releases from this program include RCAT Bobcat, Westag 97 and RCAT Legacy.

The Ontario Oil and Protein Seed Crop Committee directs the methods for registration

and performance testing of soybean cultivars. Ontario Soybean Variety Trials are grown annually at locations determined in cooperation with the Committee. The Ridgetown program conducts seven of the sixteen trials across the province with AAFC, Kemptville College and the University of Guelph picking up the rest.

Altered Oil Profiles for Healthier Foods

The major objectives of our germplasm enhancement program are improved oil quality and morphological traits. In cooperation with the University of Guelph's Department of Plant Agriculture, the oil quality program continues to identify fatty acid profiles which differ significantly from that of typical soybeans. A desirable profile is one which has low linolenic, low palmitic and high oleic fatty acid traits.

Linolenic acid is a polyunsaturated fat. Lower concentrations enhance the flavour stability of the oil. Palmitic acid is a saturated fat, lower concentrations of which are desirable from the health point of view. Oleic acid is a monounsaturated fat, of which higher concentrations are acceptable, again from the health viewpoint.

At present, soybean oil is altered physically and chemically to produce the required fatty acid profile for various food products. The goal of our breeding program is to achieve some of these changes genetically. Cultivars with modified fatty acid profiles are not yet available for commercial use in Canada. However, traits which were developed in the early nineties are now being incorporated into high-yielding cultivars. The low linolenic breeding line, RCAT 9507, is routinely crossed with a number of high yielding, adapted, normal oil composition cultivars to improve the yield potential.

Structural Improvements for Photosynthetic Efficiency

The goal of our germplasm enhancement research in morphological traits is to build a better soybean for Ontario's growing conditions.

We are currently evaluating 'semi-determinate', 'tall determinate' and 'dense



PHOTO: ONTARIO SOYBEAN GROWERS MARKETING BOARD

pubescence' traits. Most soybeans grown in Ontario are indeterminate types, meaning that they grow vegetatively for six to eight weeks, followed by a four to five week period of both vegetative and reproductive growth. After that, growth is strictly reproductive until they mature. In semi- and tall determinate types, there is less overlap of the vegetative and reproductive growth phases. This may allow for a greater proportion of the photosynthate produced to be directed towards the reproductive structures.

The 'pubescence' traits in soybeans refer to the small hairs on the stem, leaves and pods of the soybean plant which are gray or light brown. The gray hairs are quite shiny, and we suspect that increasing the density of the pubescence might increase the light reflecting among the hairs on the plant, its photosynthetic efficiency, and hence its growth.

The research on morphological traits is still in experimental stages. Our primary strategy is to incorporate these traits into cultivars that have a strong genetic background for yield, maturity and resistance to disease, rot and lodging, and then evaluate how well they will work in Ontario.

This method allows these germplasm enhancements to be effectively transferred to commercial cultivars and then to the soybean industry in Ontario. By combining research in both cultivar and germplasm development, the most important improvements can quickly be made available for the benefit of soybean growers, processors and consumers. ■

FIGHTING FUSARIUM

in ONTARIO

Biotechnology Builds

Ear mold disease caused by the fungus *Fusarium graminearum*, is the single most serious threat to the Ontario corn crop. The fungus produces mycotoxins on the grain that are a major concern to livestock producers and the milling industry. Mycotoxin-free corn would be a great asset to Ontario farmers.

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Existing corn transformation technologies are inefficient. They are only effective with a limited number of genotypes and can produce transgenic plants with unstable gene expression. For greater applicability in Ontario, we are concentrating our efforts on developing transformation procedures for Ontario corn germplasm. Once these procedures are developed, we can use them to insert a broad range of agronomically valuable genes, such as genes that increase *Fusarium* resistance, into Ontario corn hybrids.

We are focusing on an *Agrobacterium*/corn co-cultivation system. The potential advantages of this natural, bacterial, genetic engineering system over physical systems such as the particle gun, include higher transformation frequencies and less complex insertion. The result is more stable expression of the transgene. We will test *Agrobacterium* co-cultivation with corn inbreds currently used in Ontario hybrids and superior germplasm from the corn breeding program at Guelph.

Genotype Responsiveness and Compatibility Important for Transformation

Two factors have limited the development of an *Agrobacterium*-based system. For transformation to occur, corn genotypes must be both highly responsive to tissue culture and compatible with *Agrobacterium*. We have screened about 40 corn inbreds and hybrids from Ontario in a variety of tissue culture systems and have identified approximately 10 genotypes that are highly responsive.

Because of the complex interaction between the bacterial pathogen *Agrobacterium tumefaciens*, and the plant host, transformation is also very sensitive to the genotype of the plant. We have begun to screen the regenerable genotypes for their compatibility with *Agrobacterium*. We are doing this with strains of the bacteria that carry genes (like antibiotic or herbicide resistance, or reporter gene (GUS) activity) that have strong phenotypes and are relatively easy to select for in tissue culture, or to identify in regenerated shoots. This will

CORN

Disease Resistance



allow the development of protocol with end points that are easy to recognize.

The first tissue cultures and shoots with reporter gene (GUS) activity have been produced from these experiments. Our current efforts are focused on increasing the frequency of GUS gene expression in corn shoots and obtaining a complete transformed plant by this procedure.

Introducing Classes of Genes for Disease Resistance

After this has been achieved, we will be ready to introduce classes of genes, known to be effective in reducing disease incidence in plants, such as chitinases, glucanases or fungitoxic peptides, into corn by the *Agrobacterium* method. These have already shown the ability to reduce the susceptibility of other plants, like tomato, to *Fusarium*. Our collaborators at Agriculture and Agri-Food Canada (Ottawa) have isolated genes that inhibit the action of the mycotoxin DON in test plants, like tobacco. We plan to test these genes in Ontario corn inbreds as well.

Testing for resistance requires a reproducible disease rating system. Disease resistance scoring in field trials is complex and

time-consuming. Scores can differ from year to year because of uncontrollable environmental factors. Instead, we will identify molecular markers linked to the natural *Fusarium* resistance in corn, and then use the presence or absence of the markers to decide whether to use a plant for further rounds of breeding. There are several advantages to marker-assisted selection. The analysis is done at the seedling stage, small samples of tissue from the plant are sufficient, and the analysis can usually be done in two days.

The final product in the current research will be disease-resistant corn for Ontario farmers, but the development of efficient transformation technologies and the information gained about gene function will have long-term value for a variety of corn improvements in the future. ■

Acknowledgements

This research has been made possible through the cooperative efforts of many diverse groups, such as the Ontario Corn Producers, the Pork Producers of Ontario, OMAFRA, OAFT, OREP, Can Adapt, many seed companies and others.

HEALTHY HYDROPONICS: GETTING A HEADSTART ON DISEASE CONTROL IN GREENHOUSE CROPS



PHOTO: DON HAMILTON

Fig. 1. The University of Guelph research group on health management in hydroponic crops includes (l-r) grad student Melanie Johnstone, Prof. John Sutton, research technician Rodger Tschanz and Prof. Bernie Grodzinski.

Business is booming in the Ontario greenhouse industry, but environmentally-friendly disease control tops the list of research needs. In the recirculating hydroponic systems, optimal growing conditions and high plant density set the scene for explosive disease outbreaks. Early diagnoses promise better treatment results. Our research group at the University of Guelph (Fig. 1) is trying to put their collective finger on metabolic signals of pathogen attack, so that treatment can begin days before more damage is done.

Several types of growth systems are used in greenhouse agriculture. The 'nutrient film' methods circulates nutrient solution over the roots of plants positioned in troughs. The troughs are arranged in a parallel pattern, interconnected with tubing, and covered with plastic film to exclude light from the root zone.

In the 'ebb and flow' system, plants grow in supportive material in trays into which nutrient solution is pumped at regular intervals and then allowed to drain. In both systems, computer-controlled methods regulate nutrient composition, pH, ion concentration and flow rate of the nutrient to provide optimal plant growth conditions and high yields.

The diseases that plague these systems are usually caused by pathogenic fungi which cause root rots and wilts.

Root rots are caused by species of *Pythium*

and *Phytophthora* that produce significant browning of the roots, leading to plant wilting and collapse. Motile spores of the root-rot fungi infect young roots in minutes and produce a new generation of millions of spores in only a day or two. Repeated cycles of spore production and rapid dispersal of spores among roots by recirculating nutrients combine to increase incidence of the disease.

The second group of fungal disease agents, species of *Fusarium*, invade but do not seriously damage plant roots. Instead, the fungi grow into the xylem, the water-conducting vessels of the plant. From there they move quickly to the stems and leaves and produce irreversible wilting. Wilt epidemics develop more slowly than do root rots, but infection means death.

The first step in controlling root rots and

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wilts is to prevent, or at least minimize, contamination of the hydroponic system. Important measures include using water and seeds that are free from pathogenic fungi, sanitizing the production system between crops and maintaining rigorous cleanliness in the greenhouse.

Should the system become contaminated, it is vital to quickly control the spread of the disease. There are physical, chemical and biological means to achieve this. Physical methods for sterilizing the nutrient solution include filtration, ultraviolet (UV) radiation and heat.

Fungicides, ozone and hydrogen peroxide are in the chemical roster of defenses. But there has been concern about the health and environmental aspects of fungicide use.

Biological control is a relatively new area of research involving antagonistic microorganisms, many of which are not yet commercially available. Plant varieties that combine disease resistance with desirable horticultural characteristics are available in a few instances to control wilt, but not root rot.

Increased restrictions on fungicide use and release of nutrient solutions into the greenhouse environs are making it even more difficult for Ontario growers to control root rots and wilts. The industry needs additional methods of disease control for hydroponic systems to remain economically viable and competitive.

With this in mind, a research group was established at the University of Guelph in 1997 to study disease progress and control, and crop physiology and productivity in hydroponic systems scaled for research purposes. Photosynthesis and respiration rates are used as measures of plant health and vigour. Early monitoring of the gas exchange rates of infected plants acts as diagnostic signal of

pathogen attack. By learning more about when and how these primary metabolic processes occur we can predict the onset of disease several days before the appearance of any visible symptoms.

In one model system that we have developed for studying the epidemiology of the disease and the role of remediation technologies, we are infecting single plants growing hydroponically in aerated nutrient solutions. The metabolism of the plants is being monitored during early stages of pathogen attack. A more complex experimental systems has also been designed to simulate the growing conditions found in commercial greenhouses. For UV investigations, we are using a collimated beam apparatus to treat solutions in both ebb and flow and nutrient flow technique systems.

Results from this research will allow us to time, target and integrate treatments for better disease management in recirculating hydroponic systems. This, in turn, should allow Ontario's greenhouse industry to produce crops more profitably and competitively. ■

Acknowledgements

Our group is collaborating with investigators at the Protected and Processing Crops Research Centre of Agriculture and Agri-Food Canada (AAFC), Harrow and at OMAFRA and AAFC centres in Vineland. The Guelph group is supported by OMAFRA, Flowers Canada (Ont.) Inc., the Ontario Greenhouse Vegetable Producers Marketing Board, CRESTech, Trojan Technologies Inc., and CEA Technologies International.



Fig. 2. Lettuce grown in hydroponic troughs with recirculating nutrient solution.



Fig. 3. Hydroponic lettuce with root rot caused by *Pythium*, a pathogenic fungi with spores that rapidly spread among roots through recirculating nutrient solutions.



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A NOVEL & NATURAL SOURCE OF LYSOZYME

Editor's note: Agricultural raw materials – plants and livestock – are becoming building blocks in a whole range of industrial and consumer products. Corn and soybeans are already used as construction materials, lubricants, inks and fuels, just to name a few. These natural resources generally offer the advantages of being more environmentally friendly, renewable, and reduce our reliance on petrochemicals.

Livestock will also provide new products beyond food. With advances in transgenic technologies, the humble chicken may have a promising future as a source of novel proteins, deposited in the egg.

Conventional breeding has produced some very successful poultry lines. But it is limited in its potential for further advances because it can only make use of existing genetic variability. Partnered with transgenic technology however, the opportunity exists for introduction of specific, purified and characterized single or multiple genes directly into the genetic material of the most favourable stock.

Constructing transgenic poultry lines is more difficult than producing transgenic mammals. In mammals, foreign genes are usually introduced into the mammalian genome at the single-celled embryo stage, which is readily accessible. The single-celled avian embryo, unfortunately, is not. By the time an egg is laid (and the embryo is then more accessible for manipulation), the embryo already consists of approximately 30,000 to 60,000 cells.

We presented our approach to producing transgenic chickens in an earlier issue of this magazine (Vol. 19, No. 2). The greatest challenge in the production of transgenic animals is ensuring that the foreign DNA is efficiently and effectively incorporated into the host genome. We want the foreign gene to function in the transgenic animal exactly as expected. Therefore, improving the levels of incorporation of the foreign DNA into the genome is a major focus of our current research. Achieving this goal would facilitate routine production of transgenic animals, including poultry. As it is, commercially useful transgenic chickens should be in the hands of primary breeders within the next five years.

One of the objectives we hope to make possible through transgenic technology is the

deposition of novel components in the eggs. Lysozyme is an egg white protein of particular interest. Lysozyme was discovered over 70 years ago and plays an important role in food production as an antimicrobial agent. It effectively destroys bacteria and many food-borne yeasts by degrading components of their cell walls. Lysozyme's natural function is to reduce microbial contamination within the incubating egg.

Harvesting an Effective Antibacterial Agent

Humans have consumed lysozyme, in eggs, as part of their diet for thousands of years. With consumers demanding high quality food containing fewer of the traditional synthetic preservatives, lysozyme offers an attractive alternative. It is used with such common foods as milk products, fish, shellfish, cooked meats, fruits, fresh vegetables, salads, and bean curd. Lysozyme can also reduce the thermal requirements for preserving canned foods, thus improving the flavour, texture and colour of the product.

Lysozyme is a selective antimicrobial agent, destroying harmful organisms while allowing beneficial microorganisms to survive. The cheese industry uses lysozyme at concentrations that do not inhibit the growth of the beneficial organisms involved, yet inhibit the growth of undesirable bacteria like *Clostridium tyrobutyricum*. Italian wines may contain lysozyme as a preservative.

Toothpaste, mouth wash and eye care products may all contain lysozyme, and it is useful as an antimicrobial agent for treating coliform infections of the bladder. The aquaculture industry is also looking at the potential of lysozyme to control infection in fish populations. A growing concern to the medical community is the increasing

Lysozyme is a naturally occurring anti-bacterial agent used in many foods and consumer products.

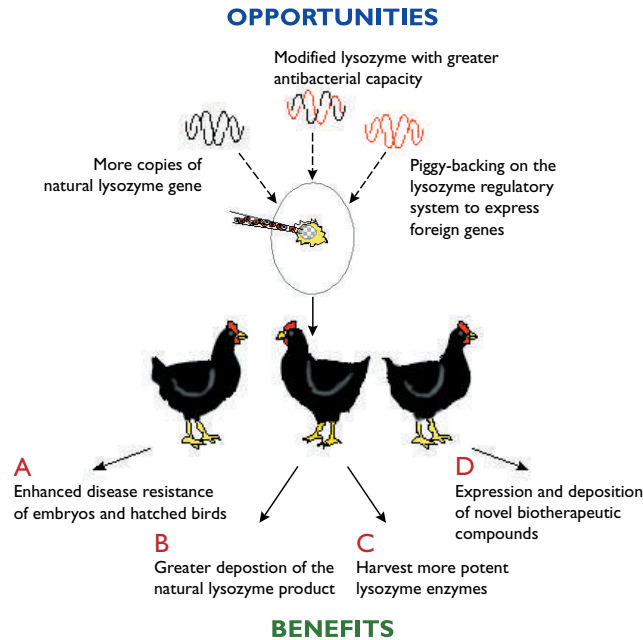


Fig. 1. Variants of the Chicken Lysozyme Gene.

resistance of pathogenic bacteria to the current range of antibiotics. Modified forms of lysozyme, developed as new antibiotics for treating human disease, offer a valuable alternative.

Our research has two objectives. One is to insert extra copies of the chicken lysozyme gene into the chicken genome to increase levels of natural lysozyme in the egg white. The other is to use genetic modification to produce novel versions of the lysozyme gene that encode modified lysozyme enzyme with a broader range of activity against disease-causing or food-spoilage bacteria.

Our work in the laboratory is progressing. The lysozyme gene is very large, and modifications to the gene have required the development of special techniques to construct a range of gene variants. We have also developed cell culture systems for testing the modified genes. Once we are satisfied the genes can express the modified proteins in cell culture systems, we will then be ready to introduce them into chickens and establish transgenic lines.

Cost and availability are major obstacles to the more wide-spread application of lysozyme. Canada currently produces 15% of the world supply. As lysozyme's approval for use in food preservation increases worldwide, supplies will become limited. There are tremendous market applications for our research if we can significantly increase our production capabilities.

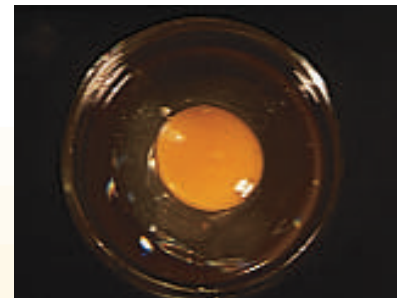


PHOTO: L. NORMAN GIBBINS

Fig. 2. Using the chicken as a bioreactor, normal components of the egg could be modified to increase their quantity or improve functional properties. More dramatically, totally novel products such as human therapeutic proteins could be deposited in the egg.

Mosaic Arthroplasty: Equine Joint Repair is

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away

With the emphasis on healthier and more active lifestyles, competitive sports are attracting people well into their later years. Horse owners are expecting the same of their mounts. Many hard working hunt, dressage and three-day event horses can be highly competitive in their late teens and early twenties. Injuries, however, are a fact of life for both people and horses. This is particularly true for those involved in competitive sports. Often these injuries occur early in life and are career-limiting. Now, with specialized mosaic arthroplasty techniques, the rehabilitation of many competitive horses with previously untreatable joint injuries, is possible.

Joint injuries are a major cause of economic loss in the equine industry. Even a small cartilage injury in a critical spot can end a successful career. While the knee is the most problematic site for the human runner, injuries of the equine carpal and fetlock joints in the front limb are limiting for the horse. Cartilage in these high motion joints does not heal in adult horses. Medications in the glucosamine sulfate, non-steroidal anti-inflammatory and hyaluronate families are ineffective treatments for the kind of cartilage and bone injury that we often see in competitive horses.

Another approach to joint injury is to resurface the site with a graft. Synthetic materials and crude cartilage grafts have been tried, but both types were easily damaged and had problems with attachment to the underlying bone. In human orthopedics, grafting is relatively

successful in difficult cases where a tumour or injury has destroyed all or part of a joint. These large grafts are sometimes harvested from another part of the same joint as an autograft, or as an allograft from an organ donor program.

Arthroscopic surgery for some previously untreatable injuries

A more recent innovation in sports medicine is using small cylindrical autografts to resurface a symptomatic cartilage defect, in the manner that small tiles make up a mosaic. This technique, called mosaic arthroplasty, is being refined by Dr. Anthony Miniaci, an orthopaedic surgeon at the Toronto Hospital (Western Division), with our Comparative Bone and Joint Disease Laboratory research group at the University of Guelph.

We deliver the grafts to precisely drilled



to the races

holes in the damaged area as small cylinders, like dowels, in an evenly spaced pattern. Our research has shown that a group of three larger diameter grafts (4.5 or 6.5 mm) was more successful than a group of smaller diameter ones (2.7 mm). The smaller grafts were fragile and the bony portion of the graft healed poorly. Larger grafts were easier to position to reconstruct complex, curved contours.

Accuracy of graft delivery is essential in areas where the cartilage is thin, such as in the equine carpus. We must exactly match the levels of graft and host cartilage, otherwise mechanical damage and loosening of the graft result. In another study, we left the grafts just proud of the joint surface and allowed joint loading to reposition the grafts into the recipient hole, even with the joint surface.

The greatest advantage of mosaic arthroplasty for horses is that the entire procedure can be done arthroscopically without the need for a large incision. As a result, previously untreatable injuries in the carpus, fetlock, hock and stifle are now potentially treatable. Two research associates, Drs. Simon Pearce and Molly Ferris, are currently experimenting with allografts for mosaic arthroplasty. Allografts, taken from another recently euthanatized or slaughtered horse, have the advantage of

being an exact match for the damaged site, but do have the potential complication of immune rejection.

Small, focal injuries most suitable candidates

Through our research we have developed specialized mosaic arthroplasty techniques and instruments for the horse. Preliminary results in the equine carpal joint are encouraging and, like Dr. Miniaci, we are offering mosaic arthroplasty to carefully selected patients in our surgical practice. The people and horses that are most likely to benefit are those with small, focal, joint cartilage injuries. Patients with chronic long-standing injuries, osteoarthritis and unstable joints are unsuitable candidates for this technique.

Longer term studies and clinical trials are beginning. The success of cartilage transplantation in controlling joint disease in horses is of significant interest. Reducing the cost of treating chronic joint disease, which includes anti-inflammatory and intra-articular medication, as well as lost training days and lost winnings, would mean real savings to horse owners. ■



PHOTO: GRANT HEILMAN PHOTOGRAPHY

Super Plants

MEAN Supper

PHOTO: CARROLL AND CARROLL



The development of transgenic plants containing a mammalian gene capable of expressing a novel protein, first occurred a decade ago and was a major advance in plant biotechnology. It opened the door for the development of safe, cost-effective vaccines through production of viral antigens in plants. Transgenic plants, as efficient, low-cost bioreactors, have enormous potential to enhance the health and productivity of Canada's farm animals.

Sows exposed to transmissible gastroenteritis develop mucosal immunity and secrete IgA into their milk, conferring passive immunity to their suckling piglets.

For example, novel proteins produced in plants can benefit pigs in several ways. They can act as oral vaccines and provide immunity to intestinal viruses, such as TGEV (transmissible gastroenteritis virus) and PRRS (porcine respiratory and reproductive syndrome). As antibiotics they can offer protection from other intestinal pathogens and parasites, and as growth factors they can improve gut development and function.

Challenge #1: Sufficient Protein Expression

There are challenges to making this technology work, and overcoming these is the focus of our research. An important requirement is obtaining sufficient expression of the foreign protein in the plant. Expression levels are often too low to be useful, and one of the

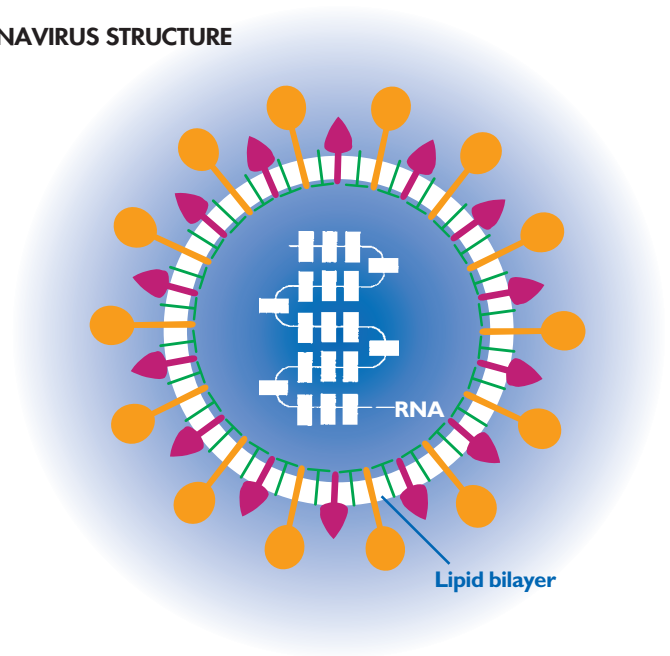
reasons has to do with codon usage.

A codon is composed of three nucleotides which specify a particular amino acid. That amino acid, however, may be represented by more than one codon. For example, lysine can be represented as AAA or AAG. An organism may use both codons for the same amino acid. Often, however, the frequency of codon used differs between plants and animals. The sequence of codons in a molecule of DNA specifies the sequence of amino acids in the protein. In order for the plant to express the mammalian protein correctly and in sufficient quantities, we need to rebuild the mammalian gene to suit the codon usage in the plant. The task becomes increasingly complex with increasing size of the gene. Through it all, however, we are gaining considerable knowledge about gene expression in plants.

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CORONAVIRUS STRUCTURE



Pigs

Challenge #2: Protein Survivability

Another challenge is survivability of the novel protein in the harsh conditions of the animal's gastro-intestinal tract. Our strategy is to select proteins that have evolved to resist degradation and retain biological activity until an immune response has occurred. Another is to target foreign proteins to particular cellular locations in the plant which might protect the protein from degradation in the digestive tract. Finding the appropriate delivery vehicle can have a dramatic effect on the levels and activity of novel protein expression.

Challenge #3: Stimulating Immune Response

A third challenge is presenting the novel protein to the animal's immune system so that it looks like a pathogen. If the novel protein resembles a food protein to the immune system, it will simply be digested as any food protein. Generally, these novel proteins must be in a particulate form, such as a bacteria or pathogen, to elicit a strong immune response.

The synthesis and delivery of vaccines, growth factors and antibiotics as plant-based feed is a simple, cost-effective approach to animal health and productivity. This is particularly important for increasingly large livestock operations where disease control is of

paramount importance and often difficult to achieve. One benefit to consumers is a reduced threat to human health from animal-borne pathogens such as *Salmonella*, *E. coli* and *Cryptosporidium*. Another benefit is the absence of residues in products produced for human consumption, since these novel proteins are naturally occurring proteins that ultimately degrade in the gut.

The successful application of genetic engineering to plant agriculture depends on an efficient gene transfer system, as well as appropriate gene constructs. The June 1996 issue of this magazine (Vol. 19, no. 2) presents some of this work. Our research is multidisciplinary in nature and involves several departments at the University of Guelph – Plant Agriculture, Ontario Veterinary College and Animal and Poultry Science – as well as the Laboratory Services Division.

The technology and products resulting from this research have tremendous market application. We will be working with established feed and animal health companies to determine the best delivery mechanism for the specialty feed products being developed. Patent preparation is underway for our inventions, offering us an important tool for the transfer of technology and return on the research investment. ■

'Super' pigs will have immunity to intestinal viruses such as the coronavirus (structure shown above) which causes transmissible gastroenteritis (TGE).

ENRICHED MILK WITH THAT OH-SO- IMPORTANT OMEGA-3



By now you've probably seen omega-3 eggs in your grocery store, so named because they're rich in omega-3 polyunsaturated fatty acids. The term 'omega-3' relates to the chemical structure of a class of fatty acids, which can reduce the risk of heart disease, as well as having other important functions in the human body. At the University of Guelph, we are now looking at developing 'omega-3 milk' with the addition of an omega-3 fatty acid known as dietary docosahexaenoic acid or DHA.

DHA is an essential dietary nutrient for both infants and children. It occurs in abundance in the brain and retina and high levels are needed for optimal learning ability and visual performance. Research has shown that infants on breast milk perform better in visual acuity and intelligence testing than those on North American formulae, which lack DHA. Mothers' breast milk contains, on average, 0.2% DHA.

Dietary DHA is also helpful in treating attention-deficit disorders, depression and anxiety disorders, and in protecting against breast cancer in post-menopausal women.

DHA occurs in abundance in fish and fish oils. The human body can convert linolenic acid (LNA), another omega-3 fatty acid, to DHA, but only to a limited extent. LNA is present in moderate amounts in soybean oils, which are a component of North American infant formulae. However, we now know that infants are not able to synthesize DHA from LNA at optimal levels for their visual and mental development. Infant formula manufacturers are conducting various experiments and clinical trials, adding DHA directly to test formulae.

Making DHA Available in Milk

Cows' milk is devoid of DHA and has only very modest amounts of LNA. If we could alter cows' milk to contain optimal levels of DHA, this important dietary nutrient would be easily available to all children. Our research is attempting to do just that by introducing DHA directly, through the feed, into dairy cows' milk and corresponding dairy fats. The dairy fats could then be assimilated into a wide variety of dairy products, such as butter, cheese and ice cream.

In the feeding experiments we conducted, we provided cattle with a basal diet of corn silage, high-moisture corn grain and straw. This is an intentionally low crude protein diet and one we knew, by itself, wouldn't result in any production of DHA in the milk. We then added to this basic diet three different concentrations of a custom-blended fishmeal supplement. Samples of milk were analysed for their content of fat, protein, lactose and DHA. The results showed that we were able to

produce 'omega-3' milk with the desired concentrations of DHA by altering the formulation of the feed.

The next step was to test whether human consumption of these omega-3 products would raise their DHA levels. Two groups of nine healthy young adults included either 750 ml/day of the control milk and 100g/day of the control cheese or similar amounts of the treatment omega-3 products in their regular diets. The experimental period ran for two weeks. We monitored DHA levels through blood sampling throughout the duration of the study to correlate fatty acid levels with plasma levels of omega-3 fatty acids, including DHA. The results are still being analysed.

Our final step will be to refine a dietary supplement for cattle. We are currently able to achieve the desired levels of DHA in the milk and are now working on a commercial formulation. The natural introduction of DHA into milk and dairy products could make this essential nutrient with so many important functions, more widely available for the benefit of children and all consumers. ■

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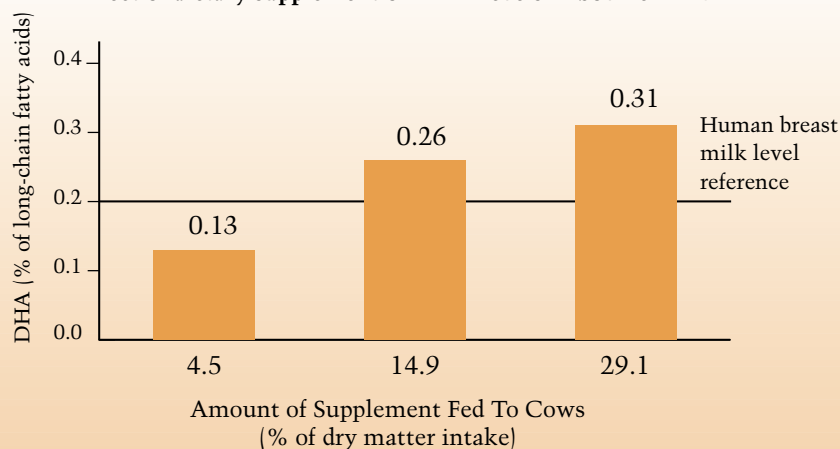
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Effect of dietary supplement on DHA levels in bovine milk



MODELING TO MINIMIZE PRE-HARVEST MICROBIAL CONTAMINATION



Food safety is a top priority for the beef industry. The 1993 outbreak of *E. coli* O157:H7 in a U.S. hamburger chain had an immense impact on public confidence in the safety of meat. It and other outbreaks since then have fundamentally changed the food safety policies of many farming, processing and retail industries, as well as the activities of public agencies responsible for food safety.

The search for better quality control measures resulted in the development of Hazard Analysis and Critical Control Point (HACCP) systems. Beef producers, processors and public health officials all recognize the need for an integrated beef safety risk

management system to minimize the pathogen load at each step of the production chain. Food service and processing industries at the post-harvest stage have been making significant advances through application of the HACCP approach to food safety. However, there is

still a need for practical and effective on-farm food safety HACCP programs at the pre-harvest stage.

At the Ontario Veterinary College, we are helping develop some of these programs. The

beef production and slaughter system is complex, and the hazards and costs of working with a pathogen such as *E. coli* O157:H7 are considerable. Because of these factors, computer modeling offers distinct advantages and is an important part of our research.

What we want to be able to do is quantitatively assess the risk associated with a particular level of disease in a particular herd of animals. There are numerous variables and uncertainties associated with this type of research. They occur with the tests and diseases themselves, with which animals are selected from a particular herd for testing, and with the history of the animal prior to reaching the abattoir. This type of assessment is best evaluated through stochastic simulation modeling, a type of modeling which gives a probability of a particular outcome.

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Les recherches en bref

Technologies de production végétale

Évaluation de la réduction des risques de l'usage de pesticides

Gord Surgeoner

L'un des principaux objectifs du programme Production alimentaire 2002 de l'Ontario consiste à réduire de 50 % l'usage de pesticides tout en maintenant la productivité agricole. Tout programme de réduction des pesticides devrait prévoir une réduction équivalente des risques. Le risque dépend de la toxicité du produit chimique et de l'exposition à ce produit. Nous avons établi trois catégories générales de risque : les risques pour les travailleurs agricoles, pour les consommateurs et pour l'environnement. Nous avons mesuré les risques que posent les pesticides au moyen d'un système de pointage élaboré à Cornell University, appelé Environmental Impact Quotient (EIQ) («quotient d'impact sur l'environnement»).

De 1983 à 1993, l'usage de pesticides a chuté de 28,5 %. Cette réduction a été réalisée en bonne partie du côté du maïs et du soya, qui représentent une forte proportion des emblavures, mais qui posent un risque relativement faible par tonne de production. Le risque associé aux pesticides par hectare était élevé pour le tabac, les fruits et les légumes, bien que la quantité de pesticide utilisé ait été beaucoup plus faible.

De nouvelles technologies ont favorisé la réduction de l'usage de pesticides, notamment la mise au point de nouveaux pesticides nécessitant une dose beaucoup plus faible d'ingrédient actif par hectare, des méthodes améliorées d'épandage et l'alternance des cultures. En outre, les producteurs eux-mêmes ont modifié leurs habitudes en ce sens.

Le risque global, mesuré au moyen de l'EIQ, a baissé de 29 % de 1982 à 1993. La baisse la plus forte a été enregistrée du côté des risques pour les travailleurs agricoles (41 %), mais les risques pour les consommateurs et l'environnement ont également diminué (de 28 % et 21 % respectivement).

Cependant, une importante question demeure toujours sans réponse. Devrions-nous poursuivre nos améliorations du côté des grandes cultures, qui présentent un risque relativement faible par hectare, ou devrions-nous plutôt concentrer nos efforts sur les cultures fruitières et légumières, qui posent un risque élevé par hectare, même si elles ne représentent que 11 % de

l'usage de pesticides? Nous avons réduit de près de 50 % les risques pour les travailleurs agricoles.

Peut-être que nous devrions maintenant chercher à créer des pesticides ou des méthodes de production qui permettraient de réduire les risques pour les consommateurs et l'environnement.

Normalisation des éléments utiles contenus dans les produits à base d'herbes médicinales

Praveen Saxena, Susan Murch, Kristen Choffe

En 1997, les consommateurs canadiens ont consacré près de 340 millions de dollars à l'achat de produits à base d'herbes médicinales. Des millions de personnes ont profité des effets bénéfiques de ces produits, qui causent moins d'effets secondaires que les médicaments traditionnels. Les consommateurs sont de plus en plus soucieux de leur bien-être et considèrent les plantes comme des solutions de rechange ou des compléments sûrs à la médecine conventionnelle; c'est pourquoi les marchés nord-américains des produits à base d'herbes médicinales sont en expansion.

À l'heure actuelle, la plupart des préparations d'herbes médicinales ne sont pas normalisées. Pour assurer l'expansion future de l'industrie des herbes médicinales en Ontario, il est essentiel de mettre sur le marché des produits uniformes et normalisés. Cependant, l'industrie phytopharmaceutique doit d'abord surmonter diverses difficultés.

Par exemple, la qualité des récoltes varie d'année en année, et la teneur en éléments actifs varie d'une plante à l'autre. Des insectes, bactéries et champignons peuvent contaminer le matériel végétal et les produits, et ceux-ci peuvent contenir des espèces de plantes mal identifiées. Enfin, faute de normalisation, le consommateur s'expose à la fraude et à des risques pour sa santé.

En vue de résoudre ces problèmes, nous élaborons de nouvelles technologies permettant le microbouturage de plantes médicinales. Les cellules ou tissus végétaux, comme l'hypocotyle ou les tissus de la tige, sont cultivés dans un milieu de culture enrichi d'éléments nutritifs et de concentrations optimales de régulateurs de croissance des plantes. Nous cultivons ces régénérats dans des conditions nutritionnelles et environnementales idéales, sans aucune contamination par des bactéries, des

champignons ou des insectes. Les plantes présentant une teneur exceptionnelle en composés médicinaux actifs sont sélectionnées, puis produites en série par voie de clonage au moyen de techniques de culture de tissus.

Le microbouturage représente un moyen efficace de production végétale présentant des caractéristiques uniformes et normalisées. Cette normalisation permettra d'améliorer la confiance des consommateurs. Nos recherches représentent une première étape en vue de fabriquer des produits à base d'herbes médicinales normalisés et de haute qualité pour l'Ontario et les marchés internationaux.

Conservation des fleurs coupées - élimination de l'éthylène au moyen du 1-MCP

Lisa Skog, Theo Blom, Wayne Brown

L'éthylène peut endommager de nombreuses fleurs d'ornement, ce qui cause de graves pertes pour les producteurs et réduit la qualité des produits à la disposition des détaillants et des consommateurs. L'éthylène agit comme un régulateur naturel de la croissance, en accélérant le mûrissement des fruits. Les plantes malades, coupées ou endommagées peuvent produire de l'éthylène. Les plantes cultivées en floriculture sont également exposées à de l'éthylène provenant de nombreuses sources externes : gaz d'échappement de véhicules, fruits mûrs, systèmes de chauffage de serres ou générateurs de CO₂ à échappement insuffisant.

Les fleurs coupées sont particulièrement vulnérables. Il est impossible d'éviter leur exposition à l'éthylène, et même de très faibles concentrations peuvent les endommager. L'industrie a besoin d'une méthode peu coûteuse, écologique et simple pour lutter contre les effets de l'éthylène.

L'industrie de la floriculture utilise le thiosulphate d'argent (STS) à cette fin. Cependant, des inquiétudes la pollution par des métaux lourds ont motivé des recherches visant à trouver d'autres méthodes. Un nouvel inhibiteur gazeux de la fixation de l'éthylène, le 1-méthylcyclopropène (1-MCP), semble une solution de rechange prometteuse. L'éthylène agit en se fixant à différentes parties de la plante et en provoquant chez elle une réaction. Le 1-MCP empêche cette fixation.

Jusqu'ici, nous avons testé quatre espèces de fleurs coupées : des muflers, des alstroemères, des dauphinelles et des gypsophiles. À la température ambiante, le 1-MCP s'est révélé efficace chez ces quatre espèces. En l'absence de 1-MCP, les plantes témoin exposées à de l'éthylène n'avaient aucune fleur trois jours après le traitement.

Les recherches futures viseront à optimiser le traitement au 1-MCP. Selon des estimations modérées, les pertes causées par l'éthylène se chiffrent à 17 millions de dollars par année. Le 1-MCP pourrait donc rapporter des économies substantielles à l'industrie de la floriculture, qui cherche à prendre de l'expansion aux États-Unis et qui, en 1995, a rapporté des revenus de 332 millions de dollars par année.

Élaboration d'un pulvérisateur intelligent temps réel de haute précision

Ralph Brown et Ken Bennett

L'agriculture de précision a recours à la technologie de l'information pour adapter l'épandage à l'état du terrain et aux besoins des cultures. Les agriculteurs ontariens consomment beaucoup d'herbicides chimiques; l'adoption de techniques de pulvérisation plus efficaces leur permettrait de réaliser des économies à ce chapitre tout en protégeant l'environnement.

Nous élaborons un pulvérisateur de précision pouvant épandre un taux variable d'ingrédient actif au niveau de la buse. Le réservoir contient uniquement de l'eau; aucun pré-mélange n'est nécessaire. Le concentré d'herbicide est puisé au besoin à un réservoir distinct. Le dosage d'une petite quantité de liquide permet d'épandre l'herbicide avec plus de précision. Il est possible de régler avec exactitude le taux d'injection, sans égard à la viscosité de l'herbicide.

En 1998, nous avons mis un prototype à l'essai sur le terrain. Les essais se poursuivront en 1999 pendant toute la saison de croissance. Des modifications seront apportées au pulvérisateur afin de réduire les frais d'adaptation et d'améliorer sa fiabilité et sa polyvalence.

L'efficacité d'un herbicide repose sur différents facteurs, notamment la sensibilité de l'espèce visée, son stade de croissance, les contraintes environnementales et les conditions météorologiques. L'intégration de ces paramètres dans le modèle de prescription permettrait de réduire encore plus les dosages.

Une version future du pulvérisateur sera dotée d'un système de positionnement intégré (GPS). L'opérateur, qu'importe où il

commence dans le champ, pourra toujours savoir les zones qu'il reste à traiter.

Nous avons encore du travail à faire du côté de la télédétection et de la modélisation des prescriptions. Le rapport entre la production agricole et l'épandage est d'une complexité telle que la modélisation traditionnelle est insuffisante. Le recours à la logique floue et à des techniques de modélisation d'ensembles flous pourrait aboutir à des approches novatrices.

Il n'existe pas encore de pulvérisateur d'herbicides intelligent temps réel et de haute précision, mais nous continuerons de rechercher un système de gestion permettant de réduire les épandages d'herbicides tout en maintenant la productivité agricole et la qualité des produits alimentaires en Ontario.

L'industrie du soya de l'Ontario : un secteur prospère

Gary Ablett

L'industrie ontarienne du soya a connu une expansion phénoménale au cours des 20 dernières années. Les cultivars élaborés par l'Université de Guelph et Agriculture et Agroalimentaire Canada (AAC) occupent environ 45 % des emblavures de soya en Ontario.

Au Collège Ridgeway, le programme de sélection du soya est axé sur la création de cultivars et la valorisation du matériel génétique. Nous mettons au point des cultivars améliorés qui présentent un potentiel de rendement supérieur ainsi qu'une bonne résistance à la verse et aux maladies, en croisant des génotypes productifs provenant de diverses sources.

Le Comité de l'Ontario pour les oléagineux et les protéagineux est responsable de l'enregistrement des cultivars de soya et des tests de rendement auxquels ils sont assujettis. Le programme de Ridgeway effectue sept des 16 essais dans la province, et AAC, le Collège Kemptville et l'Université de Guelph s'occupent du reste.

Notre programme de valorisation du matériel génétique compte parmi ses principaux objectifs l'obtention d'une huile de qualité et de caractères morphologiques particuliers. Le programme de qualité de l'huile établit des profils d'acides gras qui s'écartent considérablement de ceux de graines de soya typiques. On cherche à obtenir un profil présentant une faible teneur en acide linoléique et en acide palmitique, et une forte teneur en acide oléique.

À l'heure actuelle, l'huile de soya est modifiée physiquement et chimiquement afin d'obtenir le profil d'acides gras requis

pour divers produits alimentaires. Nous cherchons à apporter certaines de ces modifications par voie génétique. Au Canada, il n'existe pas encore de cultivars d'usage commercial qui présentent un profil d'acides gras modifié. Cependant, des caractères morphologiques élaborés au début des années 90 sont actuellement intégrés dans des cultivars de rendement élevé.

Nos recherches en matière de valorisation du matériel génétique portent sur les caractères morphologiques qui ont une incidence sur la croissance de la plante. Nous intégrons ces caractères dans des cultivars dont le patrimoine génétique leur confère de bonnes qualités au plan du rendement, de la maturité et de la résistance à la maladie, à la pourriture et à la verse, et nous déterminons ensuite si ces cultivars poussent bien en Ontario. Nous y arrivons en combinant les éléments du programme qui portent sur les cultivars et sur le matériel génétique.

Des superplantes pour des superporcs

Larry Erickson

Les nouvelles protéines produites par des plantes peuvent se révéler utiles au bétail à plusieurs titres. Par exemple, elles peuvent jouer le rôle de vaccins oraux permettant d'immuniser les porcs contre des virus intestinaux qui les affligent souvent, tels que celui de la gastro-entérite transmissible du porc (GET). En tant qu'antibiotiques, elles confèrent une protection contre d'autres agents pathogènes et parasites intestinaux, et en tant que facteurs de croissance, elles peuvent améliorer la fonction et le développement intestinaux.

Pour que cette technologie soit efficace, il reste à surmonter certaines difficultés. Ainsi, nous tentons dans nos recherches d'obtenir une expression suffisante de la protéine exogène dans la plante. Le niveau d'expression est souvent insuffisant pour être d'une utilité quelconque, notamment en raison de l'usage de codons. Une autre difficulté réside dans la capacité de survie de la nouvelle protéine dans le milieu hostile que représente l'appareil gastro-intestinal de l'animal. L'utilisation d'un vecteur approprié pourrait avoir un effet spectaculaire sur le niveau d'expression de la protéine. Une troisième difficulté consiste à faire en sorte que le système immunitaire de l'animal considère la nouvelle protéine comme un agent pathogène. En général, ces protéines doivent avoir une forme particulière, comme celle d'une bactérie ou d'un autre type d'agent pathogène, pour provoquer une forte réaction immunitaire.

La technologie et les produits résultant de nos recherches présentent un excellent potentiel sur le marché. Nous collaborerons avec des fabricants d'aliments pour animaux et des sociétés d'hygiène vétérinaire bien établies en vue de trouver un vecteur idéal pour de tels produits alimentaires spécialisés. Un brevet, qui est actuellement en préparation, représentera un outil important qui permettra de transférer nos technologies et de rentabiliser nos recherches.

La lutte contre le fusarium dans les cultures de maïs de l'Ontario

Peter Pauls

La fusariose, causée par le champignon *Fusarium graminearum*, représente la plus grave menace à laquelle font face les cultures de maïs ontariennes. Les technologies de transformation du maïs, qui pourraient permettre de créer du maïs résistant à cette maladie, ne se sont pas encore révélées efficaces. Nous tentons donc d'élaborer des procédés de transformation du matériel génétique du maïs au moyen d'un système de coculture d'agrobactéries et de maïs.

Pour opérer une transformation, le génotype du maïs doit être très sensible à la culture de tissus et compatible avec les agrobactéries. Après étude d'environ 40 lignées autofécondées et hybrides de maïs de l'Ontario, nous avons relevé environ 10 génotypes sensibles.

En raison de l'interaction complexe entre les agrobactéries et la plante hôte, la transformation repose également sur le génotype de la plante. Nous avons commencé à étudier les génotypes régénérables pour vérifier leur compatibilité avec les agrobactéries. Nous utilisons des souches d'agrobactéries qui présentent des phénotypes bien définis qui sont relativement faciles à sélectionner dans le cadre d'une culture de tissus ou à identifier dans des pousses régénérées.

Ces expériences nous ont permis de produire les premières cultures de tissus et de pousses régénérées présentant une activité du gène marqueur (GUS). Nous cherchons actuellement à accroître l'expression du gène GUS dans les pousses de maïs et à obtenir une plante transformée.

Nous pourrions ensuite introduire dans le maïs, au moyen de cette méthode recourant à des agrobactéries, des catégories de gènes qui permettraient de réduire l'incidence de maladies. Nous identifierions les marqueurs moléculaires liés à la résistance naturelle du maïs au *Fusarium*, et nous nous

fonderons sur la présence ou l'absence de ces marqueurs pour déterminer si nous soumettrons une plante donnée à d'autres opérations de sélection en vue de développer sa résistance.

Le produit final des recherches actuelles sera un maïs résistant aux maladies pour les agriculteurs ontariens, mais en outre, l'élaboration de technologies de transformation efficaces ainsi que les données recueillies sur la fonction génétique appuieront une variété de projets d'amélioration du maïs dans l'avenir.

Technologies d'élevage

Une source nouvelle et naturelle de lysozyme

Ann M. Gibbins et Jan Losos

Dans la production d'animaux transgéniques, la plus grande difficulté consiste à s'assurer que l'ADN étranger est incorporé de façon efficiente et efficace dans le génome de l'hôte. Si l'on atteignait cet objectif, il serait possible de faciliter la production courante d'animaux transgéniques, et notamment de volaille. Dans l'état actuel des choses, des poulets transgéniques utilisables commercialement devraient être mis sur le marché d'ici cinq ans.

Parmi les nouvelles opérations que nous cherchons à réaliser au moyen de la technologie transgénique se trouve le dépôt de nouvelles composantes dans des œufs. Le lysozyme, une protéine contenue dans le blanc d'œuf, nous intéresse particulièrement. Il joue un rôle important dans la production alimentaire en tant qu'agent antimicrobien. Comme les consommateurs exigent des aliments de haute qualité contenant moins d'agents de conservation traditionnels, le lysozyme représente une solution de rechange séduisante.

Le lysozyme est un agent antimicrobien sélectif. L'industrie du fromage l'utilise à des concentrations qui inhibent la croissance non pas des organismes utiles, mais bien des bactéries indésirables comme *Clostridium tyrobutyricum*. La communauté médicale s'inquiète de plus en plus de la résistance croissante des bactéries pathogènes aux antibiotiques dont on dispose actuellement. Des formes modifiées de lysozyme, conçues pour agir comme antibiotiques chez l'être humain, seraient une solution de rechange intéressante.

Nos recherches visent deux objectifs. Nous voulons d'abord insérer des copies supplémentaires du gène du lysozyme du poulet dans le génome du poulet afin

d'accroître la concentration de lysozyme naturel dans le blanc d'œuf. Ensuite, nous comptons recourir au génie génétique pour produire de nouvelles versions du gène du lysozyme qui permettraient de produire un enzyme présentant une activité plus large contre des bactéries pathogènes ou des bactéries causant l'altération des aliments.

L'usage généralisé du lysozyme est freiné par son coût élevé et sa rareté. Si nous réussissons à accroître de façon importante notre capacité de production, nos recherches revêtiront un potentiel énorme sur le marché.

L'arthroplastie en mosaïque : remplacement d'articulations chez les chevaux

Mark Hurtig

Les lésions articulaires représentent une importante cause de pertes financières dans l'industrie équestre. Même une légère lésion du cartilage à un endroit critique peut mettre un terme à une brillante carrière. Les médicaments comme le sulfate de glucosamine, les anti-inflammatoires non stéroïdiens et les hyaluronates sont généralement inefficaces chez les chevaux de compétition.

Une autre méthode de traitement des lésions articulaires consiste à réparer le site au moyen d'une greffe. On a tenté des greffes au moyen de matériaux synthétiques et de cartilage, mais les greffons étaient fragiles et difficiles à fixer à l'os sous-jacent.

Une innovation récente en médecine sportive consiste à utiliser de petits autogreffons cylindriques pour corriger un défaut dans le cartilage comme si l'on créait une mosaïque; c'est ce que l'on appelle l'arthroplastie en mosaïque.

Cette technique peut être exécutée au moyen d'un arthroscope. Nous menons actuellement des expériences avec des allogreffons prélevés sur d'autres chevaux euthanasiés ou abattus. Ces allogreffons correspondent exactement au site de la lésion, mais peuvent donner lieu à un rejet. Dans des trous percés avec précision dans la zone endommagée, nous insérons de petits greffons cylindriques espacés également. Dans nos recherches, les greffons de gros diamètre (4,5 ou 6,5 mm) ont donné de meilleurs résultats qu'un groupe de petits greffons (2,7 mm). Il est essentiel de positionner les greffons avec précision aux endroits où le cartilage est mince.

Cette technique est mise au point en collaboration avec le Dr Anthony Miniaci du Toronto Hospital (Western Division).

Lait enrichi du fameux acide gras oméga 3

Brian McBride, Art Hill, Bruce Holub,
Hong Wang, Tom Wright

Vous avez peut-être entendu parler des œufs oméga 3. Nous envisageons actuellement de créer du «lait oméga 3» par l'ajout au lait d'acide gras oméga 3, connu sous le nom d'acide docasahexanoïque (DHA).

Le DHA est un élément nutritif essentiel pour les nourrissons et les enfants. Des recherches ont démontré que les nourrissons buvant du lait maternel, qui contient du DHA, obtiennent de meilleurs résultats aux tests d'acuité visuelle et d'intelligence que ceux qui boivent du lait pour nourrissons vendu en Amérique du Nord.

Le lait de vache est dépourvu de DHA. Si on pouvait le modifier afin qu'il présente une teneur optimale en DHA, les enfants pourraient absorber facilement cet important élément nutritif. C'est ce que nous tentons de faire en ajoutant du DHA aux aliments des vaches laitières, pour que leur lait et les matières grasses correspondantes en contiennent.

Dans nos expériences alimentaires, nous avons fourni au bétail un régime de base composé de maïs et de paille. Nous avons ensuite ajouté trois concentrations différentes d'un supplément spécial à base de farine de poisson (le poisson et les huiles de poisson sont d'excellentes sources de DHA). Enfin, nous avons analysé des échantillons de lait. Les résultats confirment que nous avons pu produire du lait «oméga 3» contenant les concentrations désirées de DHA.

La prochaine étape consiste à déterminer si la consommation de ces produits oméga 3 permet d'accroître le niveau de DHA chez l'être humain. Deux groupes de neuf jeunes adultes en santé ont ajouté à leur alimentation habituelle soit 750 mL/jour de lait témoin et 100 g/jour de fromage témoin, soit des quantités équivalentes de produits oméga 3. Les résultats sont toujours à l'étude.

À la dernière étape de nos recherches, nous mettrons au point une formulation alimentaire d'usage commercial pour les bovins. L'introduction naturelle de DHA permettra d'obtenir du lait et des produits laitiers de valeur nutritive supérieure, dont le consommateur pourra tirer profit des effets positifs sur sa santé.

Technologies de production alimentaire

Prévention de la contamination microbienne et chimique du bœuf

Scott McEwen

En 1993, la contamination massive de hamburgers par la bactérie *E. coli* O157:H7 aux États-Unis a fortement ébranlé la confiance du public dans l'innocuité de la viande. Ces poussées de contamination ont transformé du tout au tout les politiques de nombreuses industries de conditionnement des aliments en matière de sécurité alimentaire, de même que les activités des organismes publics responsables.

La volonté de mettre en œuvre des procédures améliorées de contrôle de la qualité a entraîné la création de systèmes d'analyse des risques et de maîtrise des points critiques (HACCP). Les industries de la transformation des aliments ont réalisé des progrès importants grâce à la mise en œuvre de tels systèmes. Cependant, il reste à mettre en place des programmes efficaces pour les étapes préalables à la récolte.

Le système de production et d'abattage du bœuf est complexe, et il est à la fois très dangereux et coûteux de composer avec un pathogène tel que *E. coli* O157:H7. À ce titre, la modélisation informatique présente des avantages indéniables.

Nous voulons parvenir à évaluer quantitativement le risque associé à un taux d'atteinte donné dans un troupeau d'animaux. La simulation par modélisation stochastique est le meilleur moyen de réaliser cette évaluation.

Nous avons élaboré un modèle général fondé sur des techniques de Monte-Carlo pour comprendre le degré d'incertitude et de variabilité des protocoles relatifs aux épreuves de troupeau. Les résultats obtenus avec ce modèle peuvent servir à appliquer des modèles d'évaluation des risques et à répondre rapidement et de façon économique à un éventail de questions.

Les études en simulation ont toutefois leurs faiblesses. Ainsi, leur validité repose sur les connaissances dont on dispose sur le système à l'étude. C'est pourquoi les modèles de simulation ne peuvent se substituer à la recherche traditionnelle. La modélisation de simulation permet d'exploiter au maximum les ressources dont on dispose pour permettre dès aujourd'hui un meilleur contrôle de la qualité. Elle permet également de déterminer les aspects devant faire l'objet d'autres recherches en vue d'améliorer encore plus le contrôle de la qualité dans l'avenir.

La santé des cultures dans les serres hydroponiques à recirculation

Melanie Johnstone, Hai Yu, Bernard Grodzinski,
John Sutton

Les systèmes hydroponiques à recirculation sont une méthode répandue de production de plantes d'ornement et de légumes dans les serres ontariennes. Cependant, ils sont vulnérables à des flambées de maladies dévastatrices, causées généralement par deux groupes de champignons pathogènes : le pourridié des racines et la flétrissure.

Pour lutter contre ces champignons, il faut avant tout prévenir ou, à tout le moins, minimiser la contamination du système hydroponique. Pour ce faire, il est nécessaire d'utiliser de l'eau et des graines qui ne contiennent aucun champignon pathogène, de désinfecter le système de production entre les récoltes et de garder la serre impeccablement propre. En cas de contamination, il est essentiel de freiner rapidement la propagation de la maladie. On peut recourir à des techniques physiques, chimiques et biologiques; cependant, l'industrie a besoin de méthodes supplémentaires destinées spécialement aux systèmes hydroponiques afin de demeurer viable et compétitive.

Nous étudions l'évolution et le contrôle de la maladie, ainsi que la physiologie et la productivité des cultures dans des systèmes hydroponiques créés à des fins de recherche. La santé et la viabilité des plantes sont établies en fonction de la photosynthèse et du taux de respiration. Le contrôle précoce des changements métaboliques devrait permettre de signaler la présence d'agents pathogènes, et de prévoir l'apparition de la maladie plusieurs jours avant que les symptômes ne se manifestent.

Dans notre système expérimental le plus simple, nous cultivons des plantes dans des pots contenant des solutions nutritives aérées; des systèmes complexes ont été conçus pour simuler les serres commerciales. Pour les études au moyen de rayons ultraviolets, nous utilisons un appareil à faisceau collimaté pour traiter des solutions utilisées dans des systèmes à «flux et reflux» (ebb and flow) et des systèmes de culture sur film nutritif.

Les résultats de nos recherches nous permettront de concevoir des traitements ciblés, intégrés et administrés en temps voulu pour mieux lutter contre les maladies dans les systèmes hydroponiques à recirculation. Ces traitements devraient améliorer la rentabilité et la compétitivité des serres ontariennes.