



# COST863 SGM on small fruit production systems

**Instituto Nacional dos Recursos Biológicos**  
L- INIA – Polo de Oeiras

**Dates: November 08-09-10, 2007**



## L-INIA - Pólo de Oeiras

### COST 863: Euroberries

#### Oeiras (Portugal)

Dates: November 08-09-10, 2007

#### Program

#### Thursday November 08

9.00h *Welcome, registration and general remarks*

9.30-11.00h

- Survey of small fruits research in Portugal – **Luís Lopes da Fonseca, Portugal**
- Breeding and biotechnology for improving berry nutritional quality – **Bruno Mezzetti, Italy**
- The CRA-ISF strawberry breeding activity: fruit quality and healthy compounds – **Gianluca Baruzzi, Italy**

11.00h Coffee Break

11.30-13.00h

- Breeding primocane-fruiting raspberries for mild winter climates – **Teresa Valdivieso, Portugal**
- Light effects on flower bud initiation in red raspberries (*Rubus idaeus* L.) – **Pedro Oliveira, Portugal**
- An approach to study molecular aspects underlying flower bud differentiation in raspberries – **Mariana Mota, Portugal**

13.00h Lunch

14.00-16.00h

- Micrografting as a multipurpose tool in plant production – **J.L. Fernandez-Lorenzo, Spain**
- New cultural practices in the post methyl bromide era in Huelva (Spain) – **J. Lopez-Medina, Spain**



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- Strawberry sustainable production systems concerning consumers safety – **Maria Graça Palha, Portugal**
- Double-cropping of blackberries in greenhouse production – **Fanny Pitsioudis, Belgium**

16.00h Coffee Break

16.30-18.00h

- Small fruits, quality and antioxidant capacity relevant to nutrition – **Beatriz Sousa, Portugal**
- Search for plant phenolic compounds exhibiting antioxidant activity and neuroprotective properties in a neuronal cell model of Parkinson's disease – **Claudia Santos, Portugal**
- The impact of soft fruit consumption on human health – **Derek Stewart, UK**

19.30h Dinner

20.30h Departure to Odemira

### **Friday November 09**

#### **Growers Meeting at S. Teotónio, Odemira**

9:30 h Welcome

10.00 -10.45h

- Final presentation of the I&DE project AGRO 556 – **Pedro Oliveira**

10.45h Coffee Break

11.00-12.15h

- The Italian strawberry industry - **Gianluca Baruzzi**
- Round table 1 "Strawberry production systems"

Six invited strawberry growers from different Portuguese growing areas

12.15-14.30h Lunch



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14.30-15.45h

- New primocane raspberries 'Sugana' and 'Erika' - **Fanny Pitsioudis**
- Round table 2 "Production systems for berries, other than strawberry "

Six invited berry growers from different Portuguese growing areas

15.45h Coffee Break

16.15-17.30h

- Cost 863 - Euroberry for improving knowledge transfer in berry research – **Bruno Mezzetti**
- Round table 3 "Post-harvest and marketing"

Six chief executives from the main Portuguese berry enterprises

17.30h

- "Nutritional enhancement of soft fruit: recent advances" - **Derek Stewart**

18.00h Closing session

### **Saturday November 10**

9.30h Technical visit

- Herdade Experimental da Fataca
- Berryport farm

12.30h Lunch

14.30h Return to Lisbon

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### **SURVEY OF SMALL FRUITS RESEARCH IN PORTUGAL**

Luís Lopes da Fonseca and Pedro B. Oliveira  
Instituto Nacional dos Recursos Biológicos; L-INIA, Pólo de Oeiras  
Av. da República, Nova Oeiras, 2784-505 Oeiras, Portugal

A comprehensive survey of the main research work conducted by the Portuguese berries team in the last years is presented for the three main crops: raspberries, blackberries and blueberries.

From the beginning it was clear that due to the climate characteristics of the country and of the central and northern European markets, the very early or the late production where the best seasons for growers income. Research emphasis was always with that goal in mind.

It is not equally possible nowadays to manipulate the growing cycle of each species and cultivars to achieve that objective.

Today growing raspberries summer fruiting cultivars for early production and primocane fruiting cultivars for late production allows the growers to schedule the harvest period by selecting planting material (bare root or tray plants), planting dates and manipulating pruning.

Blackberries have a totally different behavior and the groups Ursini and Moreferi present different levels of potential but the optimization of scheduled production will only be achieved with the onset of primocane fruiting blackberries varieties.

The manipulation of blueberries is not, till now, fully achieved, but some Southern Highbush cultivars look promising for December production under full or partial protected cultivation.

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### **BREEDING AND BIOTECHNOLOGY FOR IMPROVING BERRY NUTRITIONAL QUALITY**

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Fruit possess different antioxidant properties depending on their content in antioxidant molecules. As a consequence of the complexity of fruit matrix, the antioxidant capacity can be interpreted as reaction carried on by a single compound and/or by a complex mixture. The final result in Total Antioxidant Capacity (TAC) and the determination of Total Phenolic Content (TPH) of a fruit, is strictly reported to the fruit sampling, procedure of extraction of antioxidant compounds from fruit and also depends on which target, free radical generator or oxidant, is used in the measurement. However, these tools can have an important application in breeding and biotech program for improving strawberry nutritional quality.

For different strawberry genotypes (wild and cultivated) a deep analyses of nutritional attributes (NA) was made for cultivar and selection that have been included in breeding programs aimed to obtain improved fruit patterns and contents. To develop new genotypes, the availability of new sources of NA is an important feature. Progenies from strawberry crosses having *F. virginiana glauca* (FVG) as a common parent showed a significant increase in their fruit quality and nutritional features, thus confirming the interest of the wild species to improve cultivated strawberry for several characters. The highest TAC and TPH combined with high quality standards were identified progenies originated from the following inter-varietal cross combinations: AN94.414.52 (FVG F1) x 91.143.5 > Cifrance x Patty > Sveva x Patty.

The genetic background in NA modifications, was also studied in strawberry fruit from genetically modified plants for two different genes involved in auxin and cytokinin metabolisms and plant development (*DefH9-iaaM* and *rolC* respectively). The increase of auxin endogenous metabolism induced in strawberry fruit – achenes by the expression of the *DefH9-iaaM* resulted with a positive effect in increasing plant productivity without altering berry quality and



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nutritional value. The increased plant cytokinin metabolism induced by the expression of *ro1C* gene had an effect on plant development (increased vigour and adaptability), but also lead to an improvement of fruit nutraceutical background. New ongoing studies are now finalized to study the effect of the expression of the DFR (dihydroflavonol 4-reductase) in improving fruit quality (colour) and TAC of GM Sveva.

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### **THE CRA-ISF STRAWBERRY BREEDING ACTIVITY: FRUIT QUALITY AND HEALTHY COMPOUNDS.**

Gianluca Baruzzi, Sabina Magnani, M. Luigia Maltoni, Marta Ranieri,  
Maura Bonoli, Walther Faedi.

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Public strawberry breeding programs, started in Italy 40 years ago, released several varieties of commercial importance for northern areas (Po Valley). Their major objective was to obtain new dessert cultivars characterized by high yield, large fruits and resistance to the main pathogens. Now, particular attention is devoted to improve fruit taste (good balance of sweetness and acidity, flavour and juisness) and recently, to the enhancement of the content of antioxidant compounds (ascorbic acid, phenolic acids, flavonoids, anthocyanins, carotenoids). Strawberry considered a source of many bioactive phytochemicals in the human diet: ellagic acid is known as a naturally occurring dietary antimutagen and anticarcinogen; ascorbic acid and phenolic acids are antioxidant compounds, which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease. There is a correlation between antioxidant activity and total phenolic content, however, no information is available on the effects of environmental factors such as cultural system on the scavenging capacity of strawberry.

The objective of the present study is to evaluate antioxidant activity in Italian strawberries and the effect of two different cultural system (integrated pest management and disease control – IPM and organic cropping system) on the content of nutritional compounds and fruits quality of three varieties (Alba, Onda and Queen Elisa). Cultivars compared for three years, were grown in Cesena area (North farm of Italy) in two different cultural systems. Cold stored plants were planted in the open field, according to four replications randomized block design and during the fruiting season, commercial production, total production, average berry weight, earliness index were recovered. Moreover twenty ripe fruits were harvested from each cultivar and were used for quality traits (skin colour, firmness, skin resistance, soluble solids content, titratable acidity, nitrates content) and healthy analysis (antioxidant

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capacity - TEAC, total polyphenols, ellagic acid, ascorbic acid content). Organic culture showed the lowest plant yield, average berry weight, and strawberry fruits had the highest soluble solids content, acidity and health-promoting compounds. These results could be used to improve market value of the strawberry organic culture.

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### **BREEDING PRIMOCANE-FRUITING RED RASPBERRIES FOR MILD WINTER CLIMATES**

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Raspberries can produce fruit all year round in Portugal using different growing systems. During the last twenty years, we have developed production systems to obtain high quality berries for the European fresh market. Autumn-winter is the most profitable season for raspberry production due to high prices in the European market.

In southern Europe low input productions systems have been developed in order to keep production costs low and assure a good market advantage. However, this approach led to a significant loss in yield mainly during January to March. In these months light sum and average temperature are low for raspberry growth and development. New genotypes are needed in order to adequate raspberries plants to these environmental conditions.

Current varieties are not adapted for Mediterranean climate (low-chilling) and no breeding line has low-chilling as main goal. In order to maintain our advantage in the market we need to develop a new breeding program in order to overcome this difficulty. We have been able to create an integrated team who are experienced in raspberry production systems, cultivar evaluation, floral biology and controlled crosses in other fruit crops (walnut and chestnut). The success of a breeding program depends on a cultivar selection and basic knowledge of flower specie morphology. Controlled crosses involves multiple factors such as an efficient emasculation and pollination during pistil receptivity in order to obtain seed formation. A new breeding program can be started with the main focus on early primocane-fruiting raspberries which should be spineless, low-chill and, short-day adapted. Raspberry varieties must also have high yield with high quality berries and be resistant to spider mites and late rust.

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### **LIGHT EFFECTS ON FLOWER BUD INITIATION IN RED RASPBERRIES (*RUBUS IDAEUS* L.)**

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Red raspberries produce biennial canes which usually initiate flowers in their first year of growth, under short days and low temperatures. However, primocane-fruiting types will initiate flowers independently of daylength and temperature, once they have reached a certain physiological maturity. Juvenile canes cannot be induced to flower, but in canes above 30 nodes, one week at 10°C with 9 hr days, will induce all genotypes to flower.

We can divide red raspberries in three flowering habits: primocane-fruiting, tip-fruiting and summer-fruiting varieties, where the first and second group flowers and fruits on first year canes and the third group only flowers on second year canes. There are different confusing results in the literature about flower bud initiation regarding these three genotypes. Some researchers postulated that buds of primocane-fruiting genotypes are only daylength insensitive at the tip of the canes, and that buds on the lower part of the cane initiate flowers only in short day conditions. Although our experiments suggest that flower bud initiation is a process that occurs down the cane and not only at the tip. Light intensity can also affect flower initiation in raspberries as observed in ornamentals were low light conditions prevented flower initiation in geranium, chrysanthemum and Spathiphyllum.

In order to clarify the main factors that affect flower bud initiation an experiment is under way with three red raspberry genotypes (Tulameen, a short day cultivar, Glen Lyon, a tip-fruiting short day cultivar and Autumn Bliss, a primocane-fruiting cultivar), three light treatments (ambient day-length and light intensity, long days and ambient light intensity, and long days and 30% shade) in three seasons (planting dates: 21 March, 7 May, and 21 June).

To establish if flower bud initiation has occurred, buds are being collected from each treatment at specific intervals, accordingly with the phenological stage. At each sampling date, every third bud from the



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bottom to the top of two canes per plot will be removed, preserved in 50% ethanol and dissected under a low power microscope.

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**MOLECULAR ASPECTS UNDERLYING FLOWER BUD  
DIFFERENTIATION IN RASPBERRIES**

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There are many reports about flowering-related genes such as LEAFY, and FLOWERING LOCUS T in *Arabidopsis thaliana* and in other plants than raspberries. These genes are considered as floral pathway integrators, as they are regulated by more than one flowering pathway and their action influences how the flower bud forms in the shoot apex. To investigate flower induction in raspberries and how it responds to environmental factors (light, temperature, root buds), morphological changes will be monitored. Also, how these genes are expressed will be analysed under different production systems so that suitable markers can be identified.

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### **MICROGRAFTING AS A MULTIPURPOSE TOOL IN PLANT PRODUCTION**

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The conference will deal with the possible applications of micrografting to plant production. These include rejuvenation (especially through serial micrografting onto juvenile rootstocks) and production of clonal plants of adult material, obtention of virus-free plants, virus indexing of plant stock, germplasm exchange, overcoming of incompatibility problems, study of graft incompatibility and breeding of specific genotypic combinations. In addition, microshoots that are difficult to root, or do not form roots at all *in vitro* can be micrografted onto rootstocks to obtain rooted plantlets.

As described by Jonard (1986), micrografting consists of grafting of a shoot apex from a mother plant or a microshoot onto a young plant grown in the greenhouse/nursery, or onto a decapitated seedling under aseptic growth conditions. In any case, some variants of the method do not correspond exactly with this description. For example, the grafts can be kept in a growth chamber to accelerate graft-take, and scion development. In other cases, scions other than shoot apex can be used. Furthermore, grafting can be undertaken onto adult rootstocks.

The success rate of the micrografts is influenced by several factors, as the type of graft used (side grafting, cleft or split grafting, horizontal grafting, etc), the size of the scion (from a meristem to a shoot apex or a nodal explant), the culture medium, the cultural conditions in which the scions were grown and the time of the year at which scions are obtained.

In the case of berries, which are susceptible to infection by a number of virus, the use of micrografting for virus indexing of plant stock *in vitro* is of interest. Scions could be grafted onto virus-free indicator rootstocks growing in tissue culture. In any case, other assays, as ELISA, should be undertaken to validate the visual symptoms. The



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method could also be used in conjunction with other methods for post-entry quarantine to identify infected material in the short term. Whenever it was necessary to initiate multiplication from infected material, thermotherapy and/or meristem grafting could allow to obtain virus-free material.

At the conference, all these items will be treated more in detail, and some practical examples of application of micrografting will be discussed.

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**NEW CULTURAL PRACTICES IN THE POST METHYL BROMIDE ERA  
IN HUELVA (SPAIN)**

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Strawberry growers have been dependent on using methyl bromide (MB) to control both insect pests and fungal diseases in the soil before planting. The loss of the major soil fumigant after 2005 (or after 2007 as critical use) has created a need to search for alternative fumigants or new and modified production systems such as: integrated production, biological control, organic production, soilless growing systems, etc.

The removal of MB has acted as a catalyst for the widespread and ongoing development of new and modified technologies. The goal is to achieve a more sustainable efficient and environmental-friendly growing system for strawberry production.

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### **STRAWBERRY SUSTAINABLE PRODUCTION SYSTEMS CONCERNING CONSUMER'S SAFETY**

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Strawberry is one of the high-value horticultural crops in the world and the major crop among the berries. Several production systems have been developed allowing the fruit to be available all the year round.

The increasing demand for strawberries has led to the development of practices that many consider unsustainable, such as monoculture systems, soil fumigation dependency (methyl bromide), and intensive use of pesticides and waste of water. The need for a change of the present practices to more sustainable management has slowly been taking along among policy makers, growers and consumers. Integrated crop management (ICM) and organic production systems are increasing, as alternatives of conventional production systems and also as response to consumer's safety and preferences.

A R&D project, in Portugal, was carried out during 4 years to evaluate and demonstrate the economical and technical viability of using strawberry integrated production systems in order to maintain yields and to obtain high quality fruits. Concerning the phase out of methyl bromide (MB), the effects of chemical and non-chemical preplant soil disinfections were evaluated on soilborne pathogens control and on crop productivity of cultivar Camarosa, grown in annual hill system. Experiments were conducted on fields subjected to monoculture and to crop rotation systems.

On fields subjected to strawberry monoculture system, soil disinfection revealed to be essential to control soilborne diseases and to improve crop productivity. In addition, raised beds solarization with black polyethylene was not also so efficient to control soilborne diseases as standard solarization with clear plastic (SSC). On fields subjected to crop rotation, strawberry production in SSC and metam sodium plots achieved plant growth and yields similar to the MB plots. The implementation of IPM as alternative to chemical control (CC) was

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satisfactory but might be insufficient in monoculture systems without soil disinfection.

These results indicate that strawberry sustainable production systems such as ICM are viable, with crop rotation inducing plant growth and yields comparable to those of conventional production systems.

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**DOUBLE CROPPING OF BLACKBERRIES IN GREENHOUSE  
PRODUCTION**

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The blackberry cultivar Loch Ness is very high yielding. If growing conditions are favourable Loch Ness develops after the primary laterals, secondary and even tertiary fruiting laterals. This observation led to first trials in 2000 to 2003 to delay harvest in a seasonal crop under rain shelter by pruning the laterals at different stages. Harvest could be delayed by 15 to 25 days with 10 to 25% loss of yield. This resulted in a less profitable culture. Further delay of the crop by cutting back the laterals later was not possible because fruit would not ripe under rain cover. Therefore the system of pruning laterals was tested on plants of an early production in a heated greenhouse. The results of 2005 and 2006 will be presented.

The laterals were pruned behind the last berry. In 2005 the laterals were cut back at three different points in time: 1. All laterals pruned at the end of the first crop (18<sup>th</sup> of July). 2. All laterals pruned on the 30<sup>th</sup> of June. 3. Laterals with maximum one berry weekly pruned starting the 6<sup>th</sup> of June. The second crop was compared with the yield of plants cold stored until the 19<sup>th</sup> of July. Total first production was 7.0 kg/m<sup>2</sup>. Earlier pruning reduced the crop with 9%.

Second crop started for all treatments the same week but 50 % harvest was up to 24 days later when laterals were pruned at the end of first harvest. The second yield of the first treatment was 3.3 kg/m<sup>2</sup>. Cutting back the laterals on the 30<sup>th</sup> of June resulted in the highest second production, 5.43 kg/m<sup>2</sup>. The yield of the cold stored plants was similar to the production of the plants pruned at the end of the first crop. The plants were harvested until the third week of December. After harvest we counted the number of unripe fruits per m<sup>2</sup>. The first treatment had 263 unripe fruits/m<sup>2</sup> which is a potential of 1,8 kg/m<sup>2</sup>. Treatment 2 had

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123 unripe fruits/m<sup>2</sup> (0.8 kg/m<sup>2</sup>) and treatment 3 133 berries (0.9 kg/m<sup>2</sup>).

Fruit quality of the second crop of the pruned plants was compared with fruit quality of the cold stored plants. Fruit size and fruit weight were less (3 mm and 1,1 g) and firmness of the berries was similar. The fruits of the pruned plants had slightly more individual discoloured drupelets.

In 2006 the trial was repeated on plants brought in the heated greenhouse on the 23<sup>th</sup> of December 2005 and the 5<sup>th</sup> of January 2006. The aim was to have an earlier first crop and loose less yield when pruning early. There was no difference in harvest period and the reduction of the yield was similar for the two starting dates. The tendency in the results of the second crop was very similar to 2005 although the cold stored plants were always yielding higher.

These results seem to be consolidated in the trials of 2007. This gives the possibility to replace the more expensive late crop with cold stored plants by pruning of laterals and have two crops in one year of the same plants. At this moment a few hectares are practiced.

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### **SMALL FRUITS, QUALITY AND ANTIOXIDANT CAPACITY RELEVANT TO NUTRITION**

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The small fruits (raspberry, strawberry, blueberry and blackberry) are gaining ever greater space in the consumer market in Portugal. There is a great diversity of produces derived from fruits and new produces for consumption are launched constantly, often without the necessary research into their activities properties and beneficial activities to health.

We are developing our activity in order to evaluate the behaviour of small fruits and the influence of harvest date and shell life mainly through quality parameters (colour, texture, microbiology, sensorial analysis and structure). The antioxidative capacities of these species will be investigated by ferric reducing antioxidant power (FRAP) and trolox equivalents (TEAC) in order to support new and agronomic production for improvement of nutritional information to the consumer.

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**SEARCH FOR PLANT PHENOLIC COMPOUNDS EXHIBITING  
ANTIOXIDANT ACTIVITY AND NEUROPROTECTIVE PROPERTIES  
IN A NEURONAL CELL MODEL OF PARKINSON'S DISEASE**

Disease and Stress biology Lab Team: Ricardo Boavida Ferreira, Cláudia Nunes dos Santos, David Barata, Dina Carrilho, Lucélia Tavares

Collaborations: Paula Alves (Technology of Animal Cells Lab, ITQB/IBET); Catarina Duarte (Nutraceuticals and Delivery Lab, IBET); Pedro Oliveira (Department of Agriculture Production, EAN); Astrid Vicente (Biopathology Centre, INSA); Dalila Espírito Santo (Ajuda Botanical Garden, ISA)

Dietary antioxidants have been reported to possess potent radical scavenging, iron-chelating, and anti-inflammatory activities. Among the various bioactive compounds, many plant extracts are particularly rich in flavonoids, a family of polyphenols found in fruits and vegetables, as well as in plant beverages, including tea, pomegranate juice, raspberry, blueberries, and red wine.

Such compounds seem to have a high antioxidant capacity in animal cells. Therefore, they may exhibit neuroprotective activity, since neurodegeneration in Parkinson's and other neurodegenerative diseases involve a complex set of oxidative reactions. These reactions lead to neuronal death and dysfunction of the ubiquitin/proteasome system with formation of inclusion bodies as a commonly observed pathology.

Parkinson's disease requires multiple drug therapy to address its varied pathological aspects. Nevertheless, the progressive neurodegeneration does not halt/slow down with the currently available strategies. Finding new therapies, preferentially natural brain permeable products, including food supplements which could help in preventing/delaying the ongoing neurodegeneration in Parkinson disease, is an important issue to be addressed.

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Our goal is to understand the effect of plant polyphenols with high antioxidant activity and to study their effect in a Parkinson's disease cell model. The polyphenol composition from tissues of Portuguese endemic plants of *Rubus* sps and their *in vitro* antioxidant capacity will be addressed. Subsequently, a toxin-induced model of neurodegeneration/Parkinson's disease in neuronal cells will be used to study: (i) cytotoxicity; (ii) intracellular radical scavenging; (iii) formation of inclusion bodies and protein metabolism, with particular emphasis on the ubiquitin/proteasome pathway; (iv) gene transcription alterations (by cDNA microarray analyses) induced by the administration of selected polyphenols to the cells.



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## **THE IMPACT OF SOFT FRUIT CONSUMPTION ON HUMAN HEALTH**

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There is an increasing interest in, and scientific support for, the beneficial effects of plant, and more specifically fruit, natural compounds. Many biomedical and clinical studies have been undertaken to support the hypothesis that fruit polyphenolics can reduce the incidence and/or initiation of a range of chronic and degenerative diseases such as atherosclerosis, heart disease, cancer etc. We will present an overview of this evidence and more specifically highlight the studies of SCRI focussed on the beneficial impact of soft fruit: blackcurrant and raspberry.



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