The sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries

Final Report Summary - EUBERRY (The sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries.)

Executive Summary:
The overall strategy of the EUBerry has been delivered through the activities of four RTD (Research and Technological Development) work packages (WP1 to 4), with additional dissemination (WP5) and management (WP6) work packages. Each WP had clear objectives (O); to achieve these objectives defined tasks were performed and verified by measurable deliverables (D) and milestones (M).

In WP1 this multidisciplinary project applied the most recent technical advances in evaluating, selecting and obtaining new genetic material with improved adaptability to cultivation conditions and systems, and exhibiting higher quality, nutritional quality and nutraceutical (human health benefits) value of the fruit. Existing breeding programs and molecular studies were integrated with the aim to combine modern genomic tools for improving breeding efficiency, specifically for genes controlling plant adaptation and fresh fruit quality. Specific and well-addressed functional studies were also used to validate, at least in strawberry, the role of genes controlling plant flowering and fresh fruit quality. The new genetic material and knowledge generated in WP1, (mainly by P1, P2, P3, P5, P6 and P13), has been transferred and used in the research programs
expected for WP2 and WP3. Data on productivity and quality of the new material were used to study the economic competitiveness (WP4) of the new cultivation systems and for scientific and technical dissemination (WP5).

WP2 optimized the integration of partner competences to develop new cultivation techniques to reduce inputs in berry production, extend plant adaptation to different and changing climatic conditions, expand production season and optimize berry quality. To guarantee high quality out-of-season berry production in south, center and north of Europe, research into plant material (type of plants and conditions of production), planting date, type of tunnels, etc. were performed. Studies focusing on plant physiology and plant adaptation/tolerance of abiotic stress caused by climate change, were performed. The reduction of environmental impact were addressed via the comparative assessment of high and low input systems (reduced water, nutrients, pesticides), as well as research on pest management by bio-control and other sustainable methods.

The focus of WP3 was to develop a set of tools to analyse and select fruits with optimised quality (organoleptic, nutritional and bioactive components). A more extensive approach was developed on strawberry, raspberry and blueberry and have been applied in a truncated format in other berries (currants and blackberry). The improvement of internal and external fruit quality was achieved for the standard sensorial quality traits. State of the art high-throughput, metabolomic, gas chromatography (GC) and liquid chromatography – mass spectrometry (LC-MS) technologies was used to maximise analytical data collation while minimising analysis time. Pre- and Post-harvest treatments were tested to improve shelf-life of strawberry, raspberry and blueberry and maximise stability of fresh fruit quality, nutraceutical and nutritional value. The effect of berries on consumer health was validated by testing and cross-comparing the fruit material showing the best performances with in vitro human cell models, known to represent human degenerative conditions.

The focus of WP4 was to evaluate how the new genotypes (WP1) and cultivation systems (WP2) developed in this project are able to optimise berry economic competitiveness and the data (in particular from WP3) were used for the development of a science-based marketing strategy to promote health benefits of berries and increase their consumption. A dissemination plan was developed in WP5 by considering a proper and logical integration of scientific, technical and public dissemination of the results generated by the project. The overall management of the project (WP6) involved all members of the consortium. It formed a pivotal link between EUBerry project activities and the Commission, society, consumers, industry and public stakeholders and end-users. In addition, WP6 oversee research, administration and financial management, IP issues and outreach related to the whole project. Key aspects of WP5 and WP6 made full use of modern technologies and recent developments.

Project Context and Objectives:
Berry market globalisation means growers need to maintain highly standardised production systems whilst sourcing and maintaining high levels of genetic resources. Berries should have a high sensory and nutritional value and be marketed as safe, produced using a minimum of pesticides. Accurate and substantiated information is needed so that consumers understand the health benefits and can make informed choices.

With this in mind, the EUBerry project provided the knowledge and tools needed to facilitate development of high-quality, consumer-desirable fresh berry fruits of high nutritional quality, optimal for human health and at a competitive cost. The team also developed and validated a set of tools to improve the competitiveness of European berry production and consumer accessibility to berry fruits.

Strawberries, raspberries and blueberries were used as model crop species, while currants, blueberries and blackberries are also on the table in the context of improving berry fruit quality and reducing production costs. This project applied the most recent technical advances to improve the sustainability of berry production and increased quality and quantity of fruit. These activities were addressed to provide to growers new cultivars and growing systems that secure high quality fruit grown under environmentally safe and economically viable conditions. Therefore, the research was focused on the improvement of berry taste, nutraceutical and nutritional quality, and plant adaptation to different climatic conditions and cultivation systems. The reduction of environmental impact (reduced water, nutrients, pesticides etc) was also amongst the aims of this project. Finally, the fresh market situation, the economical sustainability and efficiency of new cultivars and cultivation systems, the marketing strategy for new type of products, were also studied. A summary description of the project context and objectives is following reported for the 4 research and technological development Work Packages.

WP1 Improving berry varieties through the identification and utilisation of the best genetic resources
The integration and collation of phenotypic data for the main berry fruit genera was progressed through trans-national trialling and assessment by the individual partners. The online database produced as deliverable D1.1 contains a diverse range of
cultivars and advanced breeding lines from 9 partners, and provides a unique resource for the identification of the most appropriate germplasm for use in each of the climatic regions of the EUBerry partners.

Further to the collation of phenotype information, separate sub-groupings were developed in this WG specifically for breeders (D1.2) and growers/retailers (D1.4). These sub-groups, presented as databases with full online access, can enable these user groups to easily identify the most appropriate germplasm, including advanced breeding lines, for specific purposes. The breeding database gives potential parental material for yield, quality components and pest/disease resistance, to inform the breeding strategies that will deliver the new cultivars needed for the future, while the grower/retailer database gives a clear indication of the best material that is available now.

The development of marker-assisted breeding strategies was progressed in strawberry, raspberry and blackcurrant, and several putative markers for key traits were identified. These are now in the validation stages for future deployment in real breeding populations, providing collectively a major advance in the molecular breeding of berry crops. In strawberry, the marker work was focused on flowering traits, which are of fundamental significance in this crop, and markers with potential utility in the selection of everbearing strawberries were identified. Additionally, fruit quality markers were identified for both colour and volatile content, and significant steps were taken in the identification of markers linked to resistance to strawberry mildew, Verticillium wilt and Phytophthora cactorum. In raspberry, the emphasis was mainly on ripening season, berry size, fruit firmness and compositional traits, and in each case putative markers have been identified and are currently at the validation stage. For blackcurrant, the project’s focus on fresh-market traits led to the identification of putative single nucleotide polymorphism (SNP) markers for the key characteristics of berry size and anthocyanin content, but in collaborative work between partners P2 and P3, these putative markers were not fully convincing in diverse mapping populations, although associated with the relevant quantitative trait loci (QTL). Although further work using populations from P2 is scheduled for 2015, new markers linked to other quantitative trait loci associated with these traits will also be included, through continued collaborative work. The marker work for all crops was collated into a summary of markers available for each trait in the crops, and this is available online to future users.

Functional genomics work on strawberry quality led to the identification of 21 genes differentially expressed in lines with diverse content of ascorbic acid, and expression of quantitative trait loci (eQTLs) will be mapped within the population used for this work. A subset of these differentially expressed genes is also under evaluation using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Similar work on the aroma compound γ-decalactone in strawberry identified a single candidate gene controlling biosynthesis, and validation is in progress. Transgenic work to overexpress the key anthocyanin biosynthetic gene anthocyanin synthase (ANS) provided a range of lines showing differential expression profiles, confirming its role in the accumulation of anthocyanins and offering alternative means for future enhancement of this trait in strawberry.

WP2 Improved cultivation techniques
For high quality planting material, amount and timing of nutrient supply can be used to modify strawberry plant architecture in the nursery. Reduction of especially Nitrogen (N), can be used to manipulate the reproductive behaviour and plant quality. To extend harvest season, use of perforated plastic cover and fibre cover in the early spring is beneficial for early yield of raspberry plants. In the south, it is possible to extend production season of blackberry by double-cropping. With two harvests productivity is increased and season extended for the high fruit market price. Blueberry production can start as early as March with SHB (southern highbush) cultivars in the South and it can be delayed to late August with rabbiteye blueberries. Or protected cultivation, relatively simple improvements to the fittings of the tunnel, can have very significant effects on the length of growing season. Frost is most effectively delayed by limiting night-time ventilation and deploying an energy screen. A fog system will ensure maximal length of the spring growing season. Light emitting diodes can be used for supplemental light in tunnels for strawberry and raspberry in the North but to have full benefit, heat should be supplied from late September.

Climate change can increase risk for spring frost damages on berry crops. A technical innovation was developed to control spring frost with low water consumption. Studies on raspberry physiology showed that in floricanke raspberry cultivars flower initiation continues until the end of October/beginning of November. Limitation of net photosynthesis by extreme and constant high temperature reduce strongly yield and fruit quality. Still a good yield can be achieved under the high tunnel with a filter blocking UV B light, while the UV B window film could reduce the yield. The results indicate that a screening of cultivars regarding their adaptation to higher temperature seems to be necessary when increasing use of high tunnel production and of the predicted climate change. Erratic bud break and less consistent fruit yields may be expected in raspberry crops in the wake of ongoing climatic warming with declining winter chill. Inadequate winter chill may become a limiting factor for successful raspberry production in areas with mild winter climates. Insufficient chilling time is likely to limit the yield
performance of early forcing of long-cane raspberry crops in the greenhouse.

To reduce environmental impact of strawberry production and chemical residues in fruit, biological control of thrips can be enhanced by predatory mites and the predatory bug Orius majusculus. Alternative cultivation systems by application of mulching in combination with natural enemies can sharply reduce the use of chemicals to control thrips. On raspberry, repeated introductions of Neoseiulus cucumeris and Amblyseius swirskii can keep the Phylloxeropsis gracilis population on raspberry at an acceptable level during the whole season and Phytoseius persimilis effectively controls T. urticae in the tunnel. By using Decision Support systems in strawberry powdery mildew control in open field, the level of residues could be reduced with 50-75% compared to common practice. In greenhouse, UV-B light can be used to control powdery mildew infections.

In studies for drought tolerance, useful genotypic differences on strawberry could be observed by measuring the leaf gas exchange parameters. To reduce environmental impact of soilless strawberry cultures, nitrogen supply can be halved to prevent excessive nutrient consumption and leaching. Strawberry cultivars also showed different efficiency in using nutrients. As a renewable substrate, coconut coir can be used to replace peat in soilless cultivations of strawberry, stimulating the formation of more roots without effects on canopy growth, introduction of mycorrhizae can improve plant performances and bigger pots improve fruit production. It is also possible to reduce the level of nutrient input on raspberry compared to the common growing technique, without any reducing of production and fruit quality. Although raspberry is considered to be sensitive to salinity, it can tolerate suboptimal water without significant crop losses.

WP3 Fruit quality characterization and determination

The key aim of EUberry was the sustainable improvement of European berry production, quality and nutritional value in a changing environment. To do this there was an absolute requirement to establish the basal values and ranges of sensory and nutritional/health beneficial components the target fruit were across the EU as represented by the partners. To ensure reproducibility across the project, and indeed the EU fresh (and processed) fruit sector several standard operating procedures were developed (D3.1) and circulated across the consortium and to their associated networks in academia and industry. This approach encompassing simple parameter and more in depth ones, such as multiple-polyphenol characterisation, were then applied to the gamut of fruit available to, and generated by the consortium. This wealth of quality and nutritional/nutraceutical data (D3.4) highlighted the significant range important quality attributes already available to the consumer. Allied to this were the nutritional and health beneficial components that exhibited and equally broad but more objectively categorised variation, often up to 300-400%. From the perspective of the consumer and retail sector. This is desirable as it allows diversity with regard to product choice for the former and the ability to have unique selling points and product points of distinction for the latter facilitating economic growth. Indeed, the ability to actively manipulate this variation without resorting to the route of breeding was explored in some detailed by studies into pre-harvest crop manipulative strategies (D3.2). Trials on strawberry with commercial and emergent/experimental natural, non-toxic (to humans) compounds called Resistance Inducers (RIs) highlighted their utility with induce significant high levels of resistance to decay by to Botrytis cinerea, Rhizopus stolonifer and Penicillium spp decay. Indeed, in some cases this resistance equalled that of current common commercial fungicides. The mechanism of action of these Resistance Inducers rests with increased polyphenol production which means that the potential to both maintain and enhance quality in terms of microbial decay and composition are now possible. D3.4 was the basis of the many research streams into sensory and health beneficial studies, following the establishment of the analytical standard operating procedure (SOPs) (D3.1). With partner derived material and that generated from the WPs 1 and 2 the level of characterisation associated with D3.4 was significant and generally the basis of the many published papers, presentations and stakeholder. In the first year there was a concerted effort to collate fruit and to get all the partners up to speed with the SOPs to ensure cross project comparability of data and to further ensure the quality of the data for interpretation and publication.

Often aroma is a potent driver of purchase and research was done to characterise this in raspberry and to correlate this with sensory score. Using an solid-phase microextraction-gas chromatography - mass spectrometry (SPME-GC-MS) approach a detailed chemo-profile was established for the 14 lines assayed (known to have polyphenol and phenotype variation; 14 lines). This highlighted that often the more complex aroma volatile profile correlated with a sensory score of desirable. The statistical basis of this and the phytochemical driving this are still under analyses but the results will be fed back to the partner breeding programmes.

Simply having “good quality” fruit is not enough in this commercially competitive world. The fruit, once harvested, needs to be resilient across the supply chain meaning an ability to survive storage and packaging. New methods for this were explored
and the potential of hypobaric storage assessed in relation to ambient and controlled atmosphere systems (D3.5). Initially, studies in strawberry were promising in terms of sensory maintenance but more extensive studies found that in general hypobaric storage gave similar gross phenotypic results as the other strategies but that there were differences in composition leading to flavour maintenance. Extension of hypobaric studies in combination with the packaging system Xtend® highlighted that varietal and species dependency with the combined system for raspberry identified as particularly good for raspberries. Finally, the public perceive fresh fruit as having a “health halo” and the evidence based studies undertaken here support this (D3.3). Validated in vitro digestions (IVDs) of the fruit identified the impact on the health beneficial components and this was extensive and different for each fruit. These IVD extracts were then applied to cell models which highlighting the low toxicity of the fruit digestates at dietary levels. Furthermore the same studies identified the fruit digestates’ abilities to down regulate inflammatory pathways and whilst beneficially modulating the genes (and gene controllers) related to aspects of intestinal barrier function and Crohn’s disease, and golgi protein folding giving the potential for routes to reduce the impact of e.g. Alzheimer’s. With respect to neurodegeneration, cell models highlighted the ability of the fruit digestates (serum available components) to attenuate neuroinflammation and inflammatory markers, NO (nitric oxide) and tumour necrosis factor (TNF), at varying degrees. Further extension of the health beneficial work to human intervention studies resulted in the characterisation of the fruit phytochemicals’ molecular fates with some of the metabolites putatively strong markers of specific fruit intake. Another intervention study with strawberry intake highlighted that it possesses valuable properties such as counteracting exogenous oxidative stresses and improving plasma lipids profile, antihemolytic defences and platelet function in healthy subjects. All of these are supportive of a positive impact on the risk of cardiovascular diseases (CVD).

WP4 Competitiveness and marketing strategies
In this work package calculations are made of the economic viability of new production methods, the initial market situation for fresh berries in selected European countries is described and marketing as well as effective marketing strategies are developed together with SME’s (small and medium enterprises). The economic calculations are made both at the start of the project (ex-ante) in order to give the technical research a research guide of the critical economic factors for successful innovations and at the end of the project (ex-post). This ex-post calculation for strawberries together with the results of the initial market situation and the marketing strategy leads to the following conclusions:

• The economic calculations based on the technical results show promising opportunities. At this moment, these innovations for strawberry production do not lead to a higher marginal gross margin for the growers. However, these innovations have positive effects on the licence to produce and licence to deliver (less residues, longer shelf life).
• The structure of the production chain with many small growers leads to extra challenges in the chain. For example with uniformity of quality and quality itself, due to the need of an unbroken cold chain.
• Health is not a single issue to be communicated to consumers. Besides EFSA (European Food Safety Authority) regulations that restrict health claims, some consumers groups will not respond on this. A targeted communication message towards health, fun and or completed with food safety might be more successful.
• The 4 C’s strategy led to a structured approach of a tailored marketing strategy for both SME’s.
• A strategic agenda with a multi-actor as well as multi-disciplinary approach is needed.

All the activities carried out during the 42 months of the EUBerry project had a high scientific and technical impact as demonstrated by the 40 scientific papers published on peer reviewed journals, 15 technical publications and many dissemination documents, published even in different languages, 3 newsletters, different podcasts, 18 dissemination and technical meetings with different stakeholders. The partners of the project presented the results (with oral and poster presentation) in more than 50 international and national conferences and symposium. Two well-frequented berry schools were also organized. All these activities were coordinated thanks to the organization of a kick off meeting, 3 general assemblies and about 14 group meetings. An intense organizational activity was also carried out by email and with web conferences.

Project Results:
WP1 Improving berry varieties through the identification and utilisation of the best genetic resources
The work within WP1 is based on the integration of phenotypic data either already existing or collected for the 4 main crop genera (Fragaria, Ribes, Rubus, and Vaccinium), leading to the development of linkages between desirable phenotypes for fruit quality and developmental traits, and genotypes that can be selected by breeders at the earliest stages using appropriate
The approaches used differed in each crop, due to the diversity of genetic structure and differences in pre-existing resources between the crops. Additionally, work in strawberry on the development of functional genomics approaches relating to flowering and quality traits was advanced during this project.

The overarching structure and delivery from WP1 begins with the evaluation of the best germplasm currently available within the EUBerry partnership, through multi-year and multi-site phenotyping (D 1.2). This has enabled us to identify the most suitable germplasm for EU berry growers at the present time (D 1.4) but has further allowed us to identify appropriate breeding strategies, utilising the best breeding germplasm (D 1.2). The advances we have made with molecular markers and other contemporary approaches, including functional genomics (D1.3, D1.5) can support breeding of new cultivars beyond the EUBerry project. This approach will overall increase the efficiency of production of superior berry fruit cultivars within the EU, especially relating to key issues such as sustainability and environmental resilience.

D1.1 Fully integrated Databases for each genus incorporating pre-commercial germplasm
A range of berry fruit germplasm was assessed in 9 locations throughout the project, covering the main climate zones within the EUBerry partnership. Data on both agronomic and fruiting traits were collected, and this was collated by P2 to form a large database capturing the positive and negative attributes of each genotype. The total numbers of genotypes included in the assessment were as follows:
- Strawberry – 57 cultivars, 20 breeding lines (assessed in 6 locations)
- Raspberry – 26 cultivars, 14 breeding lines (assessed in 5 locations)
- Blackcurrant – 41 cultivars, 14 breeding lines (assessed in 4 locations)
- Blueberry – 10 cultivars, 10 breeding lines (assessed in 3 locations)

The assessments were made during the period of the EUBerry project up to the 2013 harvest. Efforts were made to include information from previously developed databases, namely RIBESCO (for currants) and GENBERRY (for strawberry). However, the latter had only limited utility, as it is focused on botanical rather than agronomic/production-based descriptors. The inclusion of RIBESCO data (P7) was based on a variety trial planted in 2009 as part of COST 863, and these data included agronomic, phenological and fruit quality information.

The database has been uploaded to the web in fulfilment of D1.1 (http://www.euberry.univpm.it/node/23) but further development and refinement may be possible in the future. In particular, some of the laboratory-based quality assessments can be standardised to a common value. The utility of the database for growers, breeders and researchers is based in part on the inclusion of cultivars that are grown in more than one climatic region, and also the inclusion of breeding lines gives a clear indication of the future directions in available germplasm. This latter information has been captured within D1.2 Integrated sets of characterised germplasm for strawberry, raspberry, blackberry, currant and blueberry across the climatic zones of the EU as a core collection to be used for breeding programs. A subset of data from the D1.1 database was extracted by breeders within the EUBerry partners and combined with information residing within the various partners, with breeding interests, with the aim of defining the best material for breeding across the EU regions. This breeding database also incorporated other numbered breeding lines of potential utility that are unavailable elsewhere in EUBerry, in the pursuit of key traits for new cultivars. The database was divided into crops sectors, and further divided into regions:
- Southern (Italy, Spain, Portugal)
- Central (France, Poland, Germany)
- Northern (UK, Finland, Norway)

D1.2: Integrated sets of characterised germplasm for strawberry, raspberry, blackberry, currant and blueberry across the climatic zones of the EU as a core collection to be used for breeding programs
Further to the collation of phenotype information, separate sub-groupings were developed in this WG specifically for breeders (D1.2) and growers/retailers (D1.4). These sub-groups, presented as databases with full online access, can enable these user groups to easily identify the most appropriate germplasm, including advanced breeding lines, for specific purposes, ie. the breeding database gives potential parental material for yield, quality components and pest/disease resistance, to inform the breeding strategies that will deliver the new cultivars needed for the future, while the grower/retailer database gives a clear indication of the best material that is available now.

Care was taken to ensure that all materials included in this breeding database are available to breeders, either as commercial
cultivars or through trialling agreements with the IP holders. The range of traits was more restricted, into broad areas that can then be subdivided by individual breeders according to their requirements. The input of breeders to this deliverable was crucial, with their detailed knowledge of good and bad parental choices. The dissemination of this breeding database is through the web (http://www.euberry.univpm.it/node/23) so that other non-EUBerry breeders can access the information. The overall aims of this work are to:

• improve the breeding populations across the EU regions
• increase diversity of the plant material within many breeding programmes
• improve collaborations between breeders.

The work on blueberry expected for this deliverable was developed directly by P1 and not by a subcontract, this because, as already motivated in the second reporting period, it was not possible to find an organization able to carry out this work as expected in the project.

Discussions are ongoing to determine how the breeding community within EUBerry can keep this and other EUBerry databases current and relevant.

D1.3 Development of marker-assisted breeding strategies for strawberry, raspberry and blackcurrant

Considerable progress was made in the development of molecular markers for key traits, especially those related to fruit quality, and several putative markers are now in the validation stages for deployment in breeding populations in the near future. This work will enable breeding of berry fruit crops within the EU to take significant steps forward in both efficiency and also in the timescales associated with the production of new cultivars. A compilation of the markers, putative and validated, across the various crops was published online (http://www.euberry.univpm.it/node/106) to allow breeders and researchers to gain access to the most recent information about markers for use in breeding.

Strawberry (P5, P6, P3 subcontract, P8). As expected in the project strawberry was the main crop included in this study and different traits were considered.

Development of markers linked to flowering traits (P6).

In strawberry, extension of season production represents an important challenge for increasing production, and therefore focus was mainly on developing markers for the everbearing or perpetual flowering (PF) trait. Using a reference population of Capitola (PF) and CF1116 (June-flowering), we identified a single major QTL named FaPFRU, which controls perpetual flowering in the cultivated octoploid strawberry (Gaston et al. 2013). This locus is not orthologous to the gene affecting perpetual flowering (FvTFL1) in F. vesca, therefore suggesting different genetic controls of perpetual flowering in the diploid and octoploid Fragaria species. The identification of a single QTL suggested that a single female allele controlled the trait. Initially, the closest informative/usable marker were AFLPs, and so considerable effort was made to develop SSR markers that are more easily used. After the initial identification of two new SSR markers that flanked the QTL, further work was done across other populations to validate and obtain other markers.

On testing these putative markers across a range of germplasm, two markers, Bx063 and Bx089, were identified and developed using capillary systems for high-throughput genotyping. The success of the markers in diverse populations was highly dependent on the parental genotypes within the populations: when the June-bearing parents presented the two markers, the potential for marker-assisted selection of everbearing types within the population was reduced. However, when the June-bearing parents did not present the markers, selection of the desired everbearing phenotypes was improved. Our conclusions, at the present time, are that when the PF parents showed the two markers flanking the locus FaPFRU, Bx064 and Bx089, and the June-bearing parents did not. Individuals from the crosses can be selected with an error rate around of 0% for the populations where Capitola is crossed with Junebearing genotypes without these markers, and with an error rate around 20% for the other tested crosses. Where diverse genetic resources were evaluated, all genotypes showing the absence of markers were June-bearing as expected. However, 10 of the everbearing (PF) genotypes showed these two markers. These results clearly showed the need to genotype the parents before using these two markers, but at the same time, the use of the markers can significantly enhance the selection of everbearing (PF) strawberries.
Development of markers linked to fruit quality (P5, P6)
Fruit colour is an obviously important trait for consumers. We tested across diverse genetic resources an SSR marker linked to a Myb-like gene, to evaluate its potential for selection relating to the intensity of red colour. Results showed clear potential for this marker to be used in marker-assisted selection, and as a result it was used in breeding crosses made in 2014 (results of the marker-assisted selection will be known in 2015).

For fruit volatile content, markers were sought for the active compound mesifuran, identified as a key component of fruit aroma in both Fragaria and Rubus, and for γ-decalactone, another important volatile component. Sixty-eight cultivars and species were genotyped and also phenotyped for volatile content. A marker developed from the gene FaFAD1 (an omega-6 fatty acid desaturase) was able to predict γ-decalactone production with an accuracy of over 94%, while a marker from the gene FaOMT (o-methyl transferase) was able to predict mesifuran production with over 93% accuracy.

Development of markers linked to pest and disease resistance (P2, P3 subcontractor, P6, P8)
Podosphaera aphanis Phenotyping was undertaken at both P3 subcontractor and P6 of lines of strawberry with differential resistance, as well as in segregating mapping populations of Redgauntlet x Hapil and Fragaria chiloensis x Elvira. Up to 8 putative markers linked to resistance were identified from the first population, and 5 from the second. It is unclear whether all markers are required for full resistance, or whether there are environmental influences on expression, but further analysis is in progress regarding the deployment of the markers in breeding populations.

Verticillium dahliae The collaboration between the partners was extended to include a comparison of Verticillium dahliae isolates used in resistance screening in the UK and in Poland. Two isolates BPR424 and BPR1320 were sent from Poland to EMR for genome sequencing, and segregants from the Redgauntlet x Hapil population were used to validate wilt resistance markers at P2. Up to eleven putative SSR markers have now been identified, each linked to QTL of small effect associated with resistance.

Phytophthora cactorum. Work on this pathogen by P8 focused on QTL mapping of resistance in the diploid Fragaria vesca. Using a population of Bukammen’ (resistant) x ‘Haugastøl3’ (susceptible), the parents, F1-hybrid and 92 F2-genotypes were phenotyped using the survival test and genotyped by genotyping-by-sequencing. A linkage map was produced and QTL analysis made. This will enable the future identification of linked markers for resistance, and their transferability to cultivated strawberry will then be assessed.

Raspberry (P3).
This berry was expected for this deliverable but only for some traits. With the pre-existing availability of markers linked to resistance to raspberry root rot (Phytophthora rubi), marker-assisted breeding strategies in Rubus are more closely focused on quality and developmental traits. For the latter, the identification of markers linked to ripening season was the main strand of work, while fruit quality was related to berry size (a key variable with both consumer and grower impact), fruit firmness (which impacts on shelf life) and compositional traits, notably anthocyanins (total and individual).

The initial plan was to use an association mapping approach to identify key regulatory genes and linked markers across diverse germplasm, but this approach was not entirely successful, possibly as a reflection of the high heterozygosity of the plant material. Other mapping populations were therefore used, namely the Latham x Glen Moy reference population and Autumn Treasure x Glen Fyne. QTL were mapped, and as the Glen Moy genome sequence is now available at P3, it was used to investigate the sequences underlying the QTL. A series of markers linked to ripening have been identified on linkage groups 2 and 3, with the associated genes, and this information is now in the tables at [http://www.euberry.univpm.it/node/106](http://www.euberry.univpm.it/node/106). Further associations have been identified for fruit firmness, with 4 SSRs now under validation at P3, and for berry size, with an SSR and 3 EST-based markers. The genes underlying the berry size markers were found to be aquaporins, expansins and ethylene transcription factors.

For anthocyanins, the genes underlying important individual compounds were identified, and these included flavonol synthase and various transcription factors. Further validation of these is in progress, but the prospects of marker-assisted selection for these and other fruit quality traits are looking positive.

Blackcurrant (P3 and P2)
In blackcurrant, the last berry included in this study, there is a pre-existing marker that is already deployed in breeding, linked to resistance to gall mite (Cecidophyopsis ribis). For this project, the focus was on fruit quality and nutritional traits that are
commercially important in fresh market production, rather than processing. The main quality trait that was examined was berry size, for which a variation of over 3-fold was available in the relevant germplasm. The main nutritional trait that was examined was total anthocyanin content, where many fresh-market cultivars are relatively low compared to their processing equivalents.

The linkage map for blackcurrant was enhanced with over 1.5k high-confidence SNPs developed using Genotyping by Sequencing, and the techniques used provide a model for the mapping and identification of SNPs in crop species that lack a reference genome (Russell et al., 2014). Using the reference 9328 mapping population at P3, individual traits were placed on the enhanced linkage map – regions on linkage group 5 for total anthocyanin content, and a region on LG2 for berry size. Associated SNPs were identified for evaluation in other germplasm.

For anthocyanins, a total of 96 breeding lines and commercial cultivars, with a four-fold variation in anthocyanin content, were assessed with 4 putative SNPs. Five haplotypes were identified in the germplasm, but no robust associations with anthocyanin content were evident.

Similarly for berry size, 2 SNPs were identified from the initial QTL analysis, but although 3 haplotypes were identified within the 96 breeding lines and commercial cultivars, no clear associations were made. As a result, other QTL and associated SNPs are under investigation for both berry size and anthocyanin content.

In association with P2, DNA from Polish populations was analysed at JHI. The populations are from crosses between Polish germplasm and UK material, and the genotype data have been retained for alignment with phenotype data from P2 which is due in 2015. At that time, further putative SNPs for these traits should be available for validation.

D1.4 Defined new varieties for cultivation in specific EU areas and cultivation systems (strawberry, raspberry, blackberry, blueberry, currant)

A further development from this part of WP1 is the definition of the most suitable new materials and cultivars for growing in the EU regions. By identifying the best cultivars and new releases, growers can make an informed choice of which cultivars they may want to trial on their farm, and downstream end-users can gain some ideas of new materials that may benefit their businesses.

The work within this deliverable included data on fruit sensorial and nutritional quality, and this links with deliverable D3.4 (Characterisation and quantification of germplasm quality). Tables for defined new varieties were coordinated by P1, with input from other partners, and give clear guidance on the best germplasm of strawberry, raspberry, blueberry and blackcurrant for flowering time, ripening season, yield, fruit traits (size, colour, firmness, shelf-life, sensory and nutritional) and overall pest and disease resistance. The tables are available at the following website. For strawberry, for example, the recommendations for growers in Italy are the cultivars Cristina and Romina at different harvest seasons, with differences in shelf-life and fruit size highlighted. Additionally, four advanced breeding lines are identified for future production, one of which is already released as a cultivar. Similar analyses are presented for the various partners involved in this work. It is hoped that grower groups will reference the analysis, further dissemination of this information beyond this project is planned by the individual partners. In addition, in this case the work on blueberry expected for this deliverable was developed directly by P1 and not by a subcontract, because as already motivated in the second reporting period, it was not possible to find an organization able to carry out this work as expected in the project. Data are reported in the deliverable description.

D1.5 Validate genes controlling fruit nutritional quality and plant flowering (P1, P5, P6)

This work falls into four parts:

Validation of nutritional quality-related genes (P1, P5)

This work falls into three parts:

1. Identification of genes differentially expressed in lines with low & high L-AA content
2. Identification of genes differentially expressed in lines with & w/o γ-decalactone
3. Identification of genes differentially expressed in transgenic lines overexpressing ANS

1. L-AA content

During previous projects, 3 QTLs controlling about 45% of the variation in L-AA have been detected. Two of the QTLs were detected in two or the three analysed years. We have selected contrasting progeny lines for L-AA content. Bulks (3 biological replicates) of frozen fruit tissue from 8 progeny lines with high and low content of fruit L-AA (see table 3) have been produced. Total RNA was extracted during the previous year and global transcripts quantified by RNA-seq. A total of 211 differentially
expressed genes have been identified, are been selected based in their biological function and eQTLs will be mapped in the population.

Table 8. See attached file

Currently, we are in the process of evaluating selected genes from the L-AA experience by qRT-PCR (P5). For the first 6 selected genes the correlation between the expression level measured by qRT-PCR and RNA-Seq was very high.

2. γ-decalactone
The lactone γ-decalactone has been detected in the fruits of some strawberry cultivars but not in others. In the ‘232’ x ‘1392’ mapping population, this compound was produced in fruits of parental line ‘1392’ but not in ‘232’, the segregation in the progeny matched the Mendelian 1:1 ratio (P=0.76) and the variation in this trait was mapped to LG III-2. We have analysed the global differential expression in pools of contrasting lines in the 232 x 1392 population by RNA-seq. Ripe fruits of the selected 10 progeny lines with high γ-decalactone content and 10 lines not producing the volatile compound (Table 6) were combined in two separated pools and subjected to RNA-seq. The use of a bulk segregant approach will allow normalizing the variation in transcription of the rest of genes not related to lactone production.

The analysis of the differentially expressed genes have allowed the identification of just one candidate gene as controlling the variation in γ-decalactone content. The candidate gene is now been validated by qRT-PCR in each individual of the mapping population.

3. Anthocyanin synthase (ANS)
Two independent transgenic lines generated by P1 to overexpress the ANS gene involved in the biosynthesis of anthocyanins have been compared to the control ‘Calypso’ line. Differential expression profiles of ripe fruits from transgenic strawberry lines have been evaluated and compared to the control untransformed plants by RNA-seq. These results are in the initial stages of evaluation.

The three strawberry transformed lines of cv. Calypso with ANS (anthocyanin synthase), previously tested by PCR analysis, have been subjected to Southern Blot analysis to obtain the copy number of the inserted gene.

Validation of flowering-related genes (P1, P6)
To optimize strawberry transformation trials, an experiment necessary to understand the strawberry threshold toxicity to hygromycin and kanamycin was carried out. Furthermore, a regeneration trial to test the best regeneration substrate for both varieties was made. Since January two replicates of transformation trials were carried out using a factorial experimental design comprising 2 varieties and 5 constructs (100 leaves x variety x construct). We obtained 7 rooted plants belonging from one unique line of cv. Sveva infected with a KSN gene construct and 1 regenerated plant of Sveva infected with a Ksn gene construct. Regarding Calypso we obtained 3 rooted plants (same line) and 3 regenerated lines infected with Ksn gene and 2 regenerated lines for Calypso infected with KSN gene. PCR positive transgenic lines were propagated and acclimatized. These lines, after the complete genomic characterization, will be used to study the effect of the inserted gene on plant flowering control.

WP2 Improved cultivation techniques

Within researches carried out on plant physiology and sustainable cultivation systems, strawberry plants fertilised with low levels of N induced a more rapid differentiation of a higher number of inflorescences. The effect of cultivars and techniques on strawberry and raspberry season extension was studied in different locations. Studies started on tunnel proprieties and LED lights will allow the identification of new economic techniques to modify growth conditions for season extension. Different shadowing products were tested in order to control high temperature stress, while, bending, covering with floating cover and a heating system under the cover, activated at defined minimum temperatures, were tested to control low temperature stresses. A device with low water use was prepared to control spring frost on strawberry and currants. Studies of the impacts of climate change on perennial berry plants, were carried out. The expected trials on IPM and biological based strategies were started with plant preparation and experiments setting, including the use of LED lights to control populations of arthropod pests and
Results also indicate that in mild winter regions artificial chilling can have a major impact on yield on primocane raspberries. In southern conditions, cold treatment has an important role on flowering time of primocane fruiting raspberry cultivars. The increasing use of high tunnel production and of the predicted climate change indicate that a screening of cultivars regarding their adaptation to higher temperature seems to be necessary in the context of achieving in general under the high tunnel with UV B blocking while the UV B window film could reduce the yield. The results achieved in early morning but photosynthetic rates were even more restricted for plants grown in high tunnels. Still a good yield was forcing conditions indicated that limitation of net photosynthesis by extreme and constant high temperature reduced strongly and were prepared for early fruit ripening in the next season. Ecophysiological studies with floricane cultivars under different blue nets can be profitable in higher light environments. protective and photoselective effects. Red nets can be efficient in covering strawberry plants under low light environments and growing. The application of shadowing nets is a possible technique to reduce temperature and use of coloured nets combined protective and photosensitive effects. Red nets can be efficient in covering strawberry plants under low light environments and blue nets can be profitable in higher light environments. Evaluation of floral initiation of floricane raspberry revealed that cessation of shoot growth and floral initiation are controlled by the interaction of low temperature and short-day conditions in autumn. It was evident that under German conditions floral initiation (‘Glen Ample’ and ‘Tulameen’) occurred in general between mid-September and beginning of October and continued until the end of October/beginning of November. At that time plants seemed already to reach high yield potentials and were prepared for early fruit ripening in the next season. Ecophysiological studies with floricane cultivars under different forcing conditions indicated that limitation of net photosynthesis by extreme and constant high temperature reduced strongly yield and fruit quality. In summer under open field condition high temperature reduced net photosynthesis to only few hours in the early morning but photosynthetic rates were even more restricted for plants grown in high tunnels. Still a good yield was achieved in general under the high tunnel with UV B blocking while the UV B window film could reduce the yield. The results indicate that a screening of cultivars regarding their adaptation to higher temperature seems to be necessary in the context of the increasing use of high tunnel production and of the predicted climate change. In southern conditions, cold treatment has an important role on flowering time of primocane fruiting raspberry cultivars. Results also indicate that in mild winter regions artificial chilling can have a major impact on yield on primocane raspberries.

D2.1 Develop method to use LEDs in tunnels for strawberry and raspberry and methods to avoid frost injury in berry plants. To avoid low temperature damage on berries during flowering, a technical innovation was developed to control spring frost with low water consumption. The device is a cyclone nozzle making mist of cold water using 5-10 atm water pressure. The prototype is ready for final testing. In the North, LED lights could be used successfully to extend growing season but better tunnel structures and use of additional heat would enable production of good fruit quality and yield on remontant strawberry. On primocane raspberry, humidity is not important and good quality fruits can be harvested late in October. Reduction of low-light stress in autumn fruiting raspberries in polytunnels for season extension in Northern Europe showed that supplemental light can improve the Brix value but not the fruit firmness of ‘Polka’ primocane raspberries but the effect on fruit yield may only be near significant. To have full benefit of supplemental light, heat should be supplied from late September and the plants should be grown in tunnels for winter conditions to avoid unnecessary loss of heat through the construction openings present in traditional polytunnels. LED lamps performed without failure all three years, while the SON lamps stopped functioning when the humidity increased in late September. In addition, the LED 300 W lamp introduced a higher radiation (900 μmol m-2 s-1) to the top of the canopy than the SON 400W lamps (600 μmol m-2 s-1).

D2.2 Develop methods to modify strawberry plant architecture in nursery production, to avoid summer hot temperature for strawberry and raspberry in S Europe and understanding of berry physiology affected by environment in C and S Europe, flower-bud differentiation and chilling requirement. The amount and timing of nutrient supply modifies strawberry plant architecture during nursery production and induces effects on flower differentiation in strawberry plants. The reduction of nutrient supply is possible applying 700 μS/cm fertigation and it can be an effective technique to manipulate the reproductive behaviour of plants in the nursery and finally the plant quality. Floral initiation can occur earlier in plants fertilised with low than high level of N. Early rooted tray plants show more vegetative behavior, with more nodes formed in the crown and a higher number of stolons. First appearance of flower differentiated apex was detected among the late rooted mini-tray plants. In raspberry production, freezing injuries can be avoided in a northern climate by using special trellis to bend the canes down for winter and using cover (Agrocover). Under the cover heating could be used at critical temperatures. In the experiments, bending did not injure in bent canes and the covered ones had less freezing of tip and lateral buds than the erect canes. For Summer production of berries in Southern-Central Europe, control of temperature stress is needed to achieve profitable growing. The application of shadowing nets is a possible technique to reduce temperature and use of coloured nets combined protective and photosensitive effects. Red nets can be efficient in covering strawberry plants under low light environments and blue nets can be profitable in higher light environments.

Evaluation of floral initiation of floricane raspberry revealed that cessation of shoot growth and floral initiation are controlled by the interaction of low temperature and short-day conditions in autumn. It was evident that under German conditions floral initiation (‘Glen Ample’ and ‘Tulameen’) occurred in general between mid-September and beginning of October and continued until the end of October/beginning of November. At that time plants seemed already to reach high yield potentials and were prepared for early fruit ripening in the next season. Ecophysiological studies with floricane cultivars under different forcing conditions indicated that limitation of net photosynthesis by extreme and constant high temperature reduced strongly yield and fruit quality. In summer under open field condition high temperature reduced net photosynthesis to only few hours in the early morning but photosynthetic rates were even more restricted for plants grown in high tunnels. Still a good yield was achieved in general under the high tunnel with UV B blocking while the UV B window film could reduce the yield. The results indicate that a screening of cultivars regarding their adaptation to higher temperature seems to be necessary in the context of the increasing use of high tunnel production and of the predicted climate change. In southern conditions, cold treatment has an important role on flowering time of primocane fruiting raspberry cultivars. Results also indicate that in mild winter regions artificial chilling can have a major impact on yield on primocane raspberries.

aphids of remontant strawberry and primocane raspberry. Tests with Natural Defence Stimulators (NDS) and other biocontrols showed that strawberry powdery mildew was severe on inflorescences and on fruits while it was not on leaves. Trials examining the effect of increased temperature and reduced water availability by measuring plant water use efficiency on strawberry (cv Elsanta) were carried out with 2 water management techniques on 3 substrates. Tests of renewable growth substrates and fertigation treatments were also started. Following is reported a more detailed description of the deliverables of WP2.
For a summer-fruiting cultivar cold treatments had no effect on flowering time and artificial chilling can have a detrimental effect on yield. In Northern Europe, the studies showed that the effect of the temperatures varied with the duration of the chilling period. However, even at optimal chilling temperatures, both bud burst and flowering were promoted and advanced by sustained chilling for up to 21 weeks. In conclusion, 20 or more weeks of chilling at near-freezing temperatures are required for full dormancy release and the promotion of flowering along the entire length of the raspberry cane, which is a pre-requisite for large fruit yields. Therefore, erratic bud break and less consistent fruit yields may be expected in raspberry crops in the wake of ongoing climatic warming with declining winter chill. Therefore, if present projections of future climate change (IPCC, 2007) are true, inadequate winter chill may become a limiting factor for successful raspberry production in areas with mild winter climates. Insufficient chilling time is likely to limit the yield performance of early forcing of long-cane raspberry crops in the greenhouse. It is therefore essential that plants used for this purpose are exposed to the optimum chilling conditions as early as possible after the cessation of growth and the initiation of floral primordia in Autumn. Studies on production of pot-grown raspberry long canes with high yield potential indicated that high yields can be achieved on a regular basis, even when applying intensified and cheaper production techniques. Thus, the pot size could be reduced from the previous 3.5 l, to 2.5 l and the plant spacing could be decreased without loss of cane yield potential. Even at a plant spacing of 55 cm _ 20 cm, canes with a yield potential close to 3 kg per cane were produced. It was also shown that, during the cropping year, additional one or two new canes with the same high yield potential could be raised concurrently with flowering and fruiting of the old cane, without a significant loss of yield on the old fruiting cane. When two canes were produced and cropped in each pot, yields of almost 4 kg per pot were achieved. However, long canes with such high yield potentials could only be produced under greenhouse conditions in the cool Norwegian environment. Studies on the effects of autumn temperatures on phenological characters of black currant phenotype revealed widely different seasonal timings of growth cessation and floral initiation in cultivars of different latitudinal origin. High latitude cultivars originating from crosses and selections of local, wild black currant populations from the Kola peninsula and Swedish Lapland were particularly early and had ceased growing and had initiated floral primordia by mid-June. This was approx. 5 – 6 weeks earlier than any of the other cultivars from lower latitudes. Many cultivars bred and selected in Southern Scandinavia, Scotland, and Poland did not cease growing and initiate floral primordia until late August, 9 weeks after the early, high latitude cultivars. Overall, the 19 cultivars constituted a typical latitudinal cline in their photoperiodically controlled timing of growth and flowering responses. Season extension and avoiding unfavourable climatic conditions is possible by tunnel cultivation on high-bush blueberry in North Europe. Tunnel conditions can be favorable for plant growth and advanced plant development enhancing bud break, and leaf and flower bud development, on the other hand, leaf fall can be delayed. Flowering time in tunnel is shorter but berry development lasts longer. Crop potential can be higher in plants in the tunnel: compared to open air conditions, in the tunnel conditions the number of flowers per shoot, the number of berries per shoot and the mass of berries per healthy shoot can be significantly increased. The yield of whole bushes can be double to those in the open. Tunnel conditions can also highly decrease the level of winter injuries in plants and damages of Godronia (Fusicoccum) canker.

D2.3 Methods for improved propagation, plant protection and resource use efficiency in berry.

By development of a robust system for biological control, use of chemicals can be reduced to a very low level and will improve the healthy imago of fruit consumption. Biological control of thrips can be enhanced by predatory mites and the predatory bug Orius majusculus. Also the effect of mulching by white plastic film is effective. Alternative cultivation systems by application of mulching in combination with natural enemies can improve the tolerance against thrips attack and can sharply reduce the use of chemicals to control thrips. Natural vegetation management may be an important component of biological control strategies and its importance in maintaining potential reservoir of beneficial organisms is increasing in IPM. Neighbouring vegetation may be important in conservation biological control in blackberry crop, because host weeds of mites are mostly repositories of predacious mites but on strawberry, weeds seem to be undesired as they are hosts of mite pests. For a more efficient thrips control, attention must be given to the weeds with high abundance of: abundant phytophagous thrips being also potential pests and phytophagous thrips as potential alternative prey for predators as well as thrips’ predators Aeolothrips sp. and Orius sp. predators.

Biological-based strategies for pesticide-free berry production were developed on raspberry pests. To control raspberry leaf and bud mite (RLBM, Phyllopoptes gracilis) and two-spotted spider (Tetranychus urticae) mite, selection of the best adapted
and potential biocontrol agents for polyethylene tunnel and open field raspberry in northern climatic conditions and the effect of biological control and development of release techniques for predators were studied. The results showed that with repeated introductions of Neoseiulus cucumeris and Amblyseius swirskii, the P. gracilis population can be maintained at an acceptable level during the whole season and Phytoseiulus persimilis effectively controlled T. urticae in the tunnel. The role of the naturally occurring predatory mites particularly on the RLBM is still unclear and at least the predatory mite Phytoseiusculus macropilis seem to occur particularly in the open field plots. Other mite predators found in the samples e.g. predatory midge larvae (Cecidomyiidae) seem to have some effect on mites particularly in early season.

The potential of LEDs to prolong the growing season at northern latitudes and the effects of two levels of supplemental light given in autumn by using LED with blue and red lighting were studied on remontant strawberry grown in polytunnels. The numbers of aphids and predatory mites did not differ significantly among light treatments. The regime of LED supplemental light used did not lead to higher pest populations; on the contrary, spider mite numbers in the autumn decreased. On strawberry, Botrytis cinerea and Sphaerotheca fuligina are pathogens threatening fruit production. Use of Decision Support Systems reduces the number of sprays necessary to control diseases. The effect on powdery mildew was variable, indicating that improvement of the DSS is necessary. More important, the level of residues could be reduced with 50-75% compared to common practise. The quality and quantity of the yield of the DSS strategies was comparable to practise. Although no pesticide residues were found in the untreated control the quality and quantity of yield was reduced with 33%.

In greenhouse conditions, exposure of strawberry plants to red light during night was tested in powdery mildew control. Disease severity of the naturally infected plants could be reduced significantly with time when they were exposed to 16 h daily lighting and 2 h of night interruption with red light. To use UV-B radiation in powdery mildew control, a robotic system was constructed and equipped with a blower to move the leaves below for more uniform exposure. UV-B treatment once in every three days was proved to be equally effective as daily UV-B treatments. However, daily exposure of plants to UV-B during dark showed very low level of severity and relative to 16 h of growth light, all UV-B treatments reduced powdery mildew severity below 7.8%.

Control of powdery mildew with Natural Defence Stimulators and other biocontrols were studied on strawberry. Biocontrol agents associated with fungicides can control effectively powdery mildew and they can be used in the susceptible period of harvest, when fungicide applications are not possible. This strategy will enhance limitation of fungicide use. In addition to the use of biocontrols, the use of moderately resistant variety will contribute to control powdery mildew, more particularly in everbearing variety, which presents long period of fruit production. Armicarb and Prev AM are now officially allowed in France for controlling strawberry powdery mildew, and producers routinely include these 2 products in their protection strategy. New and environmentally friendly control measures were studied on fungal diseases on strawberry, especially crown rot (Phytophthora cactorum). The study has clearly shown the difficulties when working with biological systems of many components and no constant disease control of strawberry crown rot was achieved by using micro-organisms. However, dual or triple use of microbial strains and two different composts showed quite promising results.

Evaluation of diseases on blueberry in the South revealed that the most important diseases observed were: Stem blight and dieback (Neosulcoccus spp), twig blight (Diaphorthe spp., and Pestalotiopsis sp.), leaf rust (Naohidemyces vaccinii), twig and stem blight (Botrytis cinerea) and root rot (Phytophthora cinnamomi ,Pythium undulatum and Fusarium sp). Only root rot caused by P. cinnamomi caused considerable mortality reaching 90% of the plants in two recently planted orchards. A quarantine species Phomopsis vaccinii were not found in any sample. The interest in blueberry cultivation in Portugal has increased exponentially in the last years. Information of diseases present is of utmost importance to recommend prevention and management measures to farmers.

In studies for drought tolerance, useful genotypic differences on strawberry could be observed by measuring the leaf gas exchange parameters. The growth inhibition in response to reduced water availability was higher in the above-ground portions of the plants (leaves) than in the root systems and limited irrigation resulted in significant decrease of berry yield. The study revealed significant morphological and physiological differences between the examined strawberry genotypes. ‘Elsanta’ and ‘Pink Rosa’ had high rates of net photosynthesis and a high value of water use efficiency both under favorable and water deficiency conditions. Morphological and physiological adaptations allowed them to maintain their productivity when the water availability was decreased while ‘Honeoye’ and ‘Grandarosa’ showed lower tolerance to water shortage.

Studies on nutrient use during fruit production indicated that the reduction of nitrogen supply didn’t affect the fruit yield maintaining a similar harvest pattern during the picking season and reached even higher peaks augmenting the phosphorus supply. Nutrient supply can be reduced saving fertilizers with respect to the growing technique, without losing productivity. In particular, in order to reduce environmental impact of soilless strawberry cultures, nitrogen supply can be halved to 5 mmol/l.
In double-cropping, blackberry cultivars were tested in order to develop a production system that could allow growers to establish new plantations of primocane-fruiting raspberry in order to achieve early fruiting and thus increase their production potential in the summer-autumn period. A comparative study of the ‘Meeker’ raspberry and the ‘Čačanska Bestrna’ blackberry plants propagated using the standard method and the in vitro micropropagation, did not reveal any differences in the total number of canes and the number of canes per row metre. Notwithstanding the differences in the amount of yield and some of the other monitored parameters that were in favour of the ST raspberry and blackberry plants. The many advantages of the micropropagation – supported by the fact that no deformities of the fruits as well as no genetic variability were detected, justify the recommendation that planting material propagated in this manner ought to be used on a wider scale for establishment of raspberry and blackberry commercial orchards. For the conclusion longer study of behaviour of TC plants are required.

D2.4 Season production extension
Relatively simple improvements to the fittings of the tunnel can have very significant effects on the length of growing season. Frost is most effectively delayed by limiting night-time ventilation and deploying an energy screen. Each single one of these two measures will increase minimum temperature by few/several degrees. A fog system will ensure maximal length of the spring growing season. The second best is increasing ventilation of the tunnel. Two different shading treatments and their effects on temperature and strawberry production, net assimilation and evapotranspiration were compared and the results and recommendations to modify tunnel conditions were demonstrated to growers in Sant’Orsola in 5 growers meetings.

D2.5 Develop method for crop season extension of raspberry in C and N Europe and blackberry and blueberry in S Europe. Season extension of late season (i.e. primocane) fruiting raspberry was studied with different treatments. Use of low covers, perforated plastic cover and fibre cover in the early spring advances vegetative growth and is beneficial for early yielding of raspberry plants of the tested cultivars. This cultivation method can be proposed for implementation and use on commercial plantations of primocane-fruiting raspberry in order to achieve early fruiting and thus increase their production potential in the summer-autumn period.

Blackberry (Rubus spp.) production is increasing worldwide and improved fruit firmness and shelf-life has increased consumer interest in this crop. Two different approaches were trialed in order to change production season and increase year round yield of this biennial fruiting plant. When blackberry production by floricane stem cuttings was studied, it was observed that rotting occurred both from nodes and at the base of the cuttings. The strongest roots were from the bottom part and shoots formed from axillary buds produced several flowers. For commercial use of this method, more research is needed to improve adventitious root initiation, mainly for the erect types, before the fruitful lateral expands. The containers with the new long-canoes were cold stored from January to late June (six months). After this period plants were allowed to grow in the same tunnels for the autumn crop. In double-cropping, blackberry cultivars were tested in order to develop a production system that could allow growers to
double crop (spring and autumn) using “in ground” and cold stored plants. The aim was to find the best way of growing two new canes from the same mother plant, one for the spring crop and another, in substrate, to be prepared as long-canes for next year autumn crop. The results showed that it is possible to grow two primocanes from the same blackberry plant and use one for spring and a second cane, after cold storage, for the autumn crop. With two harvests productivity is increased and season extended for the high fruit market price.

The effect of cold storage on harvest date and productivity in three Southern high-bush blueberry cultivars was determined in early substrate production. All cultivars started two and an half month earlier (79 days) in the standard-tunnel compared with the standard-open field. Cold treatments postponed harvest when compared with the standard-tunnel plants. It is also possible to confirm that blueberry production in the South can be delayed to late August with rabbiteye blueberries. Plant establishment was excellent and expected yield is high for all cultivars. Late production with cold treated northern high-bush plants was studied under tunnels and harvest in tunnel was one and an half month (48 days) before open field. Blueberry trials showed that later cultivars must be tested since harvest season for the Northern high-bush tested was too early and the target months (September-October) were not achieved. Rabbiteye blueberries can be a good option for late August in open field. A later crop should be avoided since the probability of rain is highly increased. Blueberry production in Southern Portugal can start as early as March with SHB cultivars and for some of them artificial chilling is highly beneficial.

WP3 Fruit quality characterization and determination

The work within WP3 was focussed on the key stakeholder relevant criteria: fruit appearance, quality, composition supply chain resilience (storage) and health benefits. These criterial are fundamental to the sustainability of the fresh fruit industrial sector and the consumer. For the latter many of these parameters will be the drivers of primary and continued purchase whilst for the former the parameters will constitute core fruit quality, markers of product differentiation and criteria to ensure continued supply to wider markets (supply chain resilience). To deliver on all of these in EUBerry we focussed our effort on first establishing robust and transferable standard operating procedure (SOPs) to establish fruit quality (D3.1). The SOPs were then used to assess the quality and nutritional/nutraceutical diversity of the fruit used and generated within EUBerry (D3.4). With these analytical and germplasm assessments established (or progressing) the optimisation and/or enhancement of fruit quality via novel pre-harvest approaches, including resistance induction and biocontrol, were explored (D3.2): a novel route that could give EU growers an advantage. Allied to this were the post-harvest packaging studies (D3.5) studies to ensure that once picked the fruit quality is maintained through the supply chain and with the consumer. Finally, selected fruit were used to establish species specific and cross comparative assessments of the potential health benefits to be had from the target fruit (D3.3)

D3.1 Delivery of standard operating procedure for all analytical approaches with respect to fruit quality and composition and sensory parameters.

A wide range target parameters were assessed and agreed standard operating procedures (SOPs) developed for their robust delivery. These SOPs targeted the following aspects and the details can be found at http://www.euberry.univpm.it/sites/www.euberry.univpm.it/files/euberry/documenti/SOP%20all%20partner22_12_11.pdf

- Bioactive compound extraction.
- Total Antioxidant Capacity - Trolox Equivalent Antioxidant Capacity (TEAC) and Ferris Reducing Antioxidant power (FRAP).
- Total Phenol, Anthocyanin, Flavonoid and Vitamin C Contents.
- Total Acidity, Organic acids, Sugars
- Fruit volatiles analysis
- Sensory assessment

These SOPs were then followed for the rest of the WP3 analyses. For some analyses colour, overall quality, firmness, total soluble solids (°Brix) and pH there were perfectly acceptable and industry approved (and well used) SOPs. These are ubiquitous in the industry and needed no further development. These were also used throughout the WP3 research. Given the phytochemical complexity evident in the targeted soft fruit the SOP approach was extended to include state-of-the-art approaches, here LC-MSn, by P3 to ensure that quality etc. assessment kept pace with the increasing demand of industry and the consumer for point of differentiation composition and health benefits. LC-MSn was applied to a range of the soft fruit in EUBerry (part of D3.4) and this will be expanded upon under that deliverable although a broad feel for the utilisation of the
SOPs can be found on the project site:

D3.2 Identification of strategies to maximise and/or enhance fresh fruit quality via novel pre-harvest approaches including resistance induction and biocontrol.

Considerable advances have been made in the use of Resistance Inducers (RIs) as a novel approach to address the consequences of increasing legislation to reduce or remove pesticides from the fresh fruit production chain. These compounds have nil, or limited, direct effect, on the pathogen, but promote host defence and subsequently increase resistance to the pathogen infection; in essence they bolster the existing plant immune response. Given that resources were limited as part of the call and project the resistance induction control studies were limited to strawberry, it being the key soft fruit crop and one that can be particularly devastated in some regions due to Botrytis cinerea, Rhizopus stolonifer and Penicillium spp decay.

The approach taken to establishing the efficacy of RIs was to first screen the commercially available products (Chito Plant (chitosan), Bion (benzothiadiazole), Fitocalcio, Xedabio, Abies (a fir extract from Abies sibirica), Algition and Karma) at a range of concentrations and establish a ranking of efficacy. Pre-harvest treatments and subsequent storage of the strawberries at 20 or 0°C identified that the most effective products were Chito Plant (1% w/v), Bion (0.2% w/v) and Abies (1% v/v). These compounds respectively reduced strawberry decay by 25, 20 and 42% at 20°C, and of 32, 25, and 29% when the strawberries were stored at 0°C for 7 days, then exposed to shelf life at 20°C. Re-trialling these three formulations in subsequent years, this time with a new RI, laminarin [an \( \text{\textbeta}_{1-3} \text{glucan} \)], and commercial fungicides as part of the controls for comparative purposes, highlight that the commercial and new RI were approaching the levels of disease reduction achieved by fungicide, alone and in some cases, e.g. grey mould and Chito Plant, the RI was as effective. Gene expression studies into the mechanistic aspects of this induced resistance highlighted a general uplift in the genes in the phenylpropanoids pathway. The potential for the broad scale use of this approach is significant and with further work and expansion to other environments this could be a route to address the ongoing tightening of the regulation of pesticides, part of the EU “thematic strategy for pesticides”, which is causing concern amongst growers and industry in this and other sectors.

D3.3 Evidence for the human health benefits of fresh fruit in validated human digestion, bioavailability and disease model systems.

Fresh fruit already has an accepted health halo and the aim within EUBerry was to identify more specific health benefits particularly against degenerative diseases. Indeed, epidemiological studies indicate that berries may exert a protective effect against a variety of chronic degenerative diseases in which oxidative stress is thought to play a role, because of their antioxidant properties.

Given that we are dealing with fresh fruit as part of EUBerry it was felt important that, when dealing with human health benefits and bioactivity, we be mindful, and take account of, the human digestion processes and the implications on the fruit phytochemicals. Consequently a detailed and in depth analysis of the consequences of 2 validated in vitro digestion (IVD) process on the fruit phytochemicals was undertaken: two were needed to generate digestate for subsequent appropriate disease models.

Fig. 3.1 See attached file

To deliver on this the polyphenol compositions of a total of 71 cultivars and genotypes (17 raspberry, 13 strawberry, 19 blackberry, 14 blackcurrant and 8 blueberry) were determined and from these 20 cultivars and genotypes were selected for IVD treatment. Within each berry type, cultivars and genotypes were selected that had varying contents of the different polyphenol classes (anthocyanins, flavonols, ellagic acid conjugates and ellagitannins). The LC-MS analyses of the IVD highlighted many significant changes in all the fruit phytochemical. For example, for raspberry IVD resulted in a large decrease in anthocyanins with varying recoveries, ranging from 11-16% for serum-available and 7-23% colonic-bound equivalent extracts, depending on the cultivar. Similarly, flavonols and ellagic acid (conjugates) decreased with varying recoveries, ranging from 11-32% and 18-42%, respectively, for serum associated extracts and 19-34% and 27-57%, respectively.
respectively for the colon bound ones, depending on the cultivar. Interestingly, and not noted or considered in the vast majority of intervention studies was the finding that the total amount (serum and colon) of ellagic acid increased after IVD suggesting its production from breakdown of ellagitannins.

IVD studies were accompanied by studies to establish cytotoxicity, metabolic fate and anticancer activities. Again robust and appropriate cell model systems were employed, HepG2, an immortalised liver cell routinely used for metabolic studies. In addition, an intervention trial was undertaken with a defined berry diet to establish the in vivo metabolic fate of the fruit polyphenols. The model cell studies for all IVD fruit extracts highlighted that the polyphenol digestate had no significant impact on viability after 24hrs, but prolonged exposure decreased the cell viability to 50% (48hrs) and 20% (72hrs). For all fruit extracts but only at high concentrations: indeed the cytotoxic concentrations were above those seen for the compounds in in vivo studies and greater than those seen in the intervention trial. Consequently, the dietary polyphenols can be considered ontoxic and dietary intake levels.

The allied fruit metabolic intervention used a puree of the fruits given to 9 health non-smoking individuals (see note 1). The urine from the subjects was collected and analysed by LC-MS which positively identified metabolites and sulphated, glucuronidated and methylated conjugates, some novel, with each being excreted at specific and signature times indication specific absorption points during transit through the GIT. This comprehensive study is the most comprehensive analysis of dietary polyphenolic analysis published to date and has identified potential chemical markers of intake for these fruit.

At the degenerative disease level the EUBerry studies principally focused of CVD and neurodegeneration. For CVD strawberry was the main fruit used and two studies were undertaken: the first to verify the antioxidant effects of strawberry and the second to evaluate the possible protective effects on CVD. The former study employed a rat model and studied the effect that strawberry consumption had on exogenously generated oxidative stress via doxorubicin (DOX)-induced toxicity which drastically increased lymphocyte DNA damage, liver biomarkers of protein and lipid oxidation, and mitochondrial ROS content while markedly decreased plasma retinol level, liver antioxidant enzymes, and mitochondrial functionality. After 2 months of strawberry supplementation, the negative effects of DOX were completely reversed; rats presented a significant reduction of DNA damage and ROS concentration and a significant improvement of oxidative stress biomarkers, antioxidant enzyme activities, and mitochondrial performance. A second study found that 500 g/day of strawberries supplementation for 1 month employing 23 healthy volunteers beneficially influenced the lipid profile by significantly reducing total cholesterol, low-density lipoprotein cholesterol and triglycerides levels (−8.78%, −13.72% and −20.80%, respectively; P<0.05) compared with baseline period, while high-density lipoprotein cholesterol remained unchanged. Strawberry supplementation also significant decreased serum malondialdehyde, urinary 8-OHdG and isoprostanes levels and spontaneous and oxidative haemolysis; all markers and/or consequences of oxidative stress. In summary results suggest that strawberry possesses valuable properties such as counteracting exogenous oxidative stresses, improving plasma lipids profile, antihemolytic defences and platelet function in healthy subjects. All of these are supportive of a positive impact on the risk of CVD.

Neurodegeneration aspects were dealt with by appropriate cell models systems wherein pre-incubation of the cells, with various Rubus IVDs, was followed by oxidative insults and the cells then assayed for their response. Differential cytoprotective activities were discovered across the Rubus species (and commercial cultivars) with a general diminishment of intracellular ROS levels, modulation of glutathione levels and activation of caspases, all to the cell benefit. Indeed, Blackberry metabolites were able to differentially prevent transcript alterations induced by oxidative stress, with wild blackberry more effective in reducing the number of genes altered. Extension of this to attenuation of inflammation in a neuroinflammatory cell model highlighted that IVD polyphenols from the five raspberries cultivars and breeding lines all attenuated (reduced) microglia neuroinflammation and inflammatory markers, NO and TNF-α, to varying degrees.

Targeted studies were undertaken in gut and immune function using a cell based gut model employing CaCo2 cells in layer format. IVD fruit extracts (4-5 cultivars or species/fruit) were tested for their impact on the cell layer (gut epithelial mimic) integrity and this general showed that the fruit components were not particular problematic in this regard: ideal for a dietary component. The impact of exposure to the selected extracts on cellular metabolism was studied at the gene expression level. The data from this was very complex with confounding factors and are still being mined. However, amongst the highest up regulated were genes involved in the retinoic acid synthesis (both the receptor as well as enzyme, while enzymes towards
pigment forming are reduced) which might indicate indeed a response to carotenoids and the conversion into vitamin A, which are very important for the homeostasis of gut epithelial cells. Low retinoic acids levels in epithelial cells also are related to increased risk in colon cancer. Besides that, we see many genes upregulated that showed a role in stress resistance and xenobiotic detoxification, e.g. like P450 enzymes and other monoxygenases. This was expected and a good confirmation of the models validity. Interestingly we found genes related to intestinal fibrosis which also shown to be changed in colon diseases like IDB and Crohn’s disease; we will continue to mine this data for a definitive result. Another interesting observation is the upregulation of many microRNAs which are modulators of the expression of other genes. Very often, the targets genes of these microRNAs are not known. But some we identified are also identified to have a role in intestinal barrier and Crohn’s disease. Furthermore, amongst the down regulated genes were those involved in golgi structure and function, and genes associated with golgi’s like modifications of proteins. Some of these proteins are also related to the formation of amyloid beta which could indicate that berries can reduce the risk for Alzheimer by this mechanism. Clearly down regulation of these could be potential dietary preventative routes but further well-structured research, is needed.

D3.4 Characterisation and quantification of quality and nutritional/nutraceutical data from the germplasm derived from improved cultivation studies (WP2).

This deliverable was, following the establishment of the analytical SOPs (D3.1) the basis of the many research streams into sensory and health beneficial studies. With partner derived material and that generated from the WPs 1 and 2 the level of characterisation associated with D3.4 was significant and generally the basis of the many published papers, presentations and stakeholder. In the first year there was a concerted effort to collate fruit and to get all the partners up to speed with the SOPs to ensure cross project comparability of data and to further ensure the quality of the data for interpretation and publication.

Often aroma is a potent driver of purchase and research was done to characterise this in raspberry and to correlate this with sensory score. Using an SPME-GC-MS approach a detailed chemo-profile was established for the 14 lines analysed (known to have polyphenol and phenotype variation; 14 lines). This highlighted that often the more complex aroma volatile profile correlated with a sensory score of desirable. The statistical basis of this and the phytochemical driving this are still under analyses but the results will be fed back to the partner breeding programmes. The extent of this analysis can be seen at http://www.euberry.univpm.it/sites/www.euberry.univpm.it/files/euberry/documenti/D1.1%20Data%20base/Revisione/Summary-Genotype%20characteristics%20-%20WP1%20_2_14%20last%20versionStrawberry.pdf where the target fruit (with many lines per fruit) were analysed in relation to sensory and health beneficial components. Viewing this from the perspective of the bioeconomy (industry) and consumer there is a satisfy level of compositional variation amongst the fruit. For example, in strawberry firmness, skin colour, glossiness, flesh colour, aroma, taste and volatiles as assessed on an arbitrary sensory score of 0-9 all covered at least 4 score units on the scale and sometimes 7. This means that the translatable biological variation evident is with regard to sensory aspects is significant and a resource to be exploited to create new cultivars, satisfying the consumers and industries desire for an evolution of products. At the health beneficial level, an analysis of total and specific polyphenol composition also reflected the variation seen with the sensory components (See year 2 report). Interestingly even within a single species such as raspberry, strawberry, blackcurrant and blueberry and using only cultivated and breeding lines the total phenol and anthocyanin levels varied by 283 and 400%, 285 and 254 (white fruit excepted), 154 and 267%, and 300% respectively. This is a major level of variation in what are key putative health beneficial parameter meaning that the potential is there for manipulation in new varieties at the genetic level, as well as, through cultivation methods such as LEDs (WP2) and agronomic practices.

D3.5 Shelf life maintenance of fresh fruit via the novel extension strategies of UV, O3 and controlled and reduced atmosphere treatments.

The demand for high quality fresh fruit has never been greater and with the production levels general increasing across EUBerry target soft fruit the onus is now on for increased supply chain resilience to ensure that the fruit produced is packaged, stored and transported in a manner that is both sustainable and maintains the premium fruit quality.

Within EUBerry innovation was developed in the postharvest technologies sector with respect to hypobaric treatments and packaging applications. The first of these, hypobaric treatments, atmosphere applications (particularly 0.25 atm) were
successful in initial experiments at retarding, or delaying disease following storage at 0°C for 7 days than the 20°C control. Furthermore sensory panel assessment studies highlighted that the hypobaric systems were good at maintaining strawberry, blackcurrant and raspberry fruit sensorial quality.

Fig. 3.2. See attached file

For strawberry initial studies with using 0.25 0.5 and 0.75 atm storage pressures identified 0.25 atm optimal with a significant reduction in respiration, extended shelf life and postharvest diseases. Further experiments extended the range of varieties used and the use of alternative atmospheres for comparison, such as No Atmosphere (ambient) and Controlled (CA (16% CO2+ 5% O2 and 0% CO2+ 10% O2) and the Xtend® packaging system at 0 °C. The Extend system is a tailored MAP (manufactured by Stepac) which claims to give optimal modified atmosphere, humidity and condensation control (release of excess moisture). Interestingly when stored as described above and at 0° a shelf life of 14 days was achievable with little in terms of benefits to be had. However, more detailed compositional analyses did highlight difference associated with the storage regimes such as Vit C, sugars and acids etc.

Fig. 3.3. See attached file

Extension of the above atmospheric studies to blackcurrant employed 4 varieties and 0°C storage highlighted that the varieties generally stored well under all conditions for 7 and 13 days. One variety (Ores) did exhibit limited storage performance in terms of aroma (desirability) and sweetness highlighting the generality of these technological approaches and that varietal performance needs to be tested.

Raspberry tested under similar conditions saw a separation of storage regime performance with 10 days storage highlighting that hypobaric and the Xtend® packaging gave similar good results and better than the controlled atmosphere system. The latter failed particularly in terms of flesh consistency.

WP4 Competitiveness and marketing strategies

The last RTD workpackage addressed to economic and marketing studies started with the organization of the work among partners in order to collect data useful to evaluate the effectiveness of new cultivation systems in reducing berry production costs and also on the effect of new marketing strategies in increasing consumer attraction to fresh berries with increased nutritional and nutraceutical value.

Following is reported a more detailed description of the deliverables of WP4.

D4.1 Ex-ante economic analyses of production.

A condition for implementation of sustainable culture systems by the European fruit producers is that these systems are also economically profitable. Therefore, the EUBerry project included tasks for ex ante and ex post economic evaluations of techniques that are developed to increase the sustainable production and the consumption of berries. In this economic evaluation, strawberries, raspberries and blueberries were the crop species, but the results can be projected on other berry species as well. This task, performed in 2012, concerns the ex-ante evaluation. The researchers developing the new sustainable culture systems could use its results to estimate the amount of additional labour costs, material costs (including energy costs) and capital costs that are acceptable for implementation of their techniques. At the end of EUBerry project calculations were made with the really achieved results of innovative techniques.

Results ex-ante.

Financially, raspberries with low water requirement are mainly attractive in regions where raspberries give low production and/or quality in the current situation, since not enough water is available. The marginal gross margin may increase between €7000 and €16000 per ha, if prices stay equal. If supply of tap water is possible in the current situation the advantage of new varieties is about € 160 per ha, and when surface water or groundwater is available the advantage of new varieties is even smaller.

New varieties for easier picking of raspberries are very attractive, since they reduce labour costs. If picking speed increases 30% the marginal gross margin increases between €1000 and €8500 per ha. The higher the production and the higher the
labour wages, the bigger the financial advantage.

New technologies for low residues in strawberries are only attractive if production and quality do not decrease. If the price of these strawberries increases 15% the marginal gross margin increases between €3000 and €7000/ha, or €22,000 in Italy with harvest in spring and autumn. Production losses between 15 and 25% undo the entire financial advantage of the reduced residue levels.

If enough water and nutrients are available, reduction of water and nutrients use may be important for sustainability and for the licence to produce, but have only minor effect on marginal gross margin for strawberries, raspberries and blueberries. Extension of the shelf-life of strawberries, raspberries and blueberries increases their attractiveness for retailers, what makes it expectable that the selling price for the producers increases. The level of advantage depends strongly on current production and price, and for the economic calculations, the assumption is made that production and quality (apart from shelf life) won’t be affected.

If in vitro propagation in strawberries, raspberries and blueberries results in better quality and therefore in higher prices, the financial effects are comparable with these of an extended shelf-life. If this technique results in lower prices for planting material with the same quality, the effect on marginal gross margin for the berry producers is minor.

Season extension may be very attractive for the first producers who do so. If the quality doesn’t change, the financial benefits depend on production and the difference between prices during the traditional season and the weeks before and after. If strawberries are produced five weeks earlier the effect on marginal gross margin may be between €3400 (Poland) and €4200 (Germany) per ha. For raspberries, calculated benefits are between €1600 per ha in Italy (with small differences between selling prices over time) and €140,000 in Poland (with very big differences). For blueberries the calculated benefits of 5 weeks earlier production were between €12,000 (Italy) and €28,000 (Poland) per ha. However, since it was not possible to collect weekly harvest- or supply-data from each country, calculations are based on the harvest- and supply-data that were available. This may have caused over- or underestimations of the economic effects. It should be mentioned that there are several footnotes to be made with these calculations: First: depending on the number of fruit producers starting with season extension, the selling price for off-season product will probably decrease at longer terms. In Poland, this is happening already. Polka and Polana raspberry varieties are becoming popular, due to their resistance to diseases. They also don’t need the pals and wires construction like summer varieties do. However, they are bearing from August till November and often at that time the weather is no good in Poland. Therefore, many growers use tunnels to obtain better conditions and avoid accidental frosts. Thus, they extend the time of the production. It is estimated that autumn raspberries account for more than 60% of the total raspberries production in Poland. This trend is known in other countries too but not on such a big scale. Since the degree of price dropping is not known the results in this report are the best that could be made at this time. Second: It seems possible that the yield will decrease when fruit is produced outside the traditional period. Otherwise, it is possible that total yield increases in more controlled culture systems as tunnels or greenhouses. Yield and quality changes do significantly affect the marginal gross margin and the income of the entrepreneur. Since the effect on production is not known at all, those considerations are not included in this study.

In this study, estimations are made of the financial benefits of new technologies for sustainable production of healthy berries. For fruit producers, this financial benefit equals the maximal annual costs they can afford to apply these new technologies. Annual costs may include additional labour costs, energy costs and other material costs and annual costs of fixed assets (costs of depreciation, interest and maintenance).

For investments in assets that can be used all over the farm (like machines, equipment, buildings) not the maximal acceptable investment per hectare is relevant, but the acceptable investment at farm level. In general, berries are produced at a relatively small scale, only berries in Poland and a part of the strawberries in other countries are produced on larger scale. For example: in Finland the average size of strawberry farms is 2.13 ha, raspberries 0.62 ha and blueberries 0.38 ha, and in Italy the average size of a berry farm is about 0.4 ha. The maximum acceptable investment should be calculated by multiplying the positive effect of a technique on marginal gross margin by the farm area.

Actually, producers could better reduce annual costs to a level that is significantly lower than the calculated benefits. When the supply of this higher quality fruit increases or when more fruit is produced beyond the traditional season, the selling price might lower again. As far as additional costs concern annual costs for fixed assets it will be difficult to reduce these costs after the investments are done. This risk should be priced in by a margin between the maximal acceptable investments as calculated in this study and the real investment costs.

Labour costs
For economic evaluation of the new techniques, it is important whether additional labour is done by hired workers or by the producer himself. One reason is the difference in calculated wages. However, for the calculations of the marginal gross margin it’s even more important that hired workers have to be paid, as additional costs for hired labour do affect the marginal gross margin. Additional labour costs for the fruit producer himself don’t have to be paid, do not affect marginal gross margin and are part of the entrepreneur’s income.

D4.2 Reporting fresh berry market situation

The berry fruits sector is a market segment of increasing importance over recent years. In view of the various soft fruit types there has been and still is a dominance of strawberries. Recently, also other soft fruits like blueberries and raspberries gain in significance in production area. This factor contributed to a crossing over the detection limit for data collection. In consequence, for the first time the German Federal Statistical Office carried out a secondary statistical survey with respect to other soft fruits than strawberries. This first survey was published in March 2013 and its findings were included in deliverable 4.2.

In view of production method, there is a dominance of open ground production of all fruit types. Since 2006, also data about the protected production is counted and reported. According to the pioneers of protected production, strawberries were the first berry fruit and raspberries were the second to be grown in tunnel systems.

Looking along the supply chain for soft fruits, areas with room for improvement can be identified at each level of trade. Starting with the interface between growers and grower organisations farm size, cooling facilities and the grower’s duty to supply to producers organisations can be noted. The latter concerns the rule among members of producer organisations to deliver their production quantities up to 100% or less. This means, that the growers are allowed to sell up to a certain amount fresh fruits direct to the consumers. This is accepted without problems in years with sufficient quantities. In years with small harvest volumes, this can lead to considerable insecurity in planning for the producer organisations. With respect to post harvest quality of fresh berries, a quick cooling is important. This requires cooling facilities within easy reach. Producer organisations are located within fruit growing regions and a fast delivery is possible for the majority of the growers. On the farm sites of medium and large sized farms, growers dispose on their own cooling facilities; entrepreneurs of small sized farms don’t. This might be a problem with respect to an unbroken cold chain.

A further effect of extremely small farm sizes is the difficulty of bundling the berries (lack of infrastructure). In Poland, for that reason some of the small farmers are withdrawing from the production of berries for fresh market and switching to the production of berries for processing. There are quite a few very big berries farms, which monopolized supply for hypermarkets and supermarkets in Poland. The owners of those big farms rather don’t prefer to collaborate with small ones because of the lack of standard and uncertain quality of berries coming from small farms. Therefore, there are fewer opportunities for small farms due to decreasing profitability of the production for processing every year. This will cause that within few years the number of small farms will be significantly lower in Poland. The speed of the mentioned process will depend on the scale of prices and costs changes as well as on the way in which the fresh market retail will follow. Nevertheless, there are berry producers to be found in Germany as well as in Poland, who cultivate in larger business units and are able to sell their berries to supermarkets or abroad on their own.

It continues with the interface between grower organisations and retail companies. In this exchange relationship, a problem area can be identified concerning the rules of pest management. With the cultivation of berries, growers set up an integrated resistance management. The main driver for this is to produce berries of high quality and protect the plants. The food retail companies formulate restrictions with respect to the number of plant protection products allowed within one production period. These restrictions are more severe than required by law. The main driver for this are fear of adverse publicity and worries about reputation just in case residual traces of plant protection products might be found in fruits bought in their stores. In consequence, this follows through to an opposing trend between essential protection measure and restrictions of food retail companies. The awareness of this contradiction needs to be raised and sharpened among the growers and retail traders. One could think about a personal communication platform for representatives of both groups in order to improve mutual understanding between them.

As a last link in the chain there is the interface between retail companies and consumers. Here, the field of action is linked to questions of placement and presentation within the outlets. In terms of packaging there is an accepted compromise solution with plastic packaging of different sizes with top cover.

Another aspect concerns the structural changes of food retail companies. Especially in Poland, due to continuous changes in the share of food retailers, there are fewer opportunities for small and medium sized berry producers in Poland. Especially
dynamically, the number of stores belonging to Jeronimo Martins Polska S.A. (Portugal, NL) with discounters Biedrónica and Schwarz Group (Germany) with the discounters Lidl change the Polish food market tremendously. This affects also fresh berries market. The most of the fresh berries they sell are imported. The reason for that is because their shops are relatively small and there is not enough space for placement and presentation of wide range of berries. Due to the global slowdown in economic growth, high rate of unemployment and low incomes, the majority of Polish consumers prefer groceries with low prices. This is the reason that number of discounters is growing very rapidly in Poland for the last 10 years. Now they are present already not in urban area only. This situation affects berries market by limiting the possibilities for small and medium size berries farmers to sell berries directly to local consumers. Unfortunately, under these circumstances potential consumers will shop at discounter. The proper presentation and placement of berries as well as their outstanding quality is very important in hypermarkets and supermarkets at highly populated urban area in Poland. The stands with berries are often very fancy at those places. They make it possible to sell more berries comparing to ordinary displays at fruit and vegetable at supermarkets.

Because of the many problems with fulfilling generally food quality in the past, many consumers rather prefer to buy berries at chain stores. They believe that berries there are more safety that those purchased on the local markets or those purchased directly from the producer. This is also one of the reason for successful grow of market share by discounters in Poland.

With a view to the future of fresh berry markets, the most important key challenges are food safety and communication among all actors along the supply chain. So, this applies both to the communication to the consumer (“berry business to consumer”- BBtoC) and the communication between growers and retailers (“berry business to berry business” - BBtoBB).

In terms of fresh berries, food safety is extremely important due to the short production time all types of fresh berries. Thus, the growers need to set up or hold on to an integrated pest management considering resistances. This needs to be communicated to retail companies in order to create a mutual understanding between supply and demand site. With respect to retail companies, food safety is also extremely important due their public reputation and trustworthiness. Thus, food safety can serve as a common goal.

Viewed in this light, the production methods and practices can contribute significantly to reducing pesticides inputs. As protected production of fresh berries in tunnel systems is on its way, there might also be the possibility to increase the biological pest control. Here, there is a need for further research and development of the use of beneficial organisms in order to prevent non-beneficial organisms in tunnel systems. This can lead to a win-win situation, because this will meet the claims of the retail companies and contribute to the solution of the resistance management.

In terms of fresh berries, communication to the consumer is extremely important. Which messages are meant to be conveyed? One driving force for consumption of fresh berries is their healthy image. Therefore, health benefit is one important aspect as there are consumer groups who really take care about health issues and will react on healthy arguments in a positive way. But, there are also consumer groups to whom healthy arguments could work like a morally risen forefinger and therefore they will not respond on it. As a solution, it is necessary not to focus only on healthy arguments. In addition, one can take into consideration that fresh berries are part of a modern life-style and mean enjoyment in the summer time. Health and fun are the message.

The third message, that needs to get across to the consumer, is safety. This can be done by building up trust towards the grower and the origin of the berries. Viewed in this light, a potential labelling of fresh berries could focus on the grower or the grower’s family (“berries with a face”) and the country of origin.

D 4.3 Promotion material based on marketing

Both SME’s were able to reach the target group and succeeded in reaching the aim of the tailored marketing strategy. Both SME’s explore the possibilities of social media in quite a different way, but each successful in increasing consumers awareness of the brand or berries in general. In addition, also both were able to increase quality from their own strategical perspective. SME’s communicate with the consumer about health in general terms and health benefits of berries or given as background information. Due to legislation, specific health benefits were not communicated as a single issue.

With regard to the 4 c’s each SME had their own approach, started with their own strategic plan, focussed and integrated their specific topics based on their own insights and experiences and experiences and results of other (EU) projects and research presented as input from LEI, in a way that best suits their strategy.

Concerning the consumer, each SME defined a specific target group, generally quite a broad group. Concerning the convenience, both SME focussed on quality and awareness and realized this in their own way. Concerning communication, traditional pathways are still used but both SME explore and successfully make use of several facilities of social media.
Within this process of developing a marketing strategy, in which the SME’s closely cooperate with research institutes, several valuable experiences were shared. Within this open process in which the SME’s were willing to share their confidential information a continuously process of exchange took place. The SME’s were interested in the consumer and market research they were not familiar with. In addition, they liked the way the process was structured and therefore made them conscious about the complete range of relevant issues of the whole chain.

Both SME’s were recommended to target a specific group of consumers, having a specific consumption moment in mind together with their specific product. The total concept included not only the taste and quality of the product but also it’s package. As part of this concept the communication should be in line with this, especially social media is a challenging route for this.

With regard to berries in general, the experiences of markets in UK and US and the role of government and other parties should be considered as lessons where other EU countries could learn from. Both in US and in UK, retailers placed berries at the heart of their plans. These retailers believe that the berry category is very important for turnover. Berries are an attractive product: good looking, tasty and very healthy. One should be aware that the berry consumer is quality-minded. It turned out that this berry consumer not only buys berries, but also other high quality products. Berry consumers spend more money in the store and shop more often than the average consumer. Thus, presentation of high quality berries makes consumers purchase berries again and again. This shows that retailers play an important role in increasing berries consumption for example by means of berry promotion. At the same time, some important programs have been promoted at the government level (e.g.: 5 a Day). In this way, berries consumption was given a boost.

D 4.5 Report Ex-post economic analysis and on marketing strategies.

- The economic calculations based on the technical results show promising opportunities. At this moment, these innovations for strawberry production do not lead to a higher marginal gross margin for the growers. However, these innovations have positive effects on the licence to produce and licence to deliver (less residues, longer shelf life).
- The structure of the production chain with many small growers leads to extra challenges in the chain. For example with uniformity of quality and quality itself, due to the need of an unbroken cold chain.
- Health is not a single issue to be communicated to consumers. Besides EFSA regulations that restrict health claims, some consumers groups will not respond on this. A targeted communication message towards health, fun and or completed with food safety might be more successful.
- The 4 C’s strategy led to a structured approach of a tailored marketing strategy for both SME’s.
- A strategic agenda with a multi-actor as well as multi-disciplinary approach, is needed.

Potential Impact:
The outputs from the EUBerry project affected directly on a significant number of key strategic areas including improved sustainable production, enhanced quality of life, important socio-economic factors, the knowledge-based economy, increased competitiveness and prosperity, international development as well as the advancement of fundamental science and the exploitation of knowledge. The project therefore exploited new and emerging research opportunities addressing environmental and economical challenges, the growing demand for safer, healthier and higher quality food and for sustainable use of renewable bio-resources.

In general, the EUBerry project delivered scientific excellence with impact on all the following areas:
- Improved phenotyping and Characterisation of berry Germplasm.
- Development of molecular tools for support and enhancement of berry fruit breeding.
- Validation of the role of key genes in strawberry traits.
- New cultivation techniques for season extension.
- Ensuring profitable berry production in changing climate.
- Reducing environmental impact by developing new pest and disease.
- High quality EU berry production with increased efficiency in plant growth, yield formation and cultivation techniques in different European regions, to support adaptation of production systems to changing climatic conditions.
- Fruit Organolepsis and nutritional quality. The project will establish how (micro) nutrients and beneficial human health components (predominantly polyphenols) are impacted upon by various agricultural strategies (low & conventional inputs, protected cropping etc) and how these can be associated to the standard quality traits (sugars, acids, aroma, etc.).
- New pre and post-harvest treatments for increasing stability and quality of fresh fruit.
• Validate fresh fruit value for consumer health.
• Economic viability of new berry production systems.
• Effective marketing strategies on health benefits of berries based on consumer health benefits.

Following are summarized the impacts achieved from the 4 Research and Technological Development Work Packages.

WP1 Improving berry varieties through the identification and utilisation of the best genetic resources
The sustainability of the EU berry industries depends on the availability of suitably adapted high quality germplasm to underpin production and enhance consumption. The fresh market for berry fruits within Europe is increasing across a range of crop types, but the associated industries face real challenges in the coming years, from pests and diseases, consumer demands and also from the impacts of a changing climate. As a result, there is a fundamental need for the best currently available germplasm to be clearly identified, and for breeders to be given the most appropriate tools to develop the new varieties that will be required in the future.

The deliverables in this WP have concentrated on defining the best germplasm currently available to various stakeholder groups, through trans-national trialling based in all the climatic regions of the project (D1.1 D1.2 D1.4). Through this mechanism, growers and retailers can identify the best material for their own purposes from the wide and potentially confusing range of genetic resources within the EU region. The importance of plant breeding in the future development of better cultivars and germplasm has been highlighted in several publications globally, including EU policy documents such as the Committee for Agriculture’s report ‘Plant Breeding: what options to increase quality and yields’, which stated that ‘it is exceptionally important to have an effective and competitive plant-breeding sector’. As a result, it can be seen as fundamental to the competitiveness of the European agri-food industry and the sustainability of production that breeders have access to the best sources of the traits required for the future. The EU report mentioned above also focuses on the need for ‘the development and use of new plant breeding techniques with respond to societal and agricultural demands….to enhance the competitiveness of the agricultural and horticultural sectors’. Similar views are expressed in a forthcoming European Plant Science organization (EPSO) White Paper ‘Horticulture Research in Europe – to 2020 and Beyond’, which encourages ‘the development of pre-breeding material incorporating novel desirable traits to facilitate prompt uptake by the breeding industry’.

The outcomes from the EUBerry project in developing the best contemporary technologies, including molecular breeding strategies, will play a role in ensuring the future efficiency of breeding programmes and the reduction of the significant breeding timescales involved in fruit breeding. In this project, deliverable D1.2 identified the best berry fruit germplasm for breeders to utilise, including some lines still under development, and also continued the integration of new technologies such as marker-assisted breeding strategies into the overall improvement programmes for the main berry crops, with a summary given in D1.5. A further line of development for the future was the use of functional genomics approaches in strawberry, where the results have a key role in enabling a more detailed understanding of the important traits of interest to be gained. The identification of genes and markers is part of an accumulation of genomic resources that can be accessed more widely in the future.

Overall, the EUBerry project provides a raft of resources, plant-based and genomic, that will greatly assist further development of soft fruit within the EU.

The role of plant breeding in food security is not confined to supply issues, but also requires that people have social and economic access to safe and nutritious food. In addition to the challenges for the future, the development and identification of suitable germplasm for new outlets and uses, in particular for enhanced nutritional value for dietary health and the improvement of shelf-life, to reduce waste throughout the supply chain, is both timely and necessary. Much of the impetus for this area of work is consumer-driven, with berry fruits highly regarded as part of a balanced and healthy diet. The identification of the germplasm with highest levels of antioxidants and vitamins within both WG1 and WG3 is also aligned with the mapping of key genes and marker development, where work reported in WG1 has made significant progress in this direction. The downstream value and impact of the work done in WG1 of the EUBerry project will be realised as it moves into breeding programmes at the various organisations both within and beyond the EUBerry partnership, leading to its eventual translation into new cultivars that can be grown effectively, sustainably and profitably. In several areas, such as the exchange of germplasm and also modern breeding technologies, the work of EUBerry WG1 will assist in re-establishing collaboration and integration of breeding resources and efforts between breeders and research groups, to thereby capture a ‘public good’ aspect to the development of new varieties for enhancement the supply chain throughout the EU region.
WP2 Improved cultivation techniques
The final goal for this project was to increase the safe and economically viable production of quality berries that are increasingly attractive for the consumers. WP2 focused on the modern cultivation techniques for berry season extension, on adaptation to different cultivation conditions and systems, as well as to climate change, and on reducing the impact on environment in different European regions that produce berries.

Possibilities for season extension were enhanced by developing methods to produce better planting material of strawberry with architecture and ability for higher yields by controlling nutrient use. Using covers in spring can enhance primocane raspberry harvest in spring and plant treatments like cutting delay it. In the North, light is limiting production in autumn and light emitting diode lights can be a possibility to extend season for ever-bearing strawberry cultivars and raspberry in tunnels. When prices allow, these lights can be used on berry production in greenhouses without harmful effects on predators used in biological control. Blackberry production is increasing worldwide and improved fruit firmness and shelf-life has increased consumer interest in this crop. Year-round production for blackberry fruit can be achieved with developed methods of floricanne cutting production and double-cropping in the south. Productivity is increased and season extended for the high fruit market price. For blueberry, using different cultivar types, significant season extension can be achieved in the south. Tunnel properties to ensure earlier and later production were identified: night-time ventilation and deploying an energy screen enable frost protection in late production, a fog system ensures maximal length of the spring growing season in high temperatures. Adaptation to climate change is essential for production in future conditions. With earlier growing seasons, control of low temperature stress can be very important to ensure crop. A device with low water use was designed for spring frost control and covering and bending technique to protect raspberry plants during winter. Control of high temperatures in tunnels can be achieved with shading or colored nets. In the North, tunnels can also be used to enhance plant and crop development and ensure blueberry production. Warming can be detrimental to raspberry and constant high temperatures severely reduce yield. However, tunnel covers and choice of cultivars can control these effects. Erratic bud break and less consistent fruit yields may be expected in raspberry crops in the wake of ongoing climatic warming with declining winter chill. Results also indicate that in mild winter regions artificial chilling can have a major impact on yield on primocane raspberries. In the North insufficient chilling time is likely to limit the yield performance of early forcing of long-cane raspberry crops in the greenhouse. Reducing environmental impact can be achieved in plant protection by further developing IPM methods for pest and disease control. Residue levels in fruit can be reduced by applying Decision support systems in strawberry disease control. Biological control applied against pests on strawberry and raspberry is also a competitive alternative for environmentally friendly production and combining biological and alternative control methods also reduce fungicide load in environment. In greenhouses, control systems such as UV-B light can be utilized. When biological pest control and IPM are enhanced, the natural vegetation in berry fields can be important either as a source of predators or pests.

Water is already a limiting resource for berry production in many countries and with the proceeding global warming both methods to control high temperatures and drought stress will be critical. Morphological and physiological adaptations allow some strawberry cultivars to maintain their productivity when the water availability is decreased, and they can be utilized in cultivar choice. Although raspberry is susceptible to salt, use of water with non-optimal quality is possible. To avoid leaching, nutrient input can be reduced both in nursery and fruit production, especially nitrogen use can be halved without yield loss. For a renewable substrate in soilless production coconut coir can be used to replace peat to reduce environmental impact.

WP3 Fruit quality characterization and determination
Although is difficult to access to specific and accurate EU EUBerry fruit production figures, latest figures for EU production of the EUBerry target fruit are: blackcurrant – ~200KT (2012, >90% global production, blackberry – 50KT (2005, 30% global production), blueberry 445K (fresh, 2012, 14% global production), raspberry (2012, 453K, 75% global production) and strawberry (2012, 1.3M 29% global production). Clearly there are areas for the expansion of EU production and importantly the areas for sustainable economic growth are centred on the premium fresh fruit market: if we can ensure a higher production level of premium fruit significant inroads can be made into the export markets and/or import substitution. However to do this the growers need to be confident that they know what “high or premium quality” is and how to ensure it. The outputs of WP3 have significantly underpinned this “what is premium quality” aspect by defining analytical standard operating procedures for the assessment for sensory and compositional components. We have seen these standard operating procedures taken up and utilised by the project small and medium enterprises and more widely to larger companies across Europe following the extensive dissemination activities. Furthermore, the underpinning detailed science has seen broad interest. For example, the correlation of detailed volatile and compositional data with sensory aspects allowing a more rapid
and robust objective assessment of progeny and populations in breeding programmes prior to full sensory screening has become an instrumental part of.

Allied with the aforementioned quality attributes of the fresh fruit are the nutritional and health benefits that are also driving sales. The fruit and health research undertaken here has had a significant and broad impact across the triple helix of academia, industry and government (policy). This is reflected in the publication and presentation record highlighted in the WP5 outputs. The esteem of the EUBerry outputs of the health benefits have in part resulted in the EPSO white paper “Horticulture Research in Europe – to 2020 and beyond” specifically “Fresh horticultural products are an important component of traditional diets but are also central to healthy diets of modern urban populations. They are considered as functional foods, due to their high levels of a range of valuable nutritional compounds and consequently form part of special high-value diets for infants and the elderly” with the result that “High human health value” is viewed as a priority in horticulture going forward. The results generated within the project offer great potential for dietary routes to reducing the risk of cardiovascular diseases/circulatory (the cause of mortality in the EU) and neurodegenerative/dementia diseases. It is worth noting that 2012, an estimated 8.4 million people aged 60 years and over were suffering from dementia in EU member states, accounting for 7% of the population in that age and that the direct costs of dementia account for a significant share of total health expenditure in European countries, e.g. in the Netherlands, dementia accounted for nearly 5.5% of overall health spending in 2011. Significantly, fruit studied here maintained their neuroprotective effect even following the rigours of (in vitro) digestion. Both these beneficial results have attracted significant interest from health charities, funders and policy groups keen to capitalise on fresh fruit as a relatively low cost and readily available option to reduce the risk and impact of these disease groups. Furthermore, these results have underpinned a new EU project BachBerry (www.bachberry.eu) which aims to build on the advances of EUBerry and identify key and unique fruit polyphenol biosynthetic pathways and transform these into microbes to facilitate the industrial biotechnology production and exploitation of the natural compounds in food, drink, pharma and cossmall and medium enterprisesceutical sectors.

The extensive germplasm analysis undertaken, however, gave heart to the grower and rural economies identifying significant variation within varieties and breeding lines as well as a greater variation in the wild species all of which can be exploited to develop new varieties to satisfy the industrial and consumer demand for product diversity and evolution. This compositional variation should ensure the sector is economically sustainable for some time. Allied to the germplasm assessments were studies to assess innovation in packaging and storage systems. Work with hypobaric and Xtend packaging systems identified significant shelf life benefits could be had but that they were species and variety dependent. However, some minor tailoring could see this combination leading to greater export markets for EU fresh fruit and/or import substitutions across the EU

WP4 Competitiveness and marketing strategies
The combination of both technical and economic research in one project is use full and gives extra value as the research is based on both technical and economical sustainable innovations and can lead to more successful innovations. Besides, the combined work and results can be shown to the entrepreneurs in the fruit industry and might lead to an earlier adaptation of innovations by the industry.

The use of the four C’s strategy for Small And Medium Enterprises led to a structured approach of the marketing strategy. This might lead to more successful marketing campaigns and in that case to an increased consumption of berries and other fruits. It is clear that health is not a single issue to be communicated to consumers. Besides EFSA (European Food Safety Authority) regulations that restrict health claims, some consumers groups will not respond on this. A targeted communication message towards health, fun and or completed with food safety might be more successful.

The technical innovations of the EUBerry project show promising opportunities in the economic calculations. At this moment, these innovations for strawberry production do not lead to a higher marginal gross margin for the growers. However, these innovations have positive effects on the licence to produce and licence to deliver (less residues, longer shelf life) and can therefore add to a higher demand for berries and a better situation for producers.

WP5 Impact: training and dissemination impact
Training
The most effective training activity was represented by the organization of 2 EuBerry International Berry School (IBS). In the second year, all partners were involved in the first EuBerry International Berry School (IBS). The school held at Hochschule Geisenheim University, the 5th-8th March 2013, organized by P1, and it was addressed to 25-30 early stage researchers (PhD, Postdocs) or young professional (technical staff and extension people with a bachelor or master degree) in
the berry industry. In the project webpage is available the program and the materials delivered to the student (http://www.euberry.univpm.it/node/95).

The aim of the course was to give young people an overview on: a) modern berry cultivation; b) plant physiology (including dormancy and winter hardness, flower initiation, flower mapping of strawberries) as the background for all cultivation techniques; c) healthy plant production as the basic tool for profitability of the cultivation process; d) berry quality including aroma and human health compounds, e) berry processing; and f) an international view on the berry market.

The courses of the IBS were divided in plenary lectures, seminar-like lectures and two laboratory training units. Due to the limit number of places in the laboratory courses only 28 persons out of 49 applications from 13 European countries and one overseas country were chosen according to their Curriculum to participate in the IBS. The background of the students was diverse: most of them were PhD students or work as technical staff or assistants in breeding work or research.

The students were taught by 16 teachers from 9 countries, including Adam Dale (CA) and Christoph Carlen (CH) two members of the EuBerry Advisory Board. Each subject was presented within 90 minutes.

In addition to these plenary lectures, 13 members of the EuBerry Working Groups 1 to 4, which had there yearly joint meeting from 6 -7 March also at Geisenheim, presented their current research in 30 minute talks. The complete program and material of the IBS can be found in the EUberry Webpage (http://www.euberry.univpm.it/node/95).

Different members of the Working Groups took part in the lectures and made by their long time experiences fruitful contributions to the stimulated discussions. All participants of the EuBerry International Berry School got a Certificate of their attendance.

The interest from all over Europe in the EuBerry International Berry School was very high. The students got an actually overview of the different aspects of plant physiology, berry production, berry quality including aroma, sensory evaluation and human health aspects as well as berry market and berry marketing. The talks about the current research given by the members of the EuBerry Working Groups were an excellent example of the distribution of knowledge from experienced scientists to young people working in the different part of the berry industry.

Following the success of the first IBS, in the third year, all partners were involved in the second EuBerry International Berry School (IBS) 25-28th March 2014. The school held at INRA Bordeaux, organized byP6, was addressed to early stage researchers (PhD, Postdocs) or young professional (technical staff and extension people with a bachelor or master degree) in the berry industry.

The programme overlapped thematic from lab to field. New approaches on berries concerned by genetics and genomics such as genomics on berries, molecular control of flowering or epigenetics were presented. Agronomical thematic such as the potential of the plant according to the cultural technique was developed. Biotic stresses in berries were presented. Different aspects of fruit quality and health were included in seminars.

Two laboratory training were organised on Manipulation of strawberry plant architecture and on Measurements of total antioxidant.

The different seminars were open and slides were included on the web site of the EUBerry project. In conclusion, all students were very positive on this IBS and appreciated the variety of seminars.

Dissemination

All the activities carried out during the 42 months of the EUBerry project had a high scientific and technical impact as demonstrated by the 40 scientific papers published on peer reviewed journals, 15 technical publications and many dissemination documents, published even in different languages, 3 newsletters, different podcasts, 18 dissemination and technical meetings with different stakeholders. The partners of the project presented the results (with oral and poster presentation) in more than 50 international and national conferences and symposium.

The project had a very high scientific impact demonstrated by the high number of scientific papers published on international reviewed journals. Two special issues on international refereed scientific Journals we also published. The complete list of paper published is available on the Euberry webpage http://www.euberry.univpm.it/node/19

Furthermore, a very high dissemination impact, at international level, was also achieved with large participation, with a high number of oral and/or poster presentations, of partners to many international Symposiums and Conferences, as following listed:
2014
• 29th International Horticultural Congress, IHC 2014 Brisbane, 17-22 August 2014
• Berry School, Bordeaux, 25-28 March 2014
• 8th World Congress on Polyphenols Applications, June 5-6, 2014, Lisbon- Portugal
• Summer School on Nutrigenomics, 1-5 September 2014 - Camerino, Italy
• The European Strawberry Congress 4-6 September 2013. Hoogstraten, Belgium.

2013
• EUBerry WP2 meeting at Bioforsk Grassland and Landscape Kvithamar, Stjørdal, Norway, Wednesday, September 25, 2013.
• Seminar of WP2- Tuesday 24 September 2013- Improved cultivation technique of berries - Bioforsk Grassland and Landscape Division Kvithamar, Stjørdal, Norway
• The 2nd International Strawberry Congress in Antwerp, Belgium. 4-6th September 2013
• EuBerry International Berry School (IBS) 5th-8th March 2013
• EUBerry General Assembly, Maastricht, 19th June 2012
• 10th International Symposium on Vaccinium and other Superfruits, June 17-22, 2012
• 2nd International Blackcurrant Association conference, Dundee, Scotland May 16-18 2012
• 7th strawberry Symposium, Beijing 2012
• Blueberry meeting, Amarante 2011

2011
• 2nd Management Committee & Working Group Meetings of COST ACTION FA1005, Le Croisic, France, 2011.
• 5th International Conference on Polyphenols and Health, Sitges, Barcelona, Spain, 2011.
• 1st International Conference on Food Digestion, Italy, Cesena, 2012
• 10th Symposium on Vaccinium and Other Super Fruits, Maastricht 2012

Finally, a large dissemination activity was carried out also with publication on technical journals and newspapers of national and international (eg. International Innovation Issue 118). See link on news of the EUBerry webpage
http://www.euberry.univpm.it/press
All these activities were coordinated thanks to the organization of a kick off meeting, 3 general assemblies and about 14 group meetings. An intense organizational activity was also carried out by email and with web conferences.

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