



# Dairy Product Technology 2

## NFOK16006U

**Notes taken during the course, including lectures, exercises, curriculum, and practicals**

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Last compiled: 06-11-2025

[Link to GitHub repository](#)



# Preface

These course notes have been prepared as part of the NFOK16006U course Dairy Product Technology 2 at the University of Copenhagen, covering the period from November 2025 to February 2026.

The notes compile material and reflections relevant to the course and are intended as a resource to enhance the learning experience for students. The content is shared freely and may be used as study material or as a template for structuring individual notes.

All information is provided without responsibility for its correctness, and users are encouraged to verify data, formulas, and interpretations with the original sources and course materials.

Please enjoy reading these notes, and feel free to reach out if you have any questions.

# Course Description

## Education

MSc Programme in Agriculture

## Content

The aim of the course is to give students a detailed knowledge of both theoretical and practical aspects of cheese and fermented milk production, characterisation, technology and biochemistry.

## Learning Outcome

The course is targeted to students interested in plant science (Horticulture and Agriculture) and food science students who are particularly interested in fruit and berry crops and the quality and use of the raw materials/food products these crops provide.

### Knowledge

- Understand chemistry, biochemistry, microbiology, physics and technology in the production of cheese and fermented milks.
- Understand the characteristics of, and basic differences between, cheese groups and varieties.
- Understand the use and application of dairy enzymes in cheese and fermented milks

### Skills

- Apply critical analysis of cheese and fermented milk quality and characteristics.

### Competences

- Ability to use and evaluate scientific information and knowledge concerning cheese and fermented milks, incl. all steps of production and ripening.
- Ability to comprehend the technology behind different cheese varieties and fermented milk types.
- Capacity to critically evaluate laboratory results and pilot plant trials with regard to the possible development of innovative cheese and fermented milks and/or processes.

Academic qualifications equivalent to a BSc degree is recommended.

# Teaching and Learning Methods

The course involves lectures and laboratory practicals, workshops and oral presentations, literature studies, patent searches and report writing. The student must obtain their own laboratory results and evaluate them in relation to theory given at lectures and found in literature, including critical use of electronic data bases and internet. Results from all students' practicals and literature studies will be merged during oral presentations and discussions in workshops.

## Workload

**Table 1:** A table with an overview over the workload for the course.

Category	Hours
Lectures	32
Class Instruction	15
Preparation	75
Practical exercises	75
Project work	8
Exam	1
Total	206

## Exam

**Table 2:** A table with an overview over the elaborated description of the course

Credit	7.5 ECTS
Type of assessment	Oral examination, 20 minutter
Type of assessment details	3 weeks before the examination, all examination questions (covering the essential issues dealt with in the course) will be published on Absalon. The students are expected to use all of these questions to be fully prepared for the subsequent oral exam. At the oral exam itself, one of the questions will be drawn by the student, and the examination immediately proceeds. For the question drawn, all aids are allowed during the exam (approx. 15 minutes). The last 5 minutes of the exam will be used for questions in relation to the reports prepared from the laboratory practicals.
Examination prerequisites	Attendance at all laboratory practicals is compulsory. All laboratory reports must have been approved.
Aid	All aids allowed <a href="#">Read about how to use Generative AI on KuNet</a>
Marking scale	7-point grading scale
Censorship form	<ul style="list-style-type: none"><li>• No external censorship</li><li>• Several internal examiner</li></ul>
Re-exam	Same as ordinary exam. Non-approved reports must be handed in three weeks prior to the re-exam and approved 2 weeks prior to the re-exam.

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# Chapter 1

## Lecture Notes

### 1 Introduction

The following lecture notes have been compiled by combining personal notes taken during class, personal highlights from the lecture slides, and targeted use of generative AI. The AI was provided with my notes and the highlighted slides, together with carefully designed prompts, to ensure that it focused on the most relevant and interesting aspects of each lecture. This approach aims to produce a coherent and focused summary that reflects both the course content and my individual learning perspective.

### 2 Lecture 01: Introduction - 01/09-2025

**Fruit and Berry Crop Physiology and Quality or Fruit and Berry Crop Physiology, Quality and Use**  
**Professor: Torben Toldam-Andersen, KU-PLEN**

The course aims to provide broad knowledge on the physiology and growing of fruit and berry crops (including pit-, stone-, cane-, and bush fruits, other berries, nuts, and fruit vegetables), focusing at the whole plant and organ level. Key objectives are the development of specialized knowledge on a course topic, the ability to analyze a crop to identify important aspects in growing and quality, and understanding how fruit quality is influenced, specifically balancing productivity vs. quality. The curriculum seeks to build links between the production and quality of raw materials and the food science aspects of their use, including sensory aspects. The underlying idea is to address both the Plant science and the Food Science aspects of fruits and berries, emphasizing the importance of these crops for human health and addressing lacking knowledge regarding the potentials in different genotypes (raw materials).

The course focuses primarily on temperate fruits, nuts, and berries grown mainly in open field or tunnel systems. While fruit vegetables such as tomato may be included in the individual report, the emphasis includes common commercial systems, organic growing, and relevance for small-scale/home gardening. These crops are characterized as Diverse (many species and cultivars), high value crops, demanding intensive hand labor and resources, and often requiring manipulation at the single plant level. Although often fresh consumed/used, there is a large diversity in processed uses, such as juice, jam, fruit wine, vinegar, cakes, desserts, and ice cream. They are important to human health and provide pleasure through nice aroma and taste.

The course content is structured around eight main topic areas:

1. Genetic basis and Cultivar variation

2. Yield and quality components and determinants, including Organ development and interactions
3. Allocation of dry matter and nutrients, focusing on 'Sources' and 'sinks' in fruiting plants
4. Effects of pre-harvest factors on fruit quality, such as the importance of control of plant vigour and shape
5. Content and development of secondary compounds in fruits
6. Maturation, ripening, and assessment of optimal harvest time and quality aspects
7. Post-harvest usability (fresh consumption, cooking, industry processing, especially juice)
8. Sensory aspects of quality evaluation

Pedagogic methods utilize a mix of Lectures, Exercises ('Hands on'), Written assignments, Student lectures, and Excursions. Specialization is achieved through the individual report and the student lecture, which provide case specific supplements to the general lectures. The individual report is an opportunity to develop specific competence on a free topic relevant to the course (e.g., Bud dormancy, Aroma development, Canopy management). The report structure mandates an Intro (1-2 pages), Detailed presentation and discussion based on international literature (4-6 pages), and Summary/conclusion (½ - 1 page), not exceeding 8-9 pages in total (max 10 pages including figures). Students must use their own writing and avoid 'copy-paste' and plagiarism.

The assessment is an Oral exam lasting 25 minutes in total. The exam includes a short talk about the individual report, followed by two curriculum questions: one focusing on crop physiology and one on quality aspects. The final grade is based on 50% the individual report and 50% the curriculum questions.

### 3 Lecture 02: Yield and quality components - 01/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

The overarching aims in fruit growing are to maximise fruit yield and maximise fruit quality. The tools utilized to achieve these aims include Genetic choice (cultivars) and Growing technique. Fruit science focuses heavily on the physiological background informing the growing technique. Maximized Yield is typically expressed as tons/Ha (or hekto L/ha of juice or wine) and is fundamentally a product of the components Fruit number X Fruit size. Determinants influence the size or the level of development of single components within the genetic potential. These physiological elements are connected to growth and development and can be influenced by growing techniques, climate, and growing conditions.

Maximized fruit quality is less clearly defined than yield and may vary with fruit species and final use. Important quality parameters include Colour and other aspects of appearance (especially for Fresh consumption), Taste components (sugar, acid, aroma), and 'Health components' (vitamins, phenols ..... ) which are a focus of research. Fruit size serves as both a yield and a quality component. The presence of unwanted substances such as pesticides is also an important aspect of fruit quality. The optimal compromise is crucial because physiological and technical factors may have opposite effects on yield and quality components. The environmental impact of the growing technique used, such as carbon footprint and pollution, also requires knowledge.

The physiological elements that serve as yield and quality components and determinants include:

1. Planting system and Growing system (fx Rubus, strawberry), determining Number of plants/ha.
2. Plant size and structure, including Elongation growth, shoot type development, Bud development, and Flower bud initiation.
3. Bud number/plant, bud type, and the ratio of leaf bud/flower bud.
4. Flower development, specifically Number of flowers/cluster and Flower quality (e.g., Number of seed primordia, Position in cluster).



5. Pollination, fertilization, and initial set, defining Initial fruit number/flower.
6. Fruit drop (June drop), which determines the Final fruit number/initial number of fruits.
7. Fruit growth and fruit development, the Leaf/fruit ratio, and specific factors like Number of seeds/fruit (Pollination) and Amount of flesh/seed (in Strawberries), leading to Fruit size and quality.
8. Yield/ha (Fruit number x fruit size) and Fruit quality (content).

Specific crop examples illustrate the variability: Sour cherry yields may vary drastically, from 3 to 18 tons/ha, due to factors like Bud death (sometimes reaching 90%) and Fruit set percentage. Unstable yields in sour cherry 'Stevensbær' pose a significant problem for the Market (Industry). In strawberries, vegetative growth (runner production) is influenced by long days and high temperature, while flower cluster formation is promoted by short day and low temperature. Pollination is very important in strawberries as the final fruit size depends on the Achenes/berry. For grapes, yield components include Number berries/cluster x weight/berry and Number of clusters x weight/cluster, influenced by factors like pruning, thinning, and the growing system. Analysis of 51 grape cultivars harvested in 2018 showed a high correlation ( $R^2 = 0.9516$ ) between Yield kg/plant and total sugar (Fruktose + Glukose g/plante).

## 4 Lecture 03: Bud and shoot development - 01/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

This lecture explores Bud and shoot development, covering Meristems, Bud types and shoots, and seasonal Growth patterns. Embryogenesis initiates plant development by establishing the primary meristems and determines the Apical ↔ basal axial development, including the Shoot apex, Hypocotyl (The stem), Root, Root apical meristem, and Root cap. It also establishes Radial patterning, such as Epidermal cells, Cortical tissue, and the Vascular cylinder. The initial stages of development include Cotyledons (The first leaves). Development transitions from meristem to shoot, detailing the structure of the shoot apex in 3D and the Transition from vegetative to floral meristem. Variation exists among species in how strong the xylem or wood develop, with Trees having strong development and Bushes and canes exhibiting weak development. The purposes of leaves include Production of assimilates (the source of carbon), Water transpiration resulting in uptake and transport of water and nutrients, Production of hormones, and Control of water status/cooling.

Buds are defined as locations of growth with a potential for development. The Types (fates) of buds include Vegetative, Generative, Sleeping (not dead), and Dead. Buds determine the plant's dimensions by forming shoots of different lengths, such as Short shoots (spurs), emphasizing the Importance of the shoot's position. In strawberries, the vegetative bud results in a side crown or a 'runner'. Pome fruits, exemplified by apple and pear, possess mixed buds, while Stonefruits utilize 'naked' buds. The topography of flowerbuds differs between pome- and stonefruit, which raises questions about which structure bears flowers in the terminal position on short shoots and the Importance of this difference.

Seasonal growth patterns involve a Flush of growth in spring and early summer, followed by Growth termination or indefinite growth, depending on the species. Terminal bud formation also occurs, alongside Adventive buds formed along with shoot development. The timing of terminal bud formation depends on Shoot type (short spur is early, long terminal shoot is late) and Vigour level (strong is late, weak is early). These factors interact: low vigour results in fewer and more short shoots, whereas high vigour leads to more and longer shoots.

Factors influencing seasonal growth include:

1. Shoot type and vigour.

2. Correlative inhibition within the plant, defined as 'communication among buds'.
3. Apical dominance, which influences adventive buds and inhibition. This involves Polar gravitropic transport and the activation of adventive buds upon Removal of the terminal bud. The underlying Auxin - Cytokinin balance varies among species (peach < apple < sweet cherry) and strongly influences the Branching pattern.
4. Competition for assimilates, specifically the relationship between fruits ↔ vegetative growth.
5. Number of growing meristems in top/root ratio.
6. Tree age, where Older trees produce many shoots but exhibit weak growth, and Young trees produce few and strong shoots.
7. Management and climate factors, such as Pruning, water and nutrient availability.

Development after terminal bud formation Happens suddenly, and differentiation continues inside the bud(s). A compact shoot is formed, and development continues until bud break next spring, though the intensity of this differentiation varies (dormancy).

## 5 Lecture 04: Flowers and flowerbud development - 05/09-2025

**Torben Toldam-Andersen, KU-PLEN**

The lecture addresses Flower and flowerbud development, encompassing Phases and theories, Structure - node development, Factors, and specific examples including Apples, Cherry, Currant, Raspberry, and Strawberry.

Flowerbud formation occurs across three distinct phases:

1. Induction (May - June)
2. Initiation (July - September)
3. Differentiation (July - May)

Historical theories regarding flowerbud formation include the 1822 concept that the flower bud is formed through April - June (induction) and then 'filled out' through July - October (initiation - differentiation). Later, the 1920's highlighted the C/N-ratio as decisive, while the 1970's focused on the Cytokinin/Gibberellin-ratio and Source-Sink relationships, stressing availability and activity. Node development speed influences the bud's fate: slow development results in remaining Vegetative, while relatively fast development leads to Floral buds. The time required for node formation is termed the plastochron.

The structure of the flower bud is akin to a compressed shoot. In apple, a flower bud contains 3 bracts, 6 real leaves, 3 transitional leaves, and 9 bud scales, totaling 21 nodes, with a minimum critical node number approximately 18-20. The flower organs are formed sequentially from the outside inward: sepals are formed first, followed by petals, which are formed second. The 'King flower' is formed first. Pomefruit (apple, pear) flower buds are "mixed" buds, often terminal, and are considered "strong" buds (high critical node number). Apple buds are less developed in the dormant stage, with a larger proportion of differentiation occurring in the spring. Conversely, Stonefruit (cherry, plum) flower buds are "naked" buds, always leaf buds in the terminal position, and are "weak" buds (low node number). Cherries reach an advanced stage of development before entering dormancy, which may render them more sensitive to winter damage. Sour cherry (*Prunus cerasus*) leaf buds contain 7 nodes, while flower buds contain 13 nodes.

Flower bud formation in tree fruit is affected by growing techniques related to Vigour or Source-sink dynamics:

1. Variety

2. Tree age
3. Rootstock
4. Dormant pruning
5. Root pruning
6. Light condition
7. Fruit load
8. Thinning
9. Nutrition
10. Irrigation

For shoot growth, a minimum length (e.g., >15 or preferably 20 nodes per shoot) is necessary before initiation. Initiation usually spreads upwards the shoot, except for the terminal bud, and lower buds do not form flowers. Very strong (long) shoot growth, potentially induced by Gibberellins, may counteract formation. Short shoots (spurs) easily form flowers and show greater bud density, a higher percentage of live buds, a higher percentage of flowerbuds, and more flowers/bud than long shoots. Climate factors play a role, as flower initiation normally happens if day length is <12-14 hours, and decreasing temperature has a positive effect.

In raspberries (*Rubus*), initiation requires reaching a minimum physiological stage, and is promoted by short day and low temperatures, typically starting from the top of the cane earliest in shoots where growth has terminated. Normally 2/3 of buds become flowers, but low positions on the cane are usually without flowers. For yield, the Number of laterals/cane and flowers/lateral are the most important components. Strawberries exhibit types ranging from obligate short day (June bearers), where day length is critical (<11-14 hours), to day neutral types that initiate at both short and long days. Vegetative growth (runners) is promoted by long day and high temperature. Warm periods in late autumn have positive effects on flower development, leading to more flowers/bud. This differentiation affects flower quality; for instance, strawberry 1. order flowers have 400 pistels, while 4. order flowers have only 80 pistels. In currants, the terminal flowers in the string are often poorly developed, resulting in few seeds, small berries, and a high risk of drop.

## 6 Lecture 05: Bud dormancy - 05/09-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture details Bud dormancy, covering its development, breaking, and specific studies on Sour Cherry 'Stevnsbær' buds in winter. Key concepts include Growth phases during the year, Dormancy terminology, and Metabolic activities during dormancy. The lecture introduces the concept of chilling units (CU) and Growing Degree Hours (GDH). Chilling requirement estimates for various fruit and nut species are noted. Dormancy models, including the calculation of Chill Units (Saure, 1985), and the Growing Degree Hour model are essential tools. A GDH curve was specifically developed for 'Montmorency' cherry (Anderson and Richardson 1986). Studies examining 'Redhaven' peach flowers and lateral/terminal buds explored the effect of temperature and length of chilling period on percent budbreak and GDH demand for budbreak (Scalabrelli and Couvillon 1986). Chilling periods of 600, 1340, and 2040 Chilling Hours were analyzed.

The yield components of sour cherry are multifaceted and include:

1. Tree size
2. Shoot number and type
3. Bud type
4. Number of flower buds

5. Flowers/bud
6. Bud death
7. Fruit set%
8. Number of fresh flowers in spring
9. Initial total number of fruits
10. Fruit drop
11. Final number of fruits
12. YIELD

Floral bud mortality is a critical factor. Dormancy levels in sour cherry buds can be determined by forcing shoots. Studies investigated the effects of irrigation and nutrients. Forcing of buds showed an increase in water content of forced buds over the period of October 4 to November 29 when under +drip conditions (both tip and base) compared to -drip. Shoot studies indicated a relationship between High water content and high bud death, and Low water content and low bud death. Observed damage included a missing pistil or a necrotic pistil.

Research examined the Effects of urea (0.75% and 4% urea) on sour cherry. In May 1996, the 4% urea treatment significantly reduced Living buds (79.3% compared to 91.3% in Control, LSD 4.0) and decreased Flowers/tree, Fruit set% (7.9% compared to 12.8%), and Berries/tree. Shoot length and Buds / meter shoot were not significantly affected. Furthermore, in November 1998, buds on annual shoots treated with 4% Urea showed reduced ABA content (1675 ng/drymatter versus 3948 in Control) and increased Water content (118.0% of drymatter versus 108.1% in Control), although Bud size was not significantly different. Freeze tests were conducted to determine the hardiness level in 'Stevnsbær' at -6°C (laboratory) and -7°C (field). Bud death was analyzed in relation to rootstock (Weirroot, Colt, Pavium Grundstamme) and frost exposure during winter. Counting flowers is noted as a considerable task.

## 7 Lecture 06: Flowering, pollination and fruit set - 08/09-2025

**Torben Toldam-Andersen, KU-PLEN**

The physiological processes of flowering, pollination, and fruit set are critical determinants of Yield, alongside factors such as Flower number and quality, Effective Pollination Period (EPP), Pollen quantity and quality, Pollen transfer, Initial fruit set, and June drop, leading to the Final fruit set. Flower quality is defined as "The capacity to develop into a fruit if pollinated with the right pollen at the right time". Indicators of flower quality include Ovule longevity, Flower weight, and Number of flowers per cluster (position in cluster). Stone fruit quality depends on carbohydrate reserve availability, while pome fruit quality depends on burse leaf area.

Fertility varies, categorizing species as Self fertile (Self compatible), such as apricots, peaches, strawberries, and grapes, or Self sterile (Self-incompatible), such as hazel, pear, and apple. Sterility is not absolute; in normal self-sterile conditions, own pollen mostly fails, but cross-pollination often results in faster pollen tube growth. Parthenocarpy is fruit development without fertilisation, without seeds. Some plum cultivars, such as Opal and Victoria, are self-fertile. Self-incompatibility is controlled by S alleles at the S-locus. Self-fertile sweet cherry cultivars (e.g., Stella, Lapins) are universal pollinators, often possessing a mutated S4 allele. Parthenocarpy or Apomixis (fruit or seed development without fertilisation) may be mistaken for self-compatibility.

Important flowering related terms include:

1. **Monoecious:** Having pistillate (female) and staminate (male) flowers on the same plant.

2. **Dichogamy:** Opening of male and female flowers at different times on a monoecious plant, which ensures cross-pollination.
3. **Protandrous:** Male flowers open before female flowers.
4. **Protogynous:** Female flowers open before male flowers.

Pollen tube growth requires its own reserves at the stigma surface, followed by interaction with style tissue (proteins, carbohydrates). The Effective Pollination Period (EPP) defines the time when fertilization and fruit development occur. EPP varies with flower quality and is reduced by factors such as low nutrient status and high cropping level.

The required quality of pollination depends on the species:

1. For **large fruited species** with intensive flowering, pollination does not need to be optimal, but should be optimal if flowering is weak ( $< 40 - 50\%$  of max). Heavy crop load leads to small fruits.
2. For **small fruited species**, yield is mainly determined by fruit number, and pollination should always be optimal.
3. For **species with several pistels - embryos** (where fruit size is seed-number related), pollination should be optimal.

Pollinator demands include Quantity (e.g., Malus types like M. Floribunda), Quality (compatibility, ability to germinate at low temperatures), and Timing (overlapping flowering periods). Triploid cultivars (e.g., Gråsten apple) do not produce fertile pollen.

Pollen transfer occurs via Wind (for self-fertile species like sour cherry), Insects (for cross-pollinators), or artificially. Critical distance for transfer is 10-15 m. Honeybees (47%) and Soil living bees (25%) are common pollinators. Critical flying limits are 13-14°C for Bees and 8-12°C for Bumblebees. Bumblebees visit 2-3 x as many flowers as bees. Experimental trials on 'Stevnsbær' sour cherry showed that the addition of 'artificial wind' (6 km/t) increased fruit set (%) and yield (kg/tree) compared to controls, and that early pollination (Day 1) resulted in higher fruit set than later pollination (Day 2). Pollinator branches and hand pollination also improved fruit set, demonstrating that pollination is often not good enough.

## 8 Lecture 07: Fruit Thinning - 08/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

Fruit Thinning is a necessary practice carried out in Large fruited species, specifically Apple, Pear, Plum, Peach, and Grapes (where grape clusters may be seen as similar to a big fruit). The primary objectives of thinning are to maximise fruit size distribution, prevent the breaking of branches, improve flower development, and stabilize cropping (prevent alternance). Thinning also aims to improve quality, resulting in a higher sugar%, Dry matter+acid, and potentially improved firmness, although larger fruits are naturally softer. A negative aspect is that Increased size may lead to an increase in 'Bitterpit'.

The Timing of thinning is crucial. Early thinning is important as it affects both return bloom and fruit size. Late thinning has no effect on return bloom, and the effect on size decreases with delay in time. Very late thinning (starting at ripening) primarily affects quality by increasing sugar accumulation during ripening. Thinning timing effects on size were observed in apple, and in 'Clara Frijs' pear on three different rootstocks.

Thinning can be performed using various methods:

1. Hand thinning.

2. Hormones: BA (benzyladenin/Cytokinine), Ethephon, NAA (1-naphthylacetic acid, 'Pomoxon'). (Note: NAA is Not registered in DK).
3. Fertilizers: ATS (ammoniumthiosulfate), lime sulphur.
4. Organic compounds: Oils, soaps, salts.
5. Other: shade, mechanical.

Fertilizers such as ATS (ammoniumthiosulfate) function by burning the stigma and are applied before pollination, often requiring several applications to be effective. ATS may burn leaves, but is low cost, effective, and environmental friendly. To reduce leaf damage, fast drying must be ensured by using a low amount of water and spraying on dry leaves, and the weather must stay dry after spraying. Urea is similar to ATS but less effective, and Lime Sulphur is used in plums but is less effective in apple. The general mode of action for many organic compounds tested is the burning of the stigma, often associated with leaf damage when effective.

Mechanical thinning, developed in Germany, requires optimized tree training, but risks removing the highest quality buds. Constraints include thinning delicate and fragile fruitlets at an early stage without bruising the remaining fruit or removing too many leaves and spurs. Strong and sturdy training structures are necessary, especially when using M9-style rootstocks whose trees are firmly attached to the top wire. Shade experimentally induces thinning through increased internal competition and can be applied for a short period. Leaf damage caused by fertilizers is avoided in shade methods because the resulting permanent reduction of carbon supply is not wanted.

Factors affecting the efficiency of thinners include Temperature and humidity (increased effect with high temp and high humidity), Volume of spray, Additives (surfactants, which can cause leaf damage), and the benefit of Repeated treatments. Cultivar differences exist, as Strong flowers are difficult to thin. Thinning is enhanced if trees have a weak root system, drought stress, Nitrogen deficient status, few flowers in the cluster, large yield the year before (indicating weak flowers), young trees in strong growth (high vigor), or bad pollination (few bees). Thinning is decreased if trees are in good balance (growth and nutrition), fruits sit on horizontal or weeping branches, many flowers are in clusters with well developed leaves (strong flowers), trees are open, or pollination is optimal.

Thinning serves as a growth control tool. If growth is weak, thinning should be early, especially on weak shoots. If growth is strong, thinning should be late, keeping most fruit on strong shoots. The top of the canopy can be used to judge vigour, particularly in species like Solaris which has a strong canopy. Management techniques such as Topping send carbon down to clusters, enhancing growth in shoots which are not topped and stimulating lateral development. Removing inner leaves ensures good ventilation and facilitates later 'green harvest' or thinning. Furthermore, dense clusters may be 'tipped' before they close up, and big clusters (Fx Bolero) may be reduced in size. A high crop load can be sustained on a strong root stock.

## 9 Lecture 08+09: Source-sink and carbon allocation - 08/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

This lecture addresses Source-sink and carbon allocation, focusing on definitions, effects, internal competition, seasonal changes in allocation, and the relationship between source-sink dynamics and fruit size. The basis of growth is founded on the fact that fruits generally contain about 80-90% water, and of the dry matter, at least 90%

originates from assimilates formed in the leaves. Assimilates must be translocated from leaves to fruit. The end product exported is mostly sucrose, but in Pome and Stone fruit, sorbitol is the end product.

The source strength is defined as the ability of assimilating tissue to produce and export assimilates, quantified by Source size (leaf area/leaf number) multiplied by source activity. Sink strength is the ability of importing organs to attract and metabolize assimilates, quantified by Sink size (fruit number, number of growing shoot tips) multiplied by sink activity.

Cropping significantly impacts plant physiology, as demonstrated by apple studies. High cropping stimulates Photosynthetic intensity (140-210 relative to 100 without/few fruits), Translocation intensity (160-175), and Stomata opening degree (165). However, high cropping shifts Dry matter production allocation: while total dry matter production increases slightly (112 vs 100), the dry matter allocated to roots drops sharply (<1 vs 23), and 81 relative units are allocated to fruits. The sugar + sorbitol concentration decreases during high cropping (80 relative units). Increased  $CO_2$  level leads to increased Sorbitol% (13.4 vs 12.2), Sucrose% (3.6 vs 2.7), and Transported  $14^\circ C$  (% of uptake: 59.6 vs 53.6).

Internal competition involves 'Supply - Demand'. Apple has a strong sink strength but a relatively weak source, whereas Black currant has a weak sink strength but a strong source. Internal competition, characterized by the Fruit/leaf ratio, is strongly important in large fruited species such as apple, pear, plum, and large sweet cherry, necessitating thinning. However, the effect is weakly important in small fruited species like currants, blueberry, raspberry, and sour cherry. Sour cherry 'Stevnsbær' showed no difference in total dry matter (%), sugar (%), acid, or colour when comparing low (7 kg/tree) versus normal (25 kg/tree) cropping levels.

Source and sink strength of organs vary dynamically with development and time. Source strength increases with shoot growth, light, and temperature. Types of competition include internal competition between shoots and fruits, and between fruits and flowers.

There are two primary ways to increase fruit size:

1. By changing the fruit/Leaf ratio, thereby increasing Assimilate availability, which results in a Positive correlation between size and dry matter concentration (internal quality).
2. Through an increase in sink strength (or sink 'vigour'), such as by K fertilisation, which results in a Negative correlation between size and dry matter (internal Quality).

At the tree level, no correlation exists between size and quality.

## 10 Lecture 10: Table Grapes - growing techniques and cultivars - 12/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

This lecture focuses on aspects connected to the tunnel growing of table grapes and berries, specifically within an unheated tunnelsystem. The slides cover inherent benefits and challenges of this system. The experimental tunnel was established in 2007, and is relatively small (5m wide, 2,75m tall). It uses an internal trellis system allowing training in 3 levels (low, medium, and high stem plants) for approximately 120 plants. The training system is simple and similar for all: 2 Codon on low, medium or high trunk height, typically using short 2 bud stabs later developed into double stabs with (3)-4 shoots. Ventilation is implemented in both sides and utilizes a fan.

Management priorities are defined across seasonal stages:

1. **Budbreak and the early growth:** Focus on the importance of humidity, Shoot thinning (when and how), selecting the best shoot on a spur to carry the fruit, and selecting the best shoot for next year.
2. **Flowering and fruit set:** Identifying Important factors and management, and Cluster thinning?.
3. **The summer period:** Canopy management (How are the shoots treated?), and managing Diseases (which are most important? prevention or reduction methods?).
4. **Late summer and autumn:** Fruit development (including cluster thinning and shaping), determining what to do, when, and why, and establishing When to harvest?.

The descriptor system used for testing table grape cultivars in Denmark (VitiNord Nov 2015) included 38 cultivars. The descriptor employs a scale from 1-9 for scoring parameters, where 9 is generally the best, most wanted, or most attractive. However, the scale may be modified so 1 may not always be the 'less wanted', or 5 may be the best from a growing perspective. The system uses 25 Culture technical parameters (e.g., C1 Vigor, C3 Formation of laterals, C6 Fruit-fullness from the second eye, C17 Resistance to peronospera), 36 Technological parameters (e.g., T5 Cluster weight, T9 Berry shape, T23 Sugar content when ripe, T30-32 Aroma), and 25 Morphological parameters.

Specific parameters reflect desired growing characteristics; for example, T7 (Berry weight at BBCH 89) scores 9 for Very large (>7 g), while C3 (Tendency to formation of laterals) scores 9 for Very weak occurrence (1 lateral/main shoot) because low occurrence saves Work with canopy management. C10 (Density degree of the cluster) rates both very dense (1) and very loose (1,1 or 3,3) low, while Appropriate (7) and Very appropriate (9) densities are rated high. The Relative productivity index (C25) is calculated as (cluster number/no of main shoots) x weight of 1 cluster in gram. Cultivars are ranked based on an average equivalent score derived from summing and normalizing the Culture technical and Technological parameter scores.

Highly ranked cultivars include Augustostuzi Muskotaly, Conegliano 218, New York Muscat, and Palatina. These cultivars generally achieve a high sugar content (score 7) of about 19% brix, and acid levels (score 5-7) between 6 to 6,8 g/L. The cultivar Augusta exhibits medium vigor, Above medium fruit fullness from eye no 2, and Good resistance against Oidium, Peronospera, and Botrytis. Augusta clusters are large (780 g), and the berries are very large, exhibiting a very fine crunchy structure and very high eating quality. Augusta begins harvest in week 37 in the plastic tunnel and provides a qualitative and quantitative improvement in relation to its parents, 'Italia' and 'Königin der Weingarten'.

## 11 Lecture 11+12: Fruit development of large fruited species - 15/09-2025

**Professor: Torben Toldam-Andersen, KU-PLEN**

The development of large-fruited species involves examining fruit structure, growth curves, volume changes in apple, gravity, intercellular air space, and changes in fruit shape during development. Fruit shape, as noted by Westwood (1978), is influenced by climate, with cool climates often resulting in more pronounced shapes (greater length/diameter ratio) compared to warm climates. Pear shape (e.g., 'Conference') is known to be affected by seeds, as demonstrated by parthenocarp fruit. Fruit size is determined very early, influenced by cell size and thinning practices. The relationship between fruit size and yield dictates that a significant leaf area is required per fruit; for example, 30 leaves at 20 cm<sup>2</sup> per leaf equals 600 cm<sup>2</sup> or approximately an A4 sheet/fruit.

Quality effects observed in plums (Vangdal 1982) show strong interactions between fruit load, size, and sugar content. For instance, reducing the number of fruits per spur significantly increases sugar content; fruits/spur at



0.14 resulted in 11.1% total sugar and 7.6% sucrose, compared to 0.98 fruits/spur yielding 5.6% total sugar and 2.4% sucrose. Similarly, larger fruit diameter (>40 mm) correlates with higher sugars (13.1% total sugar) than smaller fruit (30-35 mm, 11.1% total sugar). Increased skin color (80%) also shows correlation with increased sugar levels (13.2% total sugar).

Yield relations in fruit growing can be modeled as a Michaelis-Menten kinetic system. If  $Y$  is yield t/ha and  $x$  is the number of fruits/ha, then  $Y = (k \cdot x)/(K + x)$ . Fruit size is defined as  $Y/x = k/(K + x)$ . The physiological parameters  $k$  and  $K$  are crucial:  $k$  represents source activity, determined mainly by total assimilate production (potential max. yield), and  $1/K$  represents sink activity. The term  $k/K$  is the potential maximal fruit size. Comparison across different tree statuses shows that older, well-established trees typically exhibit lower sink activity ( $1/K$  lower) but possess a larger source capacity ( $k$ ) than young trees. Dense trees demonstrate lower source activity.

Internal quality, maturity, and harvest time are studied alongside external qualities like size, focusing on internal chemical changes during development. Apple C-14 labelling studies conducted end June and end July tracked chemical changes. In apple fruit development, both sorbitol (Sor) and sucrose (Suc) are unloaded into the cell wall space. Sor is taken up via sorbitol transporter (SOT). Suc is transported either directly by sucrose transporter (SUT) or converted to fructose (Fru) and glucose (Glc) by cell wall invertase (CWINV), followed by transport via hexose transporter (HT). In the cytosol, Sor converts to Fru via sorbitol dehydrogenase (SDH). Suc converts to Fru and Glc via neutral invertase (NINV) or to Fru and UDP-glucose via sucrose synthase (SUSY). Glc and Fru are phosphorylated by hexokinase (HK) and fructokinase (FK). F6P enters glycolysis/TCA cycle, and G1P is utilized for starch synthesis. Sucrose can be re-synthesized using UDPG and F6P via sucrose phosphate synthase (SPS) and sucrose-phosphatase (SPP). Finally, most non-metabolized Fru, Glc, and Suc are stored in the vacuole using special tonoplast transporters, where Suc can also be broken down by vacuolar acid invertase (vAINV).

## 12 Lecture 13+14: Fruit development of small fruited species / berries - 15/09-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture details the development of small fruited species, focusing on fruit types, growth phases and curves, size factors, chemical changes, and mechanisms for sugar and acid accumulation. Aggregate fruits are derived from many ovaries. Growth curves show that berries and stone fruits have a distinct Stage II characterized by slow size enlargement. A berry, such as a grape, gooseberry, or a currant, is a multiseeded fruit derived from a single ovary. The cell division period, specifically the first weeks, is critical in fruit development for both small and large fruited species. Fruits primarily consist of water (85-90%) and 10-15% dry matter, with about 90% of the dry matter originating from assimilates produced in the leaves.

Internal competition can be very strong during the cell division phase due to vigorous growth in all organs. Fruit drop in black currant plants was observed in shaded plants, but effects were minimal or absent in sun-exposed plants. For strawberries, size is a linear function of achene number/berry at a given achene density. Berry weight is calculated as  $(\text{total achene number/berry} - C) \times F$ , where  $C$  is a correction factor depending on the cultivar, and  $F$  is the flesh density in  $\text{g/cm}^2$ . While maximal swelling yields 6 *achenes/cm*<sup>2</sup>, it is often lower, resulting in 8-12 *achenes/cm*<sup>2</sup>, with water being probably the most important factor. In black currant, berry size is positively correlated to shoot growth intensity. Conversely, berry size is negatively correlated with the concentration of total and soluble solids, although acid content increases. This suggests the main effect on black currant composition is via sink activity. Cultivar variation is often a larger factor influencing development than growing techniques or

climate. Fruit refixation of  $CO_2$  occurs.

The relative sweetness of common compounds (scale where Sucrose =100) is Fructose: 173; Glucose: 74; and Sorbitol: 50. Relative sourness (scale 1-9; 9=most sour) is highest for Malic acid (8.2), followed by Tartaric acid (4.3) and Citric acid (3.6). Chemical analyses of black currant cultivars 'Tenah' and 'Ben Nevis' reveal changes in sugars (Sucrose, Glucose, Fructose) and acids (Citric and Malic acid) over time. A study noted that 1992 was a very warm summer. There is a relationship between water content and citrate content in black currant. Light affects fruit quality; the Sucrose/citric acid ratio in black currant was 0.28 in the Sun top compared to 0.15 in the Shade bottom.

Regarding vascular mechanisms, in the early phase (pre-veraison), the xylem is fully functional, transporting water and nutrients into the fruits. Post-veraison (developmental phase 3), the xylem loses its conductivity, leaving the fruit connected to the plant via phloem transport, which delivers sugar; the central connection to the seeds is maintained. Studies showed that when gas exchange via the berry surface is reduced by a coating with Vaseline, the sugar accumulation is impaired. Furthermore, Vaseline coated fruits combined with leaf removal resulted in fruit drop.

Fruit quality varies across berry types:

1. Black currant has the highest recorded ranges for Total dry matter (%) (17-28%) and C-vit mg/100 g (95-253).
2. Red currant exhibits the highest Acid g/100 g range (2.7-4.1).
3. Strawberry has the lowest Total dry matter (%) (7-11%) and Acid g/100 g range (0.8-1.2).

## 13 Lecture 15+16: Aromas in fruits - 22/09-2025

**Mikael Agerlin Petersen, FOOD-DCB**

This lecture explores Aroma in fruits, specifically addressing advances in volatile research, differences in aroma patterns, and the influence of the fruit/leaf ratio. Flavour is defined as the combination of basic tastes (Sour, Salt, Sweet, Bitter, Umami), odour (orthonasal and retronasal), and trigeminal stimuli. Aroma compounds are organic compounds with a certain volatility that interact with our olfactory receptors (not taste). They are effective in very small amounts; for example, 10 ppb of 2-Methoxy-3-isopropylpyrazin corresponds to mixing one teaspoon into a small swimming pool, resulting in a significant odour.

Aroma analyses are typically performed using GC-MS analyses. Gas Chromatographs (GC) cannot inject large amounts of water, lipids, sugars, or amino acids, but accept gas ( $\approx 1\text{mL}$ ) or organic solvents ( $\approx 2\mu\text{L}$ ). Headspace methods are frequently used, such as Dynamic Headspace Sampling (DHS), where headspace is continuously removed and collected/concentrated on a trap, ensuring equilibrium is never reached. The GC separates mixtures of volatiles, while the Mass Spectrometer (MS) utilizes an electron beam to create charged fragments of molecules, which are filtered by a Quadropole.

The aroma of a food is normally complex, resulting from a combination of many aroma compounds. Small peaks in a chromatogram (which may show >75 peaks) can also be important.

Fruit volatile compounds are mainly comprised of:

1. Esters
2. Alcohols
3. Aldehydes
4. Ketones

5. Lactones
6. Terpenoids
7. Some sulfur compounds

The specific content of these compounds determines, to a very high degree, the distinctive sensory quality of a fruit.

Aroma compounds are produced during ripening. Climacteric fruits show a distinct production controlled by ethylene. Studies on transgenic 'Royal Gala' apple (AO3) unable to synthesize ethylene confirmed that exposure to exogenous ethylene induces ripening. The genes involved in aroma biosynthesis are not coordinately regulated by ethylene; typically, only the first and final steps are ethylene regulated. Alcohol Acyl Transferase (AAT), which catalyzes the last step of volatile ester biosynthesis, is regulated by ethylene gene expression. Varieties like 'Golden Delicious' (high ester production) and 'Granny Smith' (low ester production) represent phenotypic extremes in volatile production.

The ethylene receptor blocker 1-MCP (1-Methylcyclopropene, "SmartFresh") binds to receptors without triggering a response. 1-MCP treatment immediately after harvest influenced the aroma profile in 'Ildrød Pigeon' apples after cold storage. 1-MCP is approved for minor use in closed storage rooms but is under re-evaluation. Aromas are generally under genetic control, but production can be influenced by Climate, Soil, and Fertilisation. The effect of the fruit/leaf ratio on aroma quality has been studied using different levels of fruit thinning (no thinning, moderate 10 cm, and heavy 20 cm) in potted apple trees.

Specific fruits contain characteristic compounds: Strawberries have one of the most complex aromas ( $\approx 350$  volatile compounds). Furanol (2,5-dimethyl-3(2H)-furanones) is a key component, and Methyl anthranilate is responsible for the typical spicy-aromatic and flowery note of wood strawberry (*Fr. vesca* L.). Banana fruity top notes derive from volatile esters like isoamyl acetate and isobutyl acetate. Citrus characteristic volatiles include aliphatic aldehydes (e.g., Octanal) and monoterpenes (e.g., Limonene). Raspberry aroma is particularly influenced by  $\alpha$ -ionone,  $\beta$ -ionone, and raspberry ketone.

## 14 Lecture 17+18: Organic growing of fruit and berries 22/09-2025

Maren Korsgaard, Inst. of Plant and Environment, mkor@PLEN.ku.dk

This lecture provides guidelines and legislative background for the organic production of fruit and berries. The main vision for organic production is built upon four foundational principles:

1. HEALTH: Sustain and enhance the health of soil, plant, animal, human, and planet as one and indivisible.
2. ECOLOGY: Based on living ecological systems and cycles, working with and emulating them.
3. FAIRNESS: Build on relationships that ensure fairness regarding the common environment and life opportunities.
4. CARE: Managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

Legislation governing organic production is based on EU 834/2007 (2021) and Guidelines of Organic agriculture in Denmark (Landbrugsstyrelsen). The EU Green Deal aims for 25% of agricultural land to be organic by 2030, rising from 10,5% in EU27 in 2022.

In Denmark, the total organic cultivated area was 295.233 ha in 2024, representing 11,1% of the Danish agri-

cultural land, showing a small decline (2,7%) related to 2023. The organic fruit and berry-acreage is increasing. The largest organic fruit areas in Denmark (2024, Ha) include Apple (616 Ha, 42,2% of total DK fruit-area) and Hazelnuts (504 Ha, 83,4%). Conventional Danish fruit production is often not profitable, and organic production is also risky business due to low yield per hectare. The organic apple-yield per hectare is approximately 5,5 ton/ha, but at least 15 ton/ha is necessary for a profitable organic apple-production. Organic production utilizes specific approved inputs. Organic fertilizers are always organic Nitrogen-fertilizers, including Farmyard-manure, Green manure, Rock phosphate, and Potassium sulphate\*. Agents allowed for pest and disease regulation include I: Azadirachtin (Neem), I: Plant oils, F: Copper-products, F: Sulphur, and F: Potassium bicarbonate (Armicarb).

Studies suggest that organic agriculture is the most environmental friendly agricultural system. Although the climate impact per kg food is the same as conventional production, organic soil contains 10% more carbon and sequesters more C (+256 kg C/ha/year), and emission of nitrous gasses is 24% lower. Local production is emphasized, as transport and cooling represent the largest environmental impact of apples. Danish apples in season have a very low climate impact (0,1 kg  $CO_2$  -eq./ kg food). Quality of organic products includes absence of pesticide-residues (apart from background pollution). Organic products also contain approximately 12% more health-promoting substances than conventional products, showing slightly higher content of total antioxidants and dry matter (taste). Organic apples have 4-7% more sugar.

To reduce risk in organic apple growing, planning should focus on dry climate locations, robust varieties, and well-drained soil. Preventing fungus diseases involves choosing weak-growing rootstocks, robust varieties, open pruning, low nitrogen-level, and potentially covering trees with a roof. Apple scab can be reduced by spraying sulphur or sodium bicarbonate. Insect damage can be prevented using beneficials by sowing flowering stripes and putting up nest boxes for insect-eating birds.

A trial involving five model organic apple orchards in 2004 found that the gross profit varied from - 83.000 kr/ha to +141.000 kr/ha, averaging 39.000 kr/ha, which was comparable to conventional production ( $\approx$  35.000 kr/ha). Main problems identified were attacks from aphids and scab.

## 15 Lecture 19: Light utilisation and canopy management 26/09-2025

**Torben Toldam-Andersen, KU-PLEN**

The physiological context of fruit production emphasizes that Biomass Yield is determined by the formula: (available light)x(% light intercepted)x(Photosynthesis)-Respiration. Of these factors, the percentage of light intercepted is the only one that can be directly manipulated. Efficient utilization of available light is of key importance in light-limited conditions, as Productivity is defined as a linear function of intercepted PAR (Photosynthetically Active Radiation). Photosynthesis and carbon allocation must be understood in this production physiological context, particularly concerning yield and quality components. A strong sink, such as a high number of fruits per  $m^2$  leaf area, stimulates a higher source activity, resulting in increasing Net photosynthesis. An optimal leaf area of  $600\text{ cm}^2$  /fruit is required to support approximately 1 g  $CO_2$  /apple/day.

Canopy management techniques are essential for manipulating light interception and distribution. Light distribution is crucial, affecting development from the single leaf up to the canopy level. The light level must not be too low to form flowers, requiring a minimum of 30% of full light for flower bud development. Light effects influence dry matter allocation, plant shape, fruit size, and colour. Data indicates that at only 10% relative light level, the percentage of large fruits drops to 22% and the percentage of fruits more than a quarter red drops to 5% (Based

on Hansen 1995). The artificial application of post-harvest light underlines the very strong effect of light on red over colour development.

Light interception is fundamentally connected to the Leaf Area Index ( $LAI = m^2 \text{ leaf area} / m^2 \text{ ground area}$ ). While light interception can be maximized through increased LAI, the optimal strategy requires a compromise between maximum interception and effective light distribution, ensuring the 30% minimum light requirement is met. An overall light interception of 50-65% and an LAI of 1.5(-2) is considered optimal. The theoretical maximum light interception ( $F_{max}$  ground area). While light interception can be maximized through increased LAI, the optimal strategy requires a compromise between maximum interception and effective light distribution, ensuring the 30% minimum light requirement is met. An overall light interception of 50-65% and an LAI of 1.5(-2) is considered optimal. The theoretical maximum light interception ( $F_{max}$ ) is reduced by light transmitted between trees to the ground ( $T_f$ ) and light transmitted through the canopy ( $T_c$ ). If LAI reaches 3, the canopy is generally deemed too dense.

Methods applied to manage light interception include:

1. Planting system (distances, orientation).
2. Canopy shape and size.

Increased light interception can also be achieved by maximizing Foliage density, using Multi-row systems, and increasing tree height in Single row systems. The leaves must be "arranged" in 3D in an optimal way. Row orientation effects show variation across cultivars and rootstocks.

The importance of shoot type is evident as the leaf area on longer shoots is not correlated to yield, but the area on spurs is. Spur leaves are critical because they fast become exporters of assimilates, supplying the developing fruit during the first 5-6 weeks after flowering, which are critical for fruit set (fruit number) and cell division (fruit size). Spurs are highly fruitful due to a high density of flowers.

## 16 Lecture 20: Canopy management/General techniques - 26/09-2025

Torben Toldam-Andersen, KU-PLEN

Canopy management encompasses methods aimed at manipulating growth and development in fruit crops. The primary aims are to control tree shape and esthetics, influence cropping and fruit development, increase fruit size and quality, facilitate the renewing of shoots, and improve overall productivity. Management is also critical for addressing space limits, improving work ease for machines and picking, and controlling tree height and shoot orientation. Furthermore, canopy management helps prevent and control diseases by removing sick shoots/parts, creating open trees or canopies, and optimizing spraying efficiency.

Growth regulating methods function by affecting different parts of the tree.

1. **Top/Root Mechanisms:** Planting density, Root stock/spur types, Pruning (top), and Root pruning affect both the Top and Root. These methods change the Top/root ratio, leading to 'root hormones' becoming dominant.
2. **Top-Only Mechanisms:** Chemical growth regulators, Arching/ringing/saw cuts (affecting Stem/transport), Bending, and Thinning (fruits, flowers) affect the Top.
3. **Root-Only Mechanisms:** Ground covers and Fertilizers/water affect the Root.

The removal of tissue (shoot or root tips) is equivalent to the removal of hormone producing tissue. Affected

hormones include Cytokinins, Gibberellins, and Auxin, with Auxin sensitivity being crucial in controlling Apical dominance. Pruning methods include the Shortening of shoot, Thinning/removal, and Shoot Positioning. Pruning intensity and timing significantly affect growth: Winter 'dormant' pruning is growth promoting, while Summer 'green' pruning is growth weakening. Summer pruning reduces root growth, delays bud break, and reduces total shoot growth, resulting in shorter shoots and more spurs. Medium late summer pruning may stimulate flower bud development, but late pruning risks reduced hardiness. The regrowth response is stronger from many small cuts than from a few larger cuts, and shortening cuts are stronger than thinning cuts (for similar mass removed). Vertical branches respond with stronger growth than horizontal branches or those at 30°.

Pruning affects source and sink dynamics. It increases light penetration, which leads to changes in leaf structure and increased Photosynthesis. Strong pruning may result in compensating growth responses, such as increasing leaf size (e.g., from 9.0 cm<sup>2</sup> /leaf (No pruning) to 16.0 cm<sup>2</sup> /leaf (Strong pruning)).

1. **Effects on Fruit Set:** Dormant pruning can be positive (due to improved water/nutrient/C availability) or negative (in case of high vigor). Early green pruning is positive due to a decrease in competing sinks.
2. **Effects on Yield:** Pruning generally delays cropping in young trees. In older trees, the decrease in fruit number caused by pruning can be compensated by increased fruit size (e.g., 181 fruits/tree at 123 g/fruit (No pruning) yields 22.3 Kg/tree compared to 146 fruits/tree at 145 g/fruit (Strong pruning) yielding 21.1 Kg/tree).
3. **Effects on Quality:** Fruit size increases due to increased light (source), increased root/top ratio (sink activity), and younger tissue/increased leaf/fruit ratio. Color development is generally improved if moderate growth is induced.

The total effect of pruning depends on the level and time of pruning, as well as the vigor level and density of the tree canopy.

Pruning systems include the Spindel (central leader) and Espalier. The Slender spindel emphasizes ensuring a balance between the top (starting strong, becoming weak later) and the basis (3-5 permanent branches). Training involves maintaining a strong top shoot in young trees, preventing branching on side shoots, and removing or spur pruning strong shoots. Growth types, such as Spur types and Tip cropping types, exhibit different branching and renewal characteristics, necessitating adjustments in management practices, such as bending or tying. The principles of Zahn (1998) state that side shoots more than 50% of the trunk diameter form a split or bushy crown.

## 17 Lecture 21: Juice processing and Quality - 29/09-2025

**Torben Toldam-Andersen, KU-PLEN**

The lecture outlines the main concepts related to Fruit Juice and their importance in juice quality, recognizes the main processes involved in fruit juice processing, and identifies the theory linked to the sensory evaluation part of the course. Fruit juice is a product derived from fruits and berries, characterized by no addition of sugar, acid, aroma, or water; however, sweetened juice exists for products like Black currant and Sour cherry. Nectar is defined as juice combined with water and sugar.

Juice processing involves a sequence of steps: Harvest, Wash, Grinding, (Enzyme treatment), Pressing, Fining, Filtration, Pasteurizing, Juice storage tank, and Concentration (resulting in Aroma Concentrate). Juice can be either clear or cloudy. Key components determining juice quality include Sugars (Fructose, glucose, (sucrose)), Acids (Malic acid, citric acid), and Phenolic compounds (Quercetin, Caffeic acid, Cinamic acid, Catechin). Important ingredients vary by fruit: apple typically contains 9-14 g/100g sugar, 0.5-1.2 g/100g acid, and 5-25 mg/100g

Ascorbic acid. Sour cherry 'Stevnsbær' contains 18-22 g/100g sugar, 2,0-2,5 g/100g acid, and 10-15 mg/100g Ascorbic acid. Black currant contains 13-14 g/100g sugar, 3,0-3,5 g/100g acid, and 65-115 mg/100g Ascorbic acid. Anthocyanin content is highest in Black currant (350-450 mg/100 g).

Sensory evaluation, specifically the correlation between the sensory impression of sugar/acid and the measured sugar/acid ratio, is critical for quality assessment, using a sensory scale from -5 (very sour) to +5 (very sweet), where 0 is the optimal sugar/acid ratio. Sensorically important aroma compounds are generally volatile (e.g., Acetateesters, Butanoateesters, Butanol) or less volatile (e.g., Benzaldehyde, Bensylalkohol, Linalool, Geraniol, Eugenol, Terpenes). Cultivars exhibit diverse aroma profiles, as shown by heat map analyses of 116 Volatile Organic Compounds (VOCs) across 8 apple cultivars.

The Pressing Yield is defined as kg juice/kg fruit. The ripening stage is important: unripe fruits, with protopectin and insoluble midtlamella intact, offer good possibilities for run off, while very ripe fruits, possessing much soluble pectin and high viscosity, result in low press yields. The vacuole must be emptied for water and solutes during pressing. Pressing can be carried out using machines like a Belt Press or small hydropres for small-scale/hobby production. The resulting juice may be separated into Free-run and Press fractions.

Enzyme treatment of the pulp increases juice yield. Pectin degrading enzymes, such as Polygalacturonase and Pectin esterase, are used. Pectin consists mainly of galacturonic acid, with some acid groups esterified with methanol ( $COOCH_3$ ). Enzyme treatment results in Lower viscosity, Better possibilities for run off, an Increase of juice yield (from 70-75% to 80-85%), Higher dry matter content, higher sugar, and Higher Methanol content.

Pasteurization, achieved by heating the juice to 90°C in 10-20 seconds, is necessary because, due to the low pH value of 3-4, only yeasts, fungus, and lactic acid bacteria can grow. Preservation methods for fruit juice include Concentration, aimed at reducing the volume from 100 l to 15-20 l. Concentration is achieved by Evaporation, Freeze-/cryo- concentration, or Reverse osmosis (membrane technology).

A major challenge during concentration by heat evaporation is the loss of volatile aroma components, as Esters, alcohols, and terpenes are more volatile than water. If only 10% of the water is evaporated, the concentration of aroma components has decreased to approximately zero. The concentration process, as illustrated, separates a 100 kg fruit juice (10% DM) into a 90 kg juice fraction (11,1% DM) and a 10 kg water + aroma fraction. Evaporation yields 20 kg concentrate (50% DM) and 10 kg water vapour + aroma components, which, via a Retification column and Condensator, yield 1 kg Aroma concentrate. Aroma components in apple juice are more volatile than those in strawberry juice, requiring a greater volume to be used as aroma fraction from strawberry juice.

Loss of anthocyanin and aroma during juice processing occurs through:

1. Loss to the press cake
2. Destruction by heating
3. Evaporation
4. Loss by clarification

The loss of the ester Ethyl butanoate and the concentration of anthocyanins decrease significantly across processing steps such as heating, enzyme treatment, pressing, clarification, and filtering. Cryo-concentration is also a method used, involving cooling curves of sucrose and glucose.

## 18 Lecture 22: Canopy management/ special techniques - 29/09-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture summarizes special techniques in Canopy management, specifically Summer pruning and Bending of shoots, which are utilized to manipulate growth and development. Summer pruning aims to open up too dense trees and is relevant for Espalier training systems. During summer pruning, unnecessary shoots are removed. Remaining shoots should be handled as follows: leaders, weak shoots and spurs are not to be touched, while others are pinched after 5-6 leaves. Several figures illustrate summer pinching techniques: Figure A shows the first summer pinching on shoot A at point b. Figure B illustrates the second summer pinching where shoot A developed shoot c, which is pinched at d. Figure C shows a second summer pinching where two shoots developed (c and d); in this case, the shoot c is removed by a cut at e, and shoot d is pinched at f, indicating that two developing shoots are considered too much. Figure D shows a similar scenario but with even stronger growth; the process involves removing the lower and weakest shoot. Figure E demonstrates a very strong growing shoot that was cut down to b in spring, resulting in all 3 buds starting to grow; the lower shoot e is pinched at g, and shoots c and d are removed with a cut at f. In problematic scenarios aimed at developing a fruiting spur (Figure F), where a spring cut at b led to three shoots (c, d, e), the lower shoot e is pinched at g, and c and d are removed at f, in the hope that the remaining shoot is weak enough to develop a spur. Another problematic scenario (Figure G) involves a shoot where lower side buds failed to develop into flowers, and only the terminal bud grew; here, the shoot is pinched short right above the base rosette of leaves as low as possible to force the lower buds to react. Figure H confirms the success of this stimulation two months later, showing a good spur development and the new shoot f pinched at g. See lecture slides for illustrations.

Bending of shoots has the primary aims of achieving Changed growth allocation and Flower bud development stimulation. Upright shoots and top exhibit the largest vigour. The degree of bending results in Reduction of vigour and Compensating growth. The degree of bending, which changes the allocation of growth and flower development, is demonstrated by quantitative data (Shoot angle, Shoot growth on bended part of branch, Shoot growth above the bended branches, Number of flowers year 2): an angle of 30° resulted in 522 cm shoot growth on the bended part and 4 flowers, while 120° resulted in 428 cm growth on the bended part and 95 flowers. Timing of bending is also crucial, as early bending results in many shoots. Studies comparing bending timing showed that March bending resulted in 28.3 shoots/tree, while bending on 1. August resulted in 8.9 shoots/tree, with shoot length varying between 32.9 cm and 37.3 cm. The mechanisms of bending involve Hormones and Apical dominance, specifically relating to Auxine and Ethylene (stress). It is important to be aware of the growth level (vigor); if the tree is weak (e.g., due to a weak root stock), bending may cause it to stop growing. Bending is an important tool in young trees.

## 19 Lecture 24: Nutrients, use and effects on development - 06/10-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture details the physiological aspects of Nutrients and fertilisation, covering Root development, Mobility, Storage, Nutrient demand and uptake, and Effects on yield components. Mobility in soil is pH dependent, where



$R_t = pH + 1/2$ . Due to nutrient uptake (ion exchange) by plants, pH will fall. Approximately 4 tons/ha of Calcium Carbonate is needed every 4 years to increase soil pH with 0.5. Optimal  $R_t$  values for nutrients range from 4.0-10.0, for instance N is 5.8-8.0 and Mo is 7.0-10.0. Mobility in plants classifies nutrients as Mobile (N, P, K and (Mg)), Less/partially mobile (S, Mo), or Immobile (Ca, B, Fe, Mn, Cu and Zn). Symptoms of deficiency depend on mobility, appearing on young leaves/top of plant, older leaves/Base of plant, or the whole plant, in addition to differences in coloring at the leaf level.

Regarding Storage, nutrients move from leaves to tree storage in Autumn. N and P storage approximates 50%, K storage is about 10%, and Ca, Mg storage is only about 1%. Early leaf drop may reduce storage N. Urea spray can increase leaf N and storage N in apple. Seasonal changes show that Arginine is the storage form, and Asparagine is the transport form of soluble N. In Spring, storage moves to new growth, causing concentration to fall. Low storage results in less shoot growth and lower fruit set. Remobilisation occurs in spring, confirming that new uptake is important, requiring early nutrient supply.

Nutrient demand and uptake correlate with seasonal growth. The leaves form the major pool for nutrients, leading to the largest demand early season. Seasonal accumulation in apple fruits shows that K, P (and N) accumulation runs parallel with fruit growth, while Ca accumulation occurs only in early season. Total uptake estimates suggest N about 60 kg/ha, K about 60 kg/ha, and P and Mg about 10 kg. The type of growth influences demand. Vegetative growing plants demand double the amount of most nutrients, except K. Fruits contain very Low Ca, but are high in K.

The effects of nutrients on yield components show that nutrient use (demand) is similar across fruit species. Small fruited species with a short period of fruit development show less impact of fruits on nutrient demand. Strawberry growers may vary nutrient solutions based on three growth phases:

1. until flowering,
2. fruit development,
3. after harvest.

Flowering intensity in apple depends on cropping level and N status the year before. Moderate N level (1.9-2.6% N in leaves) results in a higher percentage of maximal flower number than low N level (1.6% N in leaves). Fruit set (relative value of flower number) increases with N% of leaves just after flowering. For Ribes, shoot growth is linked directly to bud number, flowers, fruit number, and yield. 'Stevnsbær' and Elderberry also exhibit positive responses at high N levels, while Raspberry shows less positive responses. Strawberry response depends on the growing system: young plants in a short lifetime system need higher N levels, whereas older plants need less N to prevent them from becoming too dense.

## 20 Lecture 25: Nutrients use and effects on quality - 06/10-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture focuses on the effects of nutrients on quality components in fruit and berry crops, covering Nitrogen (N), Phosphor (P), Potassium (K), Calcium (Ca), and Magnesium (Mg), along with micro nutrients, optimal status determination, and application timing.

Nitrogen (N) negatively affects apple quality components, resulting in reduced red color (due to shade and a direct effect), more green fruits, and reduced firmness. N also reduces taste, decreases the concentration of acids and

sugars, and potentially reduces aroma. Furthermore, N reduces storability. In berries, N results in less sugar, but has no effect on acid. N affects growth and development, including vegetative growth and generative growth (flowers, fruit set). Its effects on fruit development include colour (red and green) and aroma. N effects are often indirect, dominating via the fruit/leaf ratio or carbohydrate availability, although direct effects may also exist.

Phosphor (P) is not a critical nutrient in DK but may improve storability. Potassium (K) is characterized by a high demand in fruits. However, high K and high yields can be negatively correlated to bud development. K can also increase biannual bearing and induce deficiency of Mg and Ca due to the K/Ca+Mg ratio. Thus, it is crucial to be careful not to use too much K.

Calcium (Ca) is important for storability. Studies show the percentage of bitter pit attack is a function of the calcium content of apple fruits. The incidence of 'Møsk' (tissue breakdown) is also correlated with Ca content in fruits. Ca is only mobile in the xylem and not in the phloem. Seasonal uptake in fruits of Ca occurs during development. Sprays with  $\text{CaCl}_2$  during late season may increase the Ca level. Xylem function, as observed in Braeburn' and Granny Smith' fruits assessed at 64 and 67 DAFB, shows differences in vascular function.

Magnesium (Mg) is part of Chlorophyll. Deficiency of Mg leads to necroses and leaf drop, which can be treated with leaf sprays of  $\text{MgSO}_4$  early season. Boron (B), Zinc (Zn), and Iron (Fe) are the most important micro nutrients. There is a danger of excess levels of micro nutrients, and their content may be found in fungicides (e.g., Dithane). Leaf sprays with these minerals are frequently used.

Optimal nutrient status determination using symptoms of deficiency is often seen too late. Soil samples are difficult in fruit cultures as they vary with depth, in and between rows, drip irrigation, soil types, etc.. Leaf samples are considered the basis of next years application. They should be taken in mid - late August, specifically from the middle of annual shoots.

Regarding application: N should be applied early spring, with high levels requiring 2/3 early spring + 1/3 June (mid summer). K should be applied in winter (KCL). Ca is applied via leaf sprays of  $\text{CaCl}_2$  (0.75%) late season. Micro nutrients are applied via leaf sprays early season. Nutrients should be applied in the tree row, and Fertigation is used a lot.

## 21 Lecture 23: Stresses and effects on quality - 10/10-2025

**Torben Toldam-Andersen, KU-PLEN**

This lecture addresses stresses and quality in fruit and berry crops, covering the use of water stress, damage from low and high temperature stress, prevention of damage, and damage caused by wind and biological stresses.

Low temperature stresses include freezing damage to berries (braun and dry out) and freezing in open flowers. Damage from frost (freeze) protection with water is based on the principles of high thermal mass of water and latent heat. Latent heat is the energy transferred during a phase change. The phase change from liquid water to ice transfers 80 cal of latent heat, which is crucial for frost protection. In comparison, 1 cal (4.17 Joule) is required to increase the temperature of 1 g of water by 1°C, and 600 cal of latent heat is transferred during a phase change from vapour to liquid water. Frost damage, post bloom and Winter damage are also covered.

The water vapour content of the air is important: a high content results in a slow temperature drop during the night, while a low content results in a quick temperature drop. The location near the coast is important for protection against frost damage. In spring, the minimum temperature near the coast is higher and the maximum is lower, which results in delayed Budbreak and lowered frost risk. Data for 'Red Boskoop' apples (1975-2022) indicates

that the beginning of flowering is now >3 weeks earlier than in 1975, corresponding to a temperature rise of >1.5 K since 1975. This makes frost protection by irrigation increasingly important. Frost protection methods include Overhead sprinklers and Water tubes with micro sprinklers at each tree, as well as using Heaters/burners. Radiative frost occurs on clear and calm nights and is very local. Techniques to mitigate frost include using Wind mixers (e.g., in Washington State, US) and sand cover (e.g., Soil protection in China).

High temperature and water stresses include fruit cracking due to water problems. This has been observed in Washington, US and Ullensvang, Norway. Sunburn damage results from too much sun. Hail, especially when combined with strong wind, can be very harmful. Hail can also severely damage the shoots. Rain, hail and sun protection methods are available.

Wind stress/damage requires the establishment of shelters. Pest and disease stresses lead to various negative outcomes:

1. Reduce leaf carbon assimilation and export
2. Deformities of fruits (spots, cracks, splitting)
3. Less attractive
4. Lower quality
5. Lower yield
6. Fruit rot
7. Bad taste
8. Lower storability

The shoots can also be severely damaged.

## **22 Lecture 28: Potentials in fruit and berries for fruit wine production in Denmark - 10/10-2025**

**Torben Toldam-Andersen, KU-PLEN**

This lecture explores the Potentials for (Danish) fruit wines through the 'NATVIN' Project, focusing on Apple cultivars, the content of apple juice, the importance of concentration, wine potentials, the process (How to do it), Quality impact, and the Benefits compared to alternatives to cryo concentration. For ordinary juice containing 12% Brix, the composition is typically 120 g/L sugar, 8 g acid, and 2 g sorbitol, resulting in 110 g Fermentable Sugars (FS). This concentration allows for a yield of 52 g alcohol or 6,5% vol Alc.. Wine acidity levels dictate the requirement for malolactic fermentation (MLF), defined as the conversion of malic acid to lactic acid by bacteria's (*Oenococcus oeni*). Moderate acid levels facilitate dry wines without MLF, while high levels necessitate MLF or wines with residual sugars (RS).

The implementation of cryo concentration expands wine potentials, increasing 12% Brix to 20% brix. This results in sugar content rising from 110 to 180-185 g/L, achieving 10,8% vol alc.. Acid levels concurrently rise from 5-8 g/L to 10-13 g/L, often requiring some MLF. Sparkling wines require Bottle Fermentation of added sugar to 4-5 bar, adding +1-1,2% vol alc., thereby reaching 12% total alcohol. Still wines made from 22% brix can reach 12% vol alc., generally requiring MLF. High Concentrate wines achieve 30-35% Brix, suitable for Sweet dessert wines, 'ice wines', although high acidity is a challenge, potentially exceeding >20 g/L.

The cryo concentration process involves freezing the juice, then thawing it slowly to yield concentrated juice. The resulting ice holds 2-5% Brix. Thawing often utilizes 1000 L tanks, where juice from multiple tanks is collected

into one. This method can result in an aroma explosion, with +40 aromas increased with more than 3x from control juice wines to high concentrated wines. Specific volatile increases include Dodecanoic acid, ethyl ester (314x) and Decanoic acid, methyl ester (178x). Only Ethyl lactate was observed to disappear, reduced 24x. Fufural content is also monitored, raising questions regarding Maillard reactions and/or caramellisation. Alternatives, such as Membrane filtration techniques (Reverse osmosis, Cross flow filtration), are problematic for cloudy juice due to high pectin content, necessitating viscosity reduction, and strong filtered juice may be difficult to ferment.

Berries pose unique challenges due to relative low in sugar and high or very high acid content (20-35 g/L), primarily citric and malic acid. Since these acids only form soluble salts, chemical reduction is impossible. Biological reduction via MLF is risky due to potential off flavour (Diacetyl)?, compounded by low or very low pH. Berry juice also exhibits very characteristic aromas, high phenolic content and often high in colour, and lots of pectin. Solutions involve Blends with water or a base of apple or pear. High acid species include:

1. Sour cherry 'Stevnsbær'
2. Black currant
3. Red and White Currant
4. Gooseberries
5. Rhubarb
6. Black berries

Potential techniques and styles include Sparkling, Liquor (juice+alcohol), Fortified wines, Ice wines, and Still wines. The average soluble solids in 114 Gooseberry cv's is 11,8%.

## 23 Lecture 30: Growing of wild berries - 20/10-2025

**Martin Jensen, senior scientist, [martin.jensen@food.au.dk](mailto:martin.jensen@food.au.dk)**

This lecture addresses the domestication of European Blueberry (*Vaccinium myrtillus* L.) for a new berry crop, focusing on wild fruits and the plant's morphology. The European Blueberry is an erect, woody, rhizomatous shrub that grows to a height of 5-90 cm. It exhibits tiller growth from the rhizome in a 3-angled axis, developing abundant annual shoots in a complex structure. Leaves are glabrous acute, 1-3 cm long, and flowers occur singly or in pairs in axil leaves, reaching 4-7 mm in diameter with a pink corolla. The plant forms patches through rhizome growth, with aerial shoots reaching 20-30 cm. Native wild populations of *Vaccinium myrtillus* are present in all regions of Denmark.

Wild harvested bilberries in Sweden are part of a larger yield in Nordic countries, estimated at 500 million kg per year, though only 5-8% is exploited. Traditional berry picking is often for personal consumption and local markets, involving a small and fragmented industry that exports to East Asia, with a price typically 20-30 DKK/kg. Yield in nature ranges from 0-450 kg/ha. Hand picking often employs manual rakes, with 3-4000 Asian people flying in annually to pick. For comparison, wild harvesting of lowbush blueberry (*Vaccinium Angustifolium*) in Canada, conducted as semi-cultivation on 2x50.000 ha of wild plants with a two-year production cycle, yields 99 million kg of berries per year.

European blueberry is of interest due to its status as a 'Superfruit', attractive taste, and high price. Potential health effects include lowering cholesterol and blood pressure, improving cognitive performance, and exhibiting antibacterial and vision benefits. It possesses a higher concentration of antioxidants than highbush blueberries. Currently, there is no commercial orchard production, relying only on 'natural collection'. Manual harvesting leads to high costs, limiting production and supply, and is deemed non-sustainable. Market demand is increasing

globally, and there are no reported cultivars or breeding/selection efforts done. Orchard production using selected cultivars and mechanical harvesting could create new product options due to lower costs, leveraging the fact that it is a native Danish species with available climate-adapted genetic resources.

The domestication of European Blueberry requires overcoming critical barriers:

1. Significant improved berry yield/plant is needed (selection and breeding), as maximum harvest in nature is too little (around 450 kg to 1 ton/ha).
2. Significantly more efficient and non-expensive methods of vegetative propagation are required, as past success has been minimal.
3. Development of a sustainable orchard concept/design adapted to mechanical harvesting is necessary.
4. Development of efficient mechanical harvesting is critical to reduce the cost of harvest, as Canadian solutions may not be adaptable.

The domestication project involved collecting clones, establishing a common garden trial of >100 genotypes, characterizing these genotypes for plant growth performance, berry yield, and berry quality. The established collection includes 113 clones from 40 populations across two sites, used for gene conservation, clonal comparison, and as a source of superior genetics.

Studies on fertilizer composition established that liquid fertilizers with N values of 200 and 400 ppm N damage and kill plants after 1-3 months. Optimal fertilizer concentration is 50-100 ppm N (EC = 0,3-0,6). 100 ppm N is optimal but only slightly better than 50 ppm in young seedlings, while 50 ppm was found better in cuttings. 25 ppm N suggests P deficiency (red leaf colour). A liquid fertilizer composition was developed for continuous use, using collected rainwater for irrigation, characterized by low levels of Ca, Mg, and Fe.

Berry quality analysis of 53 genotypes in 2016 showed mean total soluble solids of 9.1°Brix, total titratable acid of 0.895 g citric acid eqv./100 g, and a pH of 3.14. Total anthocyanins averaged 330.4 mg CGE\*/100 g. Superior clones identified in 2016 achieved high berry production, with the top clone yielding 115 g/plant/year, leading to a potential theoretical yield of 16.56 tons/ha/year (assuming 16 plants/m<sup>2</sup>). The average yield of all 106 clones was 3.49 tons/ha/year. Observations 11 years after planting showed top genotypes yielding 90-150 g/plant, equaling a potential yield of 8-13 tons of berries/ha (assuming 9 plants/m<sup>2</sup>). The relationship between mean berry production and the mean weight of the 10 largest berries showed a positive correlation ( $R^2=0,3553$ ). The research has demonstrated efficient and low-tech methods for cutting propagation, a liquid fertilizer composition, and characterized over 100 genotypes, pointing to possible superior genotypes for cultivation and further breeding.

## 24 Lecture 31: Bioactive compounds in fruit and berries - 20/10-2025

**Martin Jensen, senior scientist, [martin.jensen@food.au.dk](mailto:martin.jensen@food.au.dk)**

This lecture examines Bioactivity, defined by the effects or possible effects in humans from oral intake of compounds, relating to maintaining health, preventing disease, or resulting in a direct effect in the primary body or indirectly through a prebiotic or antibacterial effect. Regulatory categories include Medicine, Plant medicinal compounds, Functional foods, Supplemental foods, Food, and Novel Foods. Health claims, which must be based on scientific evidence evaluated by EFSA, include 'Function Health Claims' (related to growth, psychological functions, slimming), 'Risk Reduction Claims' (e.g., plant stanol esters reducing blood cholesterol), and 'Health Claims referring to children's development'. Documentation supporting health claims ranges from epidemiological evidence and in vitro tests to human clinical intervention tests, specifically recommending double blinded -

placebo controlled experiments for confirmation.

Examples of authorized EFSA claims include Pectins (reduction of blood glucose rise and maintenance of normal blood cholesterol) and Melatonin (alleviation of subjective feelings of jet lag and reduction of time taken to fall asleep, e.g., in sour cherry). Non-authorized claims include antioxidants from pomegranate juice and anthocyanins from elderberries.

Fruit and berries contain compounds with potential health effects, categorized as:

1. Anti-bacterial, Anti-viral, and Prebiotic effects.
2. Anti-oxidant, Anti inflammation, Anti- diabetic effects.
3. Protective against CVD - cardio vascular diseases (e.g., LDL cholesterol lowering, blood pressure lowering).
4. Anti-carcinogenic, Anti-mutagenic, Anti-toxic, detoxifying effects.

Estimated plant phenolics exceed 8000 polyphenolic compounds, including over 4000 flavonoids. Antioxidant capacity (FRAP, mmol/100 g) varies greatly, with Dog rose scoring 39.46 and Apple (Gold del) scoring 0.29. Genetic factors (cultivars), maturity, climate variation, cultivation methods, processing, and storage all cause variation in the concentration of single compounds, such as anthocyanins.

Bioavailability studies indicate that anthocyanins and large polymeric tannins generally show low uptake and low concentrations in blood. Anthocyanin metabolites reach maximum concentration after 1-2 hours and are depleted after approximately 10-12 hours. Clinical trials on single fruit species are few, often showing limited effects due to too low concentrations of active compounds in raw fruit. Research on Aronia suggests extracts or concentrates may function as functional food for disorders related to oxidative stress, but more confirmatory clinical trials are needed. A mechanism for cholesterol lowering is the ability of fruit and berries to bind and excrete bile acids in the intestines. Therapeutic effects may be achieved through additive effects in portfolio diets. Specific compounds like Galactolipid (GOPO) and two triterpene acids from Rose hip fruit are associated with pain reduction in osteoarthritis, as GOPO partly inhibits chemotaxis of human peripheral blood PMN's. Future research requires rigorous experiments, focusing on understanding compound metabolizing in the gastro-intestinal tract and modulation by bacterial flora.

## 25 Lecture 32: Water management and water stress - 24/10-2025

**Torben Toldam-Andersen, KU-PLEN**

Water is the most abundant constituent of plant tissues and functions as an excellent solvent for salts, other solutes, and gases. The majority of absorbed water is lost to the surrounding air via transpiration. Insufficient water limits horticultural and agricultural production in many areas. Future trends suggest an increase in extreme weather situations, such as intense rain events or droughts. Producers must therefore regulate their irrigation strategy based on crop needs and weather situations. Climate change is projected to increase irrigation needs; for instance, by the 2050s, eastern, southern, and central England are predicted to have greater irrigation needs than those currently experienced anywhere else in England.

Water flow through the plant (soil → plant → atmosphere) is driven by a gradient in water potential ( $\psi$ ). Water potential is defined as the energy state of water in the environment, which governs water availability. The total water potential is calculated as  $\psi = \psi_p + \psi_s + \psi_g + \psi_m$ , where  $\psi_p$  is pressure or hydrostatic potential (turgor),  $\psi_s$  is osmotic potential (binding to solutes),  $\psi_g$  is gravitational potential, and  $\psi_m$  is matric potential (binding to

surfaces). In soil, the matric and osmotic potentials are the main components, whereas in plants, hydrostatic/turgor and osmotic potentials dominate. Net photosynthesis is correlated with stomatal conductance in apple leaves under varying water stress levels.

When the soil dries, the plant initiates physiological reactions. Moderate soil drying results in a slight decrease in root water potential, an increase in ABA (abscisic acid) in roots, and increased root growth. Subsequently, ABA and pH increase in the xylem, while nitrate decreases. The leaf water potential is kept stable, although stomata gas-exchange and leaf expansion decrease, ABA in the leaf increases slightly, and nitrogen in the leaf decreases slightly. Severe soil drying causes a strong decrease in root water potential, strong increases in root ABA, and strong root growth. The leaf water potential decreases, stomata gas-exchange (including  $A_{max}$ ) decreases strongly, leaf expansion decreases, and nitrogen in the leaf decreases. Water stress affects vegetative development (root and shoot growth), flower bud formation (impacting the next year's crop), yield, and quality.

Plant water use and consumption depend on various factors:

1. Water reserves, which are determined by soil type.
2. Transpiration and evaporation, which are determined by temperature, wind, and humidity.
3. Leaf area, which depends on plant species, growing system, plant age, and plant shape.
4. Root size, which depends on plant species, growing system, plant age, and rootstock.

Cropping influences physiology in apple, leading to higher values compared to uncropped controls for photosynthetic intensity (100 vs. 140 - 210) and transpiration intensity (100 vs. 150 - 300). The water use coefficient measures L water used/m<sup>2</sup> leaf area/mm water evapotranspiration, and water use efficiency measures kg fruit/L water used.

Regulated water stress can be utilized to benefit yield and quality in fruit trees and vine production. Sensitivity varies greatly across species and cultivars. Shoot growth stops when new leaf development is reduced by water stress (increasing negative potential), which effectively controls shoot growth and induces terminal bud formation. Growers must control the water status to remain within the 'window' between the effect on shoot growth and the effect on photosynthesis to maintain dry matter production. Mild stress stimulates root growth, while strong stress reduces roots, particularly near the soil surface. Competition for water can be utilized, as green cover evaporates 50-90 mm/month (about 2-3 mm/day) compared to bare soil which evaporates 25-35 mm/month (about 1 mm/day), and covering with straw can save approximately 60 mm in a season.

Measurement techniques include Tensiometers, which use a porous ceramic cup to reach equilibrium with the soil solution's water potential, and Time Domain Reflectometry (TDR), which measures volumetric soil water content using electromagnetic waves. Water management strategies are applied to crops such as Apple and pears, Strawberry, Stone fruit, and Bush and cane fruits (e.g., Black currant and raspberry), sometimes in combination with root pruning.

Water stress reduces fruit set, berry size, and inflorescence initiation and development. The period most sensitive to stress is the first few weeks after flowering, where berry enlargement can be permanently limited. Just before ripening ('veraison'), there is little effect on fruit expansion. During ripening, mild stress enhances sugar and color content and reduces acid, promoting ripening. Strong stress during ripening can delay and reduce sugar and color. Reduced shoot growth limits competition between vegetative sinks and fruit development.

## 26 Lecture 33: Vitamins and colours in fruit and berries - 24/10-2025

**Torben Toldam-Andersen, KU-PLEN**

The internal quality of fruits, specifically concerning vitamins and colour, is influenced by climate effects and growing techniques. Fruit colours are derived from three main compound classes: Green colour is attributed to Chlorophyll a and b; Yellow colours originate from Carotenoids, including Carotins ( $C_{40}H_{56}$ ) like Beta-carotin and Lutein, and Xanthophylls ( $C_{40}H_{56}O_2$ ) such as Violaxanthin and Neoxanthin; and Red colour is due to Anthocyanins. Seasonal changes affect the concentration of the four main carotenoids in 'Golden del.' apple.

Anthocyanins are the dominant red colours found in berries. Based on data from Kaack 1975, the dominant anthocyanins include:

1. Black currant: Cyanidin-3-glucosid, Delphinidin-3-glucosid
2. Elderberry: Cyanidin-3-glucosid, Cyanidin-3-sambubiosid
3. Strawberry: Cyanidin-3-glucosid, Pelargonidin-3-glucosid
4. Cherry: Cyanidin-3-glucosid, Cyanidin-3-sophorosid

The synthesis of Anthocyanins begins with green leaves and light producing carbohydrates. Cyanin (anthocyanidin) is produced in the presence of light, and adding galactose (sugar) completes the synthesis of anthocyanin. This process involves the Pentose phosphate pathway, Shikimic acid pathway, Glycolysis, and Malonacid pathway, with glycosidation as the final step.

Colour development is regulated by factors including Fruit/leaf ratio (light indirect), Nitrogen, Light (specific via PAL), and Temperature. The ratio of Kg fruit/Kg leaves or Number of fruits/kg leaves influences the yellow and green colour of 'Golden delicious' apple. Temperature effects are observed during early and late stages of ripening. In unripe fruit, Light combined with warm temperature increases fruit growth and sugar accumulation. In mature fruit, red colour is enhanced by light and warm temperature, as well as by light and cold (night) temperature. Climate affects red colour development, demonstrated by treatments of Control, 50% shade, and Warm night conditions applied to Jonagold apples from mid-August to October. Post-harvest colour development is also influenced by artificial light exposure on bagged fruit. In Black currant, high Nitrogen (N) levels increased the concentration and content per fruit of chlorophyll and carotens. Leaf removal increased the concentration of chlorophyll and carotens, but decreased the content per fruit due to resulting smaller fruits. Anthocyanin concentration was affected only to a limited degree by N level or leaf removal, although fruits in the top of the canopy exhibited higher anthocyanin content compared to berries at the basis, which suggests the importance of light conditions and assimilate availability.

Vitamin C comprises Ascorbic acid plus De-hydro-ascorbic acid. Vitamin C content varies significantly across species in mg/100 g Fresh Weight (FW), ranging from Apple (5-10) and Pear/Grape (5) to Orange (50-60), Black currant (180), and Rose hip (400-2000). Vitamin C is formed before the ripening phase, with concentrations affected by ripening and storage effects, Light (sun/shade, south/north, peel), and other factors like fertilizers/nutrients, often through light or developmental degree. Vitamin C degrades very fast. The distribution of Vitamin C within an apple shows highest concentration in the Epidermis (70 mg/100 g fw), declining significantly in the inner flesh (6 mg/100 g fw) and core (5 mg/100 g fw), leading to the conclusion: do not peel an apple. Light exposure also affects content, with the south side of an apple fruit having 34 mg/100g fw compared to 20 mg/100g fw on the north side. The effect of development on Vitamin C is varied; it remains stable in Strawberry (e.g., 50→54 mg/100 g fw from unripe to ripe) and Apple (e.g., 10→13), but decreases in Black currant (219→140) and Sour Cherry



(20→12). Gooseberry cultivars show an average of 30.9 mg/100 g fresh weight. Storage conditions (Controlled Atmosphere, Ventilated, Cold Storage, or room temperature) also impact Vitamin C levels.

## 27 Lecture 34: Fruit harvest and postharvest - 27/10-2025

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Fruit development involves specific phases of growth and the role of ethylene, leading up to harvest. Harvest time represents a crucial compromise between optimizing internal quality and maximizing storability. Internal quality is assessed via chemical changes, while external qualities include size and colour. Seasonality significantly impacts fruit characteristics. In the early season (e.g., strawberries), a cool climate results in low sugar and high acid content, and phenology favors the first and largest fruits, with cultivar differences being notable. The main season is often warm and dry, accelerating development, which increases the danger of harvesting too late and resulting in overripe fruit. The late season (e.g., apples) is cool and humid, characterized by lack of light, and involves the last and smallest fruits, alongside increasing risks of pests, diseases, and fruit rot, impacting storage products.

Key harvest criteria are essential for balancing the goals of high eating quality and maximum storability. Important parameters include fruit size, colour change, firmness, removal force, sugar/acid ratio, starch content, aroma, and the levels of ethylene, O<sub>2</sub>, and CO<sub>2</sub>. The Streif index is calculated as  $\text{firmness (kg/cm}^2\text{)} / \text{sugar (\%)} \times \text{starch index (0-10)}$  and is dependent on both cultivar and climate. Cultivar differences are highly significant, especially regarding firmness in pears, which may change very fast. Prediction models, such as those relying on the temperature sum in 60 days after flowering for apples or the first 40-50 days determining phenology for cherries (Vitrup Christensen, 1973), aid in determining optimal harvest dates. For black currant, as fruits do not ripen uniformly, the harvest time is a compromise reached when some fruits begin to dry out and drop; machine harvest requires care to avoid damaging shoots and leaves. The optimal harvest date for storage can be related to the development of butylacetat (butyl etanoate), which is the most abundant aroma in apple juice.

Harvest and post-harvest losses can be very high, reaching 20% in Gross sale + Detail, and 20% for the consumer. Fruits are heavy, water-containing, alive, and undergo respiration and senescence, giving them short keep ability. They are fragile and sensitive to bruising and wounding, which causes ethylene release, increased water loss, and infection risks. High losses stem from damaged/reduced appearance, increased respiration and water loss, and infections. Prevention methods include careful harvest, appropriate boxing, and managing whether the stalk is included. Mechanical harvest methods include platforms for apples/pears, machinery for industry crops (e.g., 'Stevnsbær'), cane fruit harvesters, and portal harvesters for grapes in adapted growing systems.

Post-harvest physiology is strongly governed by temperature. Higher respiration equates to shorter shelf life. The Q10 ratio, which measures the respiration ratio at two temperatures 10°C apart, is typically 2-3, but can be 3-4 for fruits, emphasizing the dramatic effect of temperature. Fast cooling is necessary; for instance, strawberry Q10  $\approx$  4, meaning shelf life drops from 7-8 days at 0-2°C to about 2 days at 10°C. A delay in cooling of 6 hours can result in 50% higher water loss and loss of C-Vitamin and sugar. Fruits are categorized by ripening patterns:

1. Climacteric: Respiration and ethylene production increase (e.g., Apple, Pear, Banana).
2. Non-climacteric: Respiration gradually falls during development, indicating non-ethylene sensitive ripening (e.g., Cherry, Strawberry, Citrus).

To minimize drying out, fruits should not lose more than max 2-3% of water. Water deficit is the primary factor, heavily dependent on temperature. Water loss occurs 30% via lenticels and 70% via the cuticle. Minimization strategies include cooling, wrapping/foils, waxing, and the use of humidifiers in stores.

SmartFresh, a commercial brand of 1-MCP (1-Methylcyclopropene,  $C_4H_6$ ), is structurally related to ethylene and functions as a plant regulator to inhibit ethylene action by binding to its receptors. 1-MCP effects conclusions show that early treatment (at harvest) strongly reduces aroma development, while later treatment (after light exposure) only results in partial effects. 1-MCP maintains higher firmness. Furthermore, treatment applied before light exposure reduces the fruit's ability to develop red over colour. The application time significantly affects the concentration of specific aroma compounds like propanol and ethyl acetate in pigeon apples.

# Chapter 2

## Literature résumés

This section of the course notes is designed to streamline access to the key findings from each reading material (RM), providing a concise and accessible overview of essential information. Created through experimentation with various AI platforms, this chapter also serves to enhance my prompt engineering skills, exploring diverse methods of note-taking for maximum efficiency and clarity. The procedures for creating these summaries have varied, but all methods share a common approach: each RM has been fully read, with summaries and notes prepared after completing each respective subsection. By using these AI-co-op'ed approaches, these notes aim to be both a reliable reference and a resource for continuous improvement in capturing complex concepts.

### 1 RM for L02 - Fruit Yield and Quality. Components and Determinants

Fruit cultivation will normally be intended to maximize fruit yield and fruit quality [1]. The available tools for achieving this are variety selection (genetic selection) and choice of cultivation techniques, the physiological basis of which is treated in subsequent chapters. Maximizing fruit yield, often expressed as tonnes (or hekto litre in case of wine or juice) per hectares (ha), requires no further definition. Fruit yield is ultimately determined by fruit number per hectares and fruit size, understood as a product of several components. While component weights vary across fruit species, the main components can be summarized as shown in Table 1 and illustrated for cherry in Figure 1 [1]. Determinants define the amount or degree of development of each component within the genetic potential. Plant number per ha is primarily determined by the growing technique, mainly the selected planting system. The cultivation system may also determine factors such as the number of shots in raspberries and the number of rooted runners in strawberries. Other determinants are primarily physiological factors associated with growth and development, although they may also be influenced to some extent by growing technical interventions. The first main section of the course includes a review of these physiological determinants, serving as a basis for explaining and understanding the effects of different cultivation technical interventions.

Maximizing fruit quality is also a primary target in growing, although fruit quality cannot be defined nearly as unambiguous as fruit yield. Fruit quality includes a variety of components that vary with fruit type and fruit use, involving different weightings in different contexts. Fruit size is an important quality component, especially for table fruit. Colour and other factors related to fruit appearance are also important, particularly those factors that are part of EU standards for the grading of the various fruit species. These components are of specific interest to fruit growers because they contribute to price differences between different grades. Components attached to fruit taste and enjoyment value—e.g., sugar, acid, and aromatics content—are also important and are expected to become

even more important in the future. For industry fruit, such as sour cherries, emphasis is placed on juice colour and acidity, and for blackcurrant, on vitamin C content, despite there being no real price differences (quality premium). The concept of fruit quality can also include aspects such as the content of foreign substances like residuals from spraying of chemicals, which are of increasing importance in the complex of fruit quality. Societal priorities for health and environmental concerns, etc., also gain increasing importance in a growing technical context, and these factors are discussed in relevant, growing technical lectures.

Determinants such as flower development, and particularly fruit growth and fruit development, affect the quality components. Since physiological and growing technical factors may have opposite effects on yield and fruit quality components in some cases, the fruit grower's task is to seek the best overall compromise.

The components determining Yield per hectare and fruit quality, along with their primary determinants, follow a hierarchical path, as outlined in Table 1 [1]:

1. Number of plants / ha is determined by  $\leftarrow$  Planting System, and  $\leftarrow$  Growing System (Rubus, strawberry).
2. Bud Number / plant, bud type is influenced by  $\leftarrow$  Plant size, structure,  $\leftarrow$  Elongation growth, shot type development, and  $\leftarrow$  Bud Development.
3. Number of inflorescences / bud is determined by  $\leftarrow$  Flower initiation.
4. Number of flowers/cluster is influenced by  $\leftarrow$  Flower initiation and  $\leftarrow$  Flower Development.
5. Flower quality is determined by Number of seed primordia / Flower and Position in cluster.
6. Initial fruit set / flower is influenced by  $\leftarrow$  Pollination, fertilization, initial setting.
7. Final / initial fruit set is affected by  $\leftarrow$  Fruit drop (June drop).
8. Fruit Size is determined by Number of seeds / fruits ( $\leftarrow$  Pollination) and Quantity fruit flesh/seed, and is influenced by  $\leftarrow$  Fruit Growth, Fruit Development.

## 2 RM for L03 - Buds and Bud Development

Buds are growth points which can develop into shoots, determining the plant's future extension growth (dimension), or develop flowers, thereby determining the cropping [2]. Bud number and bud type are important components influenced by plant size and structure, which determine the number and distribution of various shoot types, consequently influencing bud number and type at the start of the growing season. Terminal buds, and sometimes the upper side buds on last year's long shoots, may develop new annual shoots. Lateral buds are formed in the axils of the leaves as the stem grows, culminating in terminal bud formation, with this elongation growth increasing the number of lateral buds. Other lateral buds on long shoots and end buds on short shoots may remain as short shoots or spurs, completing their short terminal growth with a terminal bud. Short shoots may mature well-developed lateral buds (often developing flowers), typical of fruiting spurs on cherry. Sleeping eyes (buds) only break after a strong stimulus, such as pruning. Flower buds of apple and pear are mixed buds, containing leaves and flowers, plus a new small bud (bourse-shoot bud), meaning a flower bud is usually followed by a new growth point. Stone fruit, conversely, has 'simple' or 'naked' flower buds, containing only leaves or flowers, resulting in the loss of a growth point at that position upon flowering, which can lead to bare areas on the shoot, typical of some sour cherry cultivars.

Elongation growth, and its distribution, determine bud development during the growing season. Terminal buds on short shoots are formed early (June). Lateral buds on longer annual shoots are formed in line with leaf development, with terminal bud formation occurring from June until September, depending on growth intensity. Elongation growth and bud development are subject to correlative inhibition mechanisms, including apical dominance, which involves a polar transport of growth substances (auxins, gibberellins) from terminal buds that inhibits

lateral bud growth. Heavy crop load can strongly reduce shoot elongation growth and the number of lateral buds due to competition for assimilates. Terminal bud formation occurs earlier if the ratio is high, see equation 2.1:

$$\text{Root activity} = \frac{\text{Root mass} \times \text{Root activity}}{\text{Number of Growth Points in the Top}} \quad (2.1)$$

Mature trees tend to have earlier terminal bud formation (possibly June-July) than young trees (often not before September). Elongation growth is enhanced by increasing temperature and by increased nitrogen and water supply (see sections 19.2.1 and 20.2.3).

The formation of a terminal bud is an abrupt process where bud scales develop instead of leaves, forming a compressed shoot inside with primordials for transitional leaves, true leaves, and possibly flowers. Bud break in the same growing season (re-growth) may occur exceptionally where correlative inhibitions are broken sharply, such as by removing shoot tips or performing early summer pruning. This is only possible until a certain date; with apple, for example, up to approximately 1<sup>st</sup> August, after which buds enter endo-dormancy.

Buds enter the endo-dormant phase when they can no longer break due to strong influences on correlative inhibitions. Bud dormancy is lifted by a species- and variety-specific period of low temperature, termed 'chilling'. Efficient chilling temperature is usually calculated as temperatures below +7°. In the temperate climate zone, the buds go through a physiological period of dormancy where there is no visible growth, which, if not met, can cause poor development or bud fall. The duration of dormancy varies by species (Table 1-2 shows Black currant ending dormancy in December, Apple in March). The depth and length of dormancy can be influenced by growth conditions in summer and geographical location, with cool summers accelerating termination. Dormancy is controlled by growth regulating substances, with Absciscic acid (ABA) having the strongest relationship with dormancy, while Gibberellins (GA) and/or cytokinins are growth-promoting substances. In areas with too little chilling, cultivation can be enabled by initiating the process with drought, followed by artificial defoliation (e.g., spraying with 10% urea) and then applying a spray to interrupt dormancy, such as 0.1% active DNOC +4% emulsified crude oil at the first watering.

Woody plants propagated by seeds must reach a certain age before flowering, termed the juvenile period or phase. This period may be short (e.g., Rubus and Ribe species), or long (e.g., 4-5 years for apple, 6-8 years for pear). The period between the end of the juvenile phase and first natural flowering is the transitional period. The juvenile period is characterized by different leaf forms and, sometimes, the transformation of short shoots into woody thorns (e.g., pear). To shorten the juvenile period in apples, experiments have reduced the period to approximately a third of normal using continuous growth in greenhouse conditions with increased day length and CO<sub>2</sub> addition.

### 3 RM for L04 - Chapter 2: Flower bud formation, flower development and flowering

A proportion of the buds formed during the growing season develop into flower buds, a process that is a very important yield component [3]. Flower bud formation begins with flower initiation, which occurs when there are no inhibitory effects. The time of initiation is typically summer - late summer, occurring in the approximate order of: Ribes, stone fruit, pome fruit, strawberry, and Rubus. This initiation is followed by flower differentiation.

Flower bud formation is most detailed studied in apple (pome fruit), primarily because it is an important crop and because it is frequently a limiting factor, especially concerning alternation (every two-year cropping). Studies show that a bud must undergo development before initiation can occur. For the apple variety 'Cox's Orange', a

critical node number of about 20 must be formed inside the bud. If the total number of nodes reaches 20 or more, a flower bud is formed, with a flower primordial in the terminal position and others in the corners of bracts (usually six to seven flower buds in total). The earlier a bud is formed (like spur buds), the longer time it has to develop, making flower formation easier. If node development is too rapid, the bud may break again in the same year (re-growth), causing delays that inhibit flower formation. Microscopically, the first sign of initiation is an increase in width of the growing point from 12-15 to 18-25 cells. Different theories exist regarding the mechanism, often involving positive and negative-acting factors, such as the Carbon/Nitrogen (C/N) ratio, where starch accumulation promotes flower bud formation. Flower bud formation may be associated with a high auxin / gibberellin content. The Source / sink ratio and flower bud formation are positively correlated. A strong negative correlation between fruit number per tree (fruit / leaf ratio) and flower bud formation explains the phenomenon of alternation. Reduced fruit growth rate, resulting in smaller fruits, leads to very large flower bud formation. Increased photosynthetic activity due to CO<sub>2</sub> addition is accompanied by an increase in flower bud formation. Increased shoot growth generally correlates negatively with flower bud formation. However, on young trees, recent studies suggest that a positive correlation can be found between shoot growth and flower bud formation on annual shoots, particularly when nutrients and water are supplied by drip irrigation. This allows the buds on annual shoots to reach their lower critical node number (14-16).

In stone fruit, alternation is typically less pronounced than in apple, possibly due to fruit size-related sink strength. In 'Stevnsbær', most buds on short shoots bloom, and the negative correlation between shoot growth and flower bud formation is less strong, primarily affecting longer annual shoots. Flower initiation may depend on the rate of development rather than a critical node number.

In *Ribes*, flower bud formation requires relatively short days (<12-14 hours) and low temperature. There is no direct effect of fruit number on flower bud formation; however, good cropping reduces shoot growth, leading to fewer buds and thus fewer flowers per bush the following year.

In strawberry (short-day types), day length is the crucial factor, with initiation occurring under Danish conditions when day length falls below 11 to 14 hours. Low temperature also promotes initiation. Vegetative growth (e.g., runner formation) is promoted by long days and high temperatures.

Flower development determines flower quality, which relates to the ability of the flower to set fruit. The differentiation of flower parts follows a sequence:

1. Sepals and petals differentiate rather quickly after initiation.
2. Stamens and carpels differentiate next.
3. Anthers (pollen sacks) and ovules often only differentiate next spring.

In cherry ('Stevnsbær'), bud death and abortion of flower primordiums can cause mortality rates between 30-80% of initial primordiums. In strawberries, the inflorescence is a cyme. With increasing flower order (primary, secondary, etc.), development becomes less complete, leading to inferior quality, characterized mainly by a decreasing number of pistils (Achenes) per flower.

Once bud dormancy is over, flowering time is mainly determined by temperature. The duration of the flowering process is also controlled by temperature; hot conditions may reduce it to one week, while cooler years may extend it beyond two weeks.

## 4 RM for L05 - A model to predict the beginning of the pollen season

The aim of the present study was to test phenoclimatographic models, comprising the Utah phenoclimatography Chill Unit (CU) and ASYMCUR-Growing Degree Hour (GDH) sub-models, on the allergenic trees *Alnus*, *Ulmus*, and *Betula* in order to provide a method to predict the beginning of the pollen season [4]. This type of model relates environmental temperatures to rest completion and bud development. Flowering is a phenological event resulting from a long period of development, beginning with the initiation and differentiation of buds into flower and vegetative buds during the summer. Falling temperatures cause a gradual change into a phase of winter rest with little or no growth activity. After a period, which length apparently depends on the climate and plant species, the plant gradually reverts to a phase of active growth in the spring.

As the phenologic parameter, 14 years of pollen counts (1977 to 1990) from a Burkard Volumetric Spore Trap in Copenhagen were used. The observed dates for the beginning of the pollen seasons were defined from the pollen counts as a fixed percentage (2.5%) of total counts. The observed dates of first bloom and pollen counts deviate profoundly from one year to another, with *Alnus* deviating from the 30<sup>th</sup> December to the 1<sup>st</sup> April, *Ulmus* from the 21<sup>th</sup> February to the 2<sup>nd</sup> May, and *Betula* from 2<sup>nd</sup> April to the 9<sup>th</sup> May. The chilling requirement was expressed using Chill Units (CU), where one CU is defined as one hour at 6°C, the optimum Chill Unit temperature for fruit trees. CU calculations began at the first day yielding positive chilling values. The experimentally determined temperatures for the GDH function included an optimum temperature of 25°C and a critical temperature of 36°C. The base temperature for GDH was changed from 4°C to 2°C, giving a slightly better correlation.

The models used were:

1. A fixed day model, using only the GDH model with 1<sup>st</sup> January as fixed initiation point.
2. A CU/GDH model, with a fixed sum of Chill Unit requirement as the initiation point for subsequent GDH accumulation.
3. A dynamic CU/GDH model, based on a dynamic relationship between CU and GDH.

In the ordinary CU/GDH model, the statistically estimated requirements for the trees were: *Alnus*: CU = 1550, GDH = 200; *Ulmus*: CU = 1850, GDH = 700; and *Betula*: CU = 1900, GDH = 2446. The minimum standard deviation for *Betula* was obtained at a CU estimate of 1900, giving an average of 2446 GDH. The dynamic CU/GDH model, equation 2.2, adjusted the necessary GDH to budbreak according to the CU obtained, proposing a non-linear S-curve function:

$$GDH = C + \frac{(D - C)}{1 + \exp\left(\frac{CU - CU_0}{A}\right)} \quad (2.2)$$

When compared with observed dates, the dynamic model provided the best predictions: within 2-4 days for *Alnus*, 8-10 days for *Ulmus*, and 3-5 days for *Betula*. This deviation size is acceptable given the large temporal variation from year to year. It is concluded that the CU and GDH relationships defined for fruit trees are generally applicable and give a reasonable description of the growth processes of other trees. *Alnus* tends to be completely regulated by temperature, but other parameters like the photoperiod apparently must be involved for *Ulmus* and *Betula* if predictions are to be further improved. The involvement of a dynamic relationship, though complicating the model, tends to improve the simulation of gradual changes during dormancy and budbreak. Furthermore, the results indicate that frost damage, detected using an LT<sub>50</sub> submodel (e.g., in 1981 and 1988), might be an important factor that can strongly affect total pollen counts.

## 5 RM for L06 - Physiology of Temperate Zone Fruit Trees

Fruit set plays an important role in modern fruit production, as a large yield of fruit is only expected if conditions for pollination and fruit set are favorable. Fruit set is a complex series of physiological events where no single element can be limiting to the overall process [5]. This overall process begins with the transfer of viable pollen to a receptive stigma, followed by germination, pollen tube growth, nutrient supply, and the successful fertilization of the mature embryo sac, which must occur before subsequent growth of the embryo. The events involved in fruit set are grouped into three main categories: the flowering process itself; circumstances that influence pollination and fertilization; and conditions conducive to fruit set without fertilization. All processes connected with fertilization are influenced by regulators, nutrition, and rootstock type, all of which are controllable by the grower [5].

The biology of bloom describes the process in which sepals and petals slowly enlarge, and the stigma(s) and stamens are exposed [5]. Bloom is considered to begin when 12-15% of the flowers are open and ends when 95-100% of the flowers are open. Cultivars generally do not bloom at the same time, and ensuring effective pollinizers requires that the bloom period of the main variety and the pollinizer overlap sufficiently. The timing of flowering varies from year to year, regardless of the absolute date, due to the differential sensitivity of various stages of bloom development to temperature stimuli [5].

The majority of fruit trees are self-incompatible, defined by the inability of fertile hermaphrodite seed plants to produce zygotes after self-pollination. Self-pollen is usually genetically inhibited from germination or pollen tube growth, preventing fertilization. However, even in self-fruitful cultivars, cross-pollination usually sets heavier crops. Triploid cultivars contain more pollen, which is generally less viable and has less value as a pollinizer [5]. Temperature has a great influence on the amount of pollen produced, with long cold winters or cold temperatures in early spring leading to a reduction in the number and vitality of pollen grains [5]. Bees are considered the most important pollinator insect [5].

The concept of the Effective Pollination Period (EPP), introduced by Williams in 1966, describes the limited period immediately following flower opening during which fertilization is possible [5]. The EPP duration equals the longevity of the ovule minus the time required for pollen tubes to reach the embryo sac. Ovule longevity varies significantly by cultivar; for example, the ovule longevity of 'Delicious' apple was estimated at 5 days, compared with 7-8 days for 'Jonathan'. The growth rate of compatible or partially compatible pollen is dictated by temperature. Pollen tube growth responds to temperature in a linear fashion, and a temperature response index can estimate the time required for pollen tube growth, compounded based on daily mean temperatures [5].

For a good fruit set, three requirements are sequential: first, the development of a strong flower bud during the previous fall, requiring sufficient photosynthate and nitrogen supply; second, a certain temperature range during and soon after bloom to ensure good pollination, pollen tube growth, and fertilization; and third, a relatively high photosynthate supply for the young developing fruit after fertilization [5]. Failure to satisfy these factors results in a poor fruit set and early drop of the young fruit. Early shedding of fruit is a regular feature of fruit set, often appearing in four waves at 12-14 day intervals. The third and fourth drops, called the 'June drop', are more conspicuous due to the larger size of the dropping fruit and involve a complete abscission process that includes the formation of ethylene.

Parthenocarpic fruit set, where fruit sets and grows to full size without fertilization, is most widespread in pears among temperate zone fruits. Pear varieties can be classified into four groups based on their ability to set parthenocarpic fruit [5]:

1. Not parthenocarpic: 'Pap Pear'.
2. Parthenocarpic fruit is shed at the time of June drop: 'Hardy'.



3. Variably parthenocarpic: 'Bosc', 'Clapp Favorit', 'Diel', 'Madame du Pois', 'Oliver Serres', 'Bartlett'.
4. Consistently parthenocarpic: 'Arabinka', 'Hardenpont', 'Passe Crassane', 'Pringall'.

In most fruit, the seed is the source of Gibberellic acid (GA), which is sensitive to temperature, explaining the dependence of parthenocarpy on high temperature around bloom [5].

## 6 1<sup>st</sup> RM for L07 - Physiology of Temperate Zone Fruit Trees

The utilization of plant reactions to changes in the fruit/leaf ratio requires consideration of the growth phase of the fruits, which varies between cultivars. Vigor, growth form, and cluster structure are also factors to be taken into account.

In fruit science, an organ with the status of an importer, such as strong growing shoot tips or developing fruit, is termed a "**sink**," importing dry matter and energy. Conversely, exporting organs, such as fully developed leaves that produce more sugar through photosynthesis than they use, are termed a "**source**". The surplus sugar is exported to competing sinks throughout the plant. The main source capacity covering the grapes' import/sink needs is located in the bottom 2/3 of the foliage wall.

The development of berries is roughly divided into 3 phases [6]:

The effects of affecting the fruit/leaf ratio must take into account the growth phase of the fruits, which are roughly divided into 3 phases characterized by different developmental processes. Phase 1 is dominated by cellular divisions and strong berry growth, quickly increasing berry size. This phase determines both the number of berries per cluster (based on the percentage of fruit set) and the size potential of the berries; increased cell divisions result in larger potential berry size. Reducing the number of bunches during Phase 1 decreases the fruit/leaf ratio, thereby increasing available resources, including carbohydrates (from leaves) and minerals (root intake). The result is an increase in both the percentage of fruit set and the number of cells per fruit, with the effect being stronger the earlier the action is taken. Early fruit (cluster) thinning can increase fruit set if the prospect for fruit set is less than desired. Simultaneously, there is a "risk" that the size of the berries increases, potentially resulting in too dense clusters.

The availability of resources for fruit development can be supported or enhanced by tipping shoots that have reached the desired height on the foliage wall. Strong growing shoot tips are defined as "sinks" because they import dry matter and energy, as their new young leaves are unable to cover their own needs. Conversely, exporting organs, such as fully developed leaves that produce surplus sugar in photosynthesis, are "sources". When the shoot tip is removed, a competing sink is eliminated, and the plant sends more sugar resources downward to cluster development, which also has sink status. Tipping also eliminates the apical dominance of the shoot, encouraging side shoot growth, although a period exists when the resource surplus primarily benefits the development of young/new fruit clusters. In wine grape production, the goal is often concentrated grapes and a relatively large skin/fruit ratio (i.e., small berry size), so early cluster thinning is usually avoided.

For cultivars such as 'Solaris,' utilizing the ability of fruit growth activity to limit shoot growth is often preferred. Early fruit thinning in Phase 1 is generally done only if the shoot growth is less than desired (e.g., on young plants). If growth is strong, maximum fruit load is kept, and the amount of leaves may be reduced. Uneven growth rates can be evened by tipping vigorous shoots when they reach the desired height on the foliage wall, moving resources to strengthen weak shoots. Phase 1 typically occurs for approximately 5 weeks, from 'Sct. Hans' until the end of July.

Phase 2 involves decreasing cell division, dominant seed development, and acid levels reaching their maximum. Xylem transport ability decreases, and the plant/fruit development is most robust to stress influences, such as drought stress. If the fruit load is clearly higher than optimal for Phase 3 (maturation), a first coarse thinning (e.g., down to 2 clusters per shot) may be appropriate. Bunch size reductions can be considered in high-setting, high-risk varieties like 'Johanniter' or Pinot's, or in large-clustered cv's such as 'Bolero' and possibly 'Cabernet Cortis,' if the clusters have not yet closed up. This period is typically around the start to mid-August. Continuous trimming of significant summer side shoot growth is important. Cutting off a dense cluster tip may allow the grapes ample space, and it may be better to keep 2 such adjusted bunches per shot instead of 1 large and dense cluster.

Phase 3 is marked by color shift, progressing from green to more yellow shades. Berries grow in size through cellular expansion, resulting in decreased firmness and a doubling of grape size. If a first rough adjustment was not made in Phase 2, it can be done early in Phase 3. Managing this phase is an act of balance; weaker growth requires greater thinning, while stronger growth requires delaying thinning to use the fruit to slow growth. As foliage becomes denser, leaf thinning begins, focused on the grape zone, and side shoots are heavily controlled. Thinning later than 3 weeks before harvest will reduce the effect on sugar accumulation (higher brix value). The final fruit/leaf ratio should normally be established by the first week of September.

For varieties susceptible to bunch stem-necrosis, such as 'Rondo' and 'Cabernet Cortis,' gradual adjustments are particularly important to avoid/reduce shock treatments. Leaf removal usually begins early, focusing on inner leaves in the grape zone to create an "inner open box" while preserving the outer leaves as important sugar producers and for protection. Leaves are first removed on the east side in August to stimulate early morning drying. Gradually, the west side is opened to achieve the desired exposure of around 40-50% of the clusters. The main source capacity covering the grapes' import/sink needs is located in the main leaves in the bottom 2/3 of the foliage wall.

## 7 2<sup>nd</sup> RM for L07 - Manipulation of growth and development by plant bioregulators

The growth and development of deciduous fruit trees are traditionally regulated by practices such as choice of rootstock, pruning, training, fertilization, water supply, and adjustment of crop load by hand thinning. When these cultural practices are insufficient, Plant Bioregulators (PBRs) are applied. PBRs are natural or synthetic compounds that mimic or counteract naturally occurring hormones. Examples include the cytokinin BA, which promotes cell division and fruit growth, and AVG, which suppresses ethylene formation. PBR results are often variable due to a poor understanding of their mode of action, "carry over" effects in fruit trees, variation in environmental conditions affecting uptake and translocation, and the specific anatomy and metabolism of different crops and cultivars. Societal concern and high re-registration costs pose significant obstacles to the continued use and development of synthetic PBRs [7].

PBRs are generally applied as aqueous foliar sprays. A major constraint on effective delivery is penetration through the leaf surface, which is covered by the cuticular membrane (CM), a non-cellular lipoidal barrier. The effectiveness depends on the PBR's nature, concentration, formulation, droplet size, and carrier volume. Decreasing droplet size or increasing carrier volume per leaf generally increases uptake, translocation, and biological response. High-volume spray applications often provide greater consistency. Environmental factors greatly affect PBR uptake and response. For instance, uptake of NAA and NAAM in apple and pear leaves increased with temperature in the range 5°C to 35°C.

## Regulation of Fruiting (Thinning)

Excessive fruit set must be reduced because fruits may not reach adequate size and quality due to insufficient leaf area per fruit. Overcropping also reduces or inhibits flower-bud formation, which leads to a biennial pattern of cropping. Hand thinning is the most accurate method, but chemical thinning greatly reduces cost and time.

To reduce excessive fruit set, several PBR strategies can be followed:

1. The initiation of floral primordia may be partially inhibited.
2. Flowers can be killed or prevented from setting fruits at the time of blossoming (chemical flower thinning).
3. Thinning may be postponed until fruit set can be judged and, if abundant, chemicals aimed at promoting the drop of young fruitlets can be used (chemical fruit thinning).

The third method (chemical fruit thinning) is usually preferred as the least risky strategy. However, a too long delay in thinning diminishes the desired effects on fruit growth and flower-bud formation. For 'Empire' apples, fruit weight increased significantly when hand thinning occurred at bloom, 10, or 20 days after bloom compared to 40 days after bloom.

Flower thinning is crucial for cultivars that set abundantly, as later fruitlet thinning may be ineffective. Two flower-thinning strategies exist:

1. Using caustic compounds, such as ATS, that damage or severely desiccate stigmas and styles to prevent effective pollination. These compounds may also act by stimulating wound-ethylene evolution.
2. Using chemicals, such as ethephon, that enhance ethylene formation in the flowers. Ethephon application at anthesis increases ovule senescence and reduces auxin basipetal transport, leading to enhanced flower abscission.

## Flower thinning

Flower thinning success may advance fruit maturity. Thinning activity is promoted by cool, cloudy, wet periods preceding application, and is enhanced by high temperatures after application, which increases competition for assimilates between sinks (shoot tips and fruitlets). For long-term sustainability, the most promising thinning compounds are those that occur naturally (e.g., BA), degrade rapidly (e.g., ethephon), or disappear quickly (e.g., NAA, ATS).

## 8 RM for L08+09 - Chapter 5 Growth, Growth correlations and Assimilate turnover

Fruit growth is an important yield determinant and affects fruit quality. Fruits contain 80-90% water. Of the dry matter, at least 90% originates from assimilates formed in leaves by photosynthesis. In pome and stone fruit, the sugar-alcohol sorbitol is mainly formed and serves as the transport agent. Assimilate production and allocation form the basis for growth and fruit development. By incorporating radioactive CO<sub>2</sub> (<sup>14</sup>CO<sub>2</sub>), one can track assimilate use [8].

Approximately 21% of the dry matter produced in leaves in autumn builds up storage nutrients, which are stored in the tree and utilized the following spring. A significant share, 18%, is stored in the roots. Storage nutrients include starch, sorbitol, and sugar. Fall and spring are often dominated by starch, while sorbitol and sugar proportions increase in winter. Most stored reserves (17 of 21%) disappear completely, assumed used for respiration

to generate energy for spring growth. Warm spring weather may lead to enhanced respiration and consumption of reserves, resulting in low carbohydrate content, reduced ovule development, lower flower quality, and reduced fruiting ability. Early autumn defoliation reduces carbohydrate storage, which subsequently reduces shoot growth the following year. High fruit load reduces root growth and stored carbohydrate reserves. This may reduce shoot growth and thickness growth early next summer and decrease the cell number per fruit. Storage materials play a role in other species; in strawberries, starch accumulates in the roots and is utilized during leaf and flower development in spring.

In the very early growth stages, storage material is widely used; flower buds and young shoot tips receive 50-65% of their building material from reserves, with the remainder from young leaves. New foliage is initially a net importer, but leaves become net exporters when they reach around half their final size. Vigorous-growing shoot tips are strong users of assimilates, dominating competition over young fruit. High young fruit drop is evident by particularly strong growth shoots. If shoot tips are removed, the assimilate demand of the shoot is temporarily reduced, and young fruitlet drop decreases. As shoot growth decreases and terminal growth is completed, only approx. 15% of what the leaves produce are retained in the shoot. Large quantities then support fruit growth, such as in Figure 5-2e where a fruit received approx. 35% of substance produced in leaves of annual shoots further out. Strong summer pruning (in August) that removes many annual shoot leaves can reduce fruit growth and dry matter content.

Although fruits are not strong competitors in the earliest developmental stages, their ability to attract assimilates (as strong sinks) becomes very large later. A fruit in July-August consumes approx. 80% of the assimilates formed in leaves on the same spur (Figure 5-2f). If there are many fruits, they can pull assimilates from other branches over distances of 1-1.5 m; in Figure 5-2g, 68% of assimilates formed in leaves on a branch without fruit were allocated to fruits on a branch with many fruits. In strongly growing apples, at least 95% of fruit carbohydrate consumption originates from leaf assimilates. Flowers and young fruits may contribute 15-33%, mostly at the 'tight cluster' stage.

The temporal increment of dry matter often results in an S-shaped curve (Figure 5-8). Spur leaves complete growth in June, followed by annual shoot leaves, shoots, small branches, main branches, stem, and roots, which often have their main growth in September-October. The strong import ability of fruits allows them to dominate competition for assimilates. At very high cropping levels, apple fruits can utilize nearly 70% of total dry matter production in a growing season. Vegetative growth is strongly inhibited by high fruit load. In young trees, compared to similar non-bearing trees, high fruit load may reduce:

1. Leaf growth to half.
2. Shoot growth to one-third to half.
3. Branch growth to a third.
4. Root growth increment even more.

The inhibitory effect is more powerful further from the fruit, correlating with the temporal coincidence of stem and root growth with the fruits' main growth period. To achieve strong vegetative growth in young trees, fruit should be removed as early as possible. The total photosynthetic production in young apple trees may be more than two times greater per leaf area unit in bearing than in non-bearing trees in July and August.

In blackcurrant, even with a much lower share of total dry matter accumulation in fruits compared to apples (Table 5-9), reduced leaf and shoot growth are found at a good cropping level. However, differences are much smaller on branches and especially roots compared to apple. This is likely due to the earlier fruit harvest (approx. August 1), which limits the fruit's effect on late-season growth organs like roots. Unlike apple, where the root/top ratio decreases with cropping, the opposite tends to be true for blackcurrant. The strong pull in assimilates exerted by

fruits causes a greater photosynthetic intensity and more rapid transport out of leaves. Assimilate retrieval from nearby leaves to growing fruit occurs with almost no loss, possibly due to CO<sub>2</sub> re-fixation within the fruit.

## 9 RM for L10 - Table Grapes in Cold Houses

This compendium note provides detailed information on growing vines in unheated glasshouses on a month-by-month basis, assuming the vines are well established. Maximum ventilation should be provided in January to keep buds dormant. In February, start maintaining a slightly higher temperature, opening ventilators near 7°C. Syringing with tepid water should only occur on sunny days, before midday, ensuring the house and vines are dry by evening. By March, the temperature can rise to about 10°C before admitting air. A moist atmosphere must be maintained by damping, sometimes twice a day on sunny days. Borders should be examined to a depth of 45 cm; if dry, water (30-40 mm), and repeat (10-20 mm) in a week, combined with manure water immediately after (never water manure on dry soil) [9].

Mildew is a common disorder. To prevent infection, spray with a fungicide or use sulphite smoke when shoots are 5 to 7 cm long. In early April, reduce young growths to two shoots per spur, retaining the terminal one and a strong shoot near the base for short spur pruning. Before the end of April, pinch young shoots at two to four leaves beyond the bunch. In May, gradually tie laterals down to the wires using a loop-tie (Fig. 3), preventing them from touching the glass. During flowering, maintain a fairly high temperature, keeping the top ventilator open day and night unless there is an exceptionally cold spell. Drier atmospheric conditions are desirable before midday for drying pollen. To pollinate, draw the hand gently down the bunches about midday, or tap cordons sharply around 9 a.m. for shy setters.

Immediately after berry set in June, the number of bunches must be reduced, retaining a small surplus. As berries swell, thinning should start in two stages using vine scissors, first removing all seedless berries and those pointing towards the centre of the bunch, retaining all tip berries. Overcropping must be avoided; a rough estimate for vines on the single cordon system is one bunch for every foot (30 cm) of cordon from the basal spur. A mulch of farmyard manure may be applied in June, ensuring ventilation is maintained to disperse fumes. In July, the berries undergo the first swelling (BBCH 77) followed by stoning (BBCH 77-79), a critical period where temperature should not fluctuate. After stoning, a second thinning is advised, suspending shoulders with raffia to maximize swelling (BBCH 80-89).

As berries approach maturity in August, gradually reduce atmospheric moisture. For the second swelling (BBCH 83), temperature can be increased by early closure of ventilators, accompanied by copious damping's. Scalding (discolored sunken patches) is caused by hot sun on moist tissues; ventilators must be opened slightly before the sun strikes the house. Ripening is categorized using BBCH stages, with total days for BBCH 70 to 89 ranging from 75 (very early) to 100 (very late).

Pest and Disease control includes managing Mealy bug with a 5% tar-oil wash when buds are dormant, or approved sprays during the season. Shankling is a condition caused by unhealthy roots or overcropping, requiring root defect correction, crop reduction, and maintaining pH and K/Mg balance. Deficiencies, such as Magnesium (yellowish orange discoloration), can be corrected by spraying 250g magnesium sulphate in 10L of water plus spreader.

By November, bunches should be removed, and maximum ventilation given to rest the vines; sub-lateral growth should be removed to ripen laterals. Pruning should occur immediately in December after leaf fall, cutting the lateral back to two buds to reduce bleeding. Cultivars tested at Pométet, such as those in the Interspecific resistant types (Group C), are ranked based on 25 culture technical parameters and 36 technological parameters. Very high ranked cultivars generally show high sugar content (score 7)  $\approx$  19% brix and acid levels (score 5-7) between 6

to 6,8 g/L. Ventilation is crucial and should be managed using top ventilators first, avoiding drafts, and seldom requiring bottom ventilators except at flowering.

## 10 1<sup>st</sup> RM for L11-13 - CHAPTER 6 Fruit growth and fruit quality

Fruit growth determines fruit size and shape, which is an important yield and quality component. External and internal quality factors vary greatly between species. Botanically, fruit types include a pome (pear, fusion of ovary and receptacle), a drupe (peach, one-seeded from ovary), a berry (grape, multiseeded from single ovary), an aggregate fruit (blackberry, many ovaries of a single flower), and an epigynous or false berry (blueberry). Most fruit is high in water, often over 80%. Carbohydrates dominate the dry matter in most species, though nuts contain protein and fat. Fructose has the highest relative sweetness (173, with sucrose as 100). Malic acid or citric acid are most often the dominant organic acids [10].

At least 90% of the fruit's dry matter originates from assimilates formed in leaves via photosynthesis. In pome and stone fruit, the sugar-alcohol sorbitol is mainly formed and serves as the transport agent, while sucrose is the transport agent in most other species, including fruit bushes and strawberries. Approximately 21% of dry matter produced in leaves in autumn is stored as reserves (starch, sorbitol, sugar), with 18% stored in the roots. Storage reserves are crucial, as flower buds and young shoot tips receive 50-65% of their building material from them in early growth stages. Fruit growth is conceived to be affected by the assimilate level, which depends on the ratio of assimilate producing organs (sources) and utilizing organs (sinks). Source strength is the capacity to synthesize compounds for export, and sink-strength is the potential capacity for accumulation of metabolites.

**Leaf/Fruit Ratio and Assimilate Turnover** With increasing leaf/fruit-ratio, increased fruit growth, and increased concentration of total solids, soluble solids (sugar), and acid are observed in apples. Conversely, a larger fruit/leaf ratio causes a stronger "pull" in assimilates, which animates leaves to greater production (greater photosynthetic intensity) and faster transport, but results in a lower assimilate level and lower concentration of certain substances, such as fruit dry matter. The relationship between yield ( $y$ ) and number of fruits ( $x$ ) per hectare can be expressed as  $y = kx/K + x$ , potentially using  $k$  to express source activity and  $1/K$  to express sink activity. High fruit load strongly inhibits vegetative growth in young trees, reducing leaf growth to half and shoot growth to one-third to half.

### Source and Sink Activity

Increasing source activity, such as through more light or higher CO<sub>2</sub> concentration, leads to a greater "pressure" from the leaves, resulting in larger fruits and increased accumulation of soluble solids (sugar). Light is an important factor affecting source activity in the field. Increasing sink activity in the fruits, often affected by substances from the root (e.g., potassium), causes fruits to "pull" assimilates stronger, resulting in larger fruit size but a lower dry matter content. For example, a good potassium supply resulted in larger fruit (117 g/fruit) but lower soluble solids (15,1%) compared to fruits lacking potassium (98 g/fruit, 15,9% soluble solids). Cultivar differences in fruit growth rate potential, such as 'Graasten' being approximately 25% higher than 'Golden Delicious', are apparently due to genetic differences in fruit sink activity. Variation in Species

The importance of the leaf/fruit-ratio on fruit growth is less pronounced in small-fruited species. In plums, reduced fruit number increases fruit size and solids/sugar content. In small-fruited sour cherry ('Stevnsbær'), reducing fruit number gives no or only a small increase in size. In black currant, sink activity is the most important factor for fruit growth, and large fruits are negatively correlated with solids concentration. For strawberries, flower development

and quality are essential for berry size. Berry weight is a linear function of achene number per berry at a given achene density (Annex 6-23). Development of achenes (seeds) is important as they release growth substances (auxin) needed for berry growth. In strawberries, thinning effects on berry growth are small, and the impact of physiological and cultivation factors on sugar and acid content is very limited. The relative importance of key factors varies across species (Annex 6-6):

1. Leaf/fruit ratio shows a positive correlation (+) with Fruit size, Dry matter, Acid, and Yellow background color.
2. Source-activity (light) shows a positive correlation (+) with Fruit size, Dry matter, Acid, and Yellow background color.
3. Sink-activity shows a positive correlation (+) with Fruit size, but a negative correlation (-) with Dry matter (sugar) and Red over-color (apples).

## 11 2<sup>nd</sup> RM for L11-13 - CHAPTER 7 Fruit Development and fruit ripening

Fruit development is characterized by phases which gradually transition, resulting in an overall effect on the developed fruit that is quantitatively influenced by the source-sink ratio. Fruit development transforms into ripening, which is characterized by a decrease in size increment that eventually stops, as well as the synthesis of specific substances, such as colors and flavors. The fruit may be tree- or picking ripe (mature) for a longer period before becoming eating ripe (ripe), particularly with many apple and pear varieties, though berry species often unite these stages.

Size increment typically follows an S-shaped (sigmoidal) curve in apples, pears, and strawberries. Stone fruit growth follows a double S-shaped curve that can be divided into three stages:

1. Seed coat and endosperm develop.
2. Seed coat hardens (lignifies, or pit-hardening), and the embryo develops. Growth seems less powerful.
3. Growth is again rapid, and the flesh develops via cell expansion, increasing the proportion of intercellular space.

Black currants (inner fruits in the raceme) can also show a double S-shaped growth curve. Cell divisions are dominant during the first 10-20% of the fruit developmental period, equalling the first approximately 4 weeks after flowering in apple. Thereafter, the time is dominated by cell expansion, although in strawberries, cell divisions may occur over a major part of the growing period.

With fruit development, the attraction between cells decreases as changes shorten the molecules within the pectin fraction, decreasing fruit firmness. The content of solids generally increases, with soluble solids (sugar) rising particularly sharply at the end. Assimilates (at least 90% of dry matter) are transported mainly as the sugar-alcohol sorbitol in pome and stone fruit, and as sucrose in most other species, including fruit bushes and strawberries. In apples, total acidity decreases with time, partly because de novo synthesis mainly occurs early in the growing season (when sorbitol is largely transformed into acid, e.g., malic acid, by June 30), and partly because already formed acid is eventually metabolized during ripening. Sorbitol transported into the fruit later (after August 20) yields especially sugars, mostly fructose. Starch accumulates and subsequently decomposes in apples, contributing to the increase in total sugar content.

The development and maturation rate is influenced by the rate of turnover in each phase, largely affected by source-sink relationships, and the duration of each phase. Increased temperature promotes the metabolic turnover

rate and shortens the duration of individual phases, thus increasing development and maturation rate. In late apple and pear varieties, increased temperature leads to larger fruit (Annex 7-6) and a higher sugar content (Annex 7-7). For the sour cherry cultivar 'Stevnsbær', the number of days between full bloom and harvest can be calculated as  $160 - (0.13 \times \sum T_{0-40})$ , where  $\sum T_{0-40}$  is the temperature sum in the first 40 days after full bloom.

Harvest criteria in apples and pears include fruit size, change in base color from green (chlorophyll decomposition) to yellow (carotenoids), formation of red over color (anthocyanins, which require light), pip color change (white to brown), formation of the abscission layer, and firmness. High sugar content, approximated by soluble solids (refractometer), and decreasing acidity are important quality factors. The degradation of starch, followed by iodine-potassium-iodine solution staining, is also used as a harvest criterion. Because a single criterion is too risky, an index is used, such as the equation 2.3 that shows the Streif index:

$$\text{Streif index} = \frac{\text{Fruit firmness (kg/cm}^2\text{)}}{\text{Refractometer (\%)} \times \text{starch value (0-10)}} \quad (2.3)$$

The index decreases during maturation. Optimal index values vary by variety, e.g., 'Elstar' 0.30 to 0.38. Fruits for fresh consumption are still picked by hand, while fruits for industry (e.g., sour cherries, black currants) are largely harvested mechanically.

## 12 RM for L13 - Growth and development in black currant (*Ribes nigrum*)

### III. Seasonal changes in sugars, organic acids, chlorophyll and anthocyanins and their possible metabolic background

Potted black currant plants of 'Tenah' (one year old, 1992) and 'Ben Nevis' (three year old, 1993) were sampled weekly from shortly after anthesis until harvest to measure fruit growth as dry-matter increments and seasonal changes in soluble sugars, organic acids, chlorophyll, and anthocyanins. Fruit growth in *Ribes* may be divided into two major rapid growth phases separated by a relatively short transition period. The typical double sigmoid growth pattern includes a plateau between 25-38 d from fertilization. The first rapid growth period coincides with cell division and seed growth.

In the very early stage of fruit development, high concentrations of sugar, malic acid, and chlorophyll were observed. Sugar concentration was initially high, then dropped to a minimum about 3-4 weeks after full bloom. Sugars started to accumulate rapidly, followed by a declining rate during the last weeks of ripening. During ripening, sugars, especially glucose and fructose, accumulated. Fructose reached a higher level while sucrose attained much lower values.

For organic acids, the initial concentration of malic acid in young fruits was high, declining to a steady state level of about two thirds of the initial level. During ripening, malic acid concentration fell rapidly until harvest. Citric acid concentration increased rapidly along with fruit growth until harvest, resulting in citric acid dominating the acid content per fruit. The accumulation of citric acid was similar in both years/cultivars on a per fruit basis. The total acid content decreases because initial malic acid concentration is very low and malic acid dominates, initially accounting for 70-75% of total organic acids. The final malic/citric acid ratio was 1:6-10 at maturity.

The sugar/acid ratio was initially high (1.4-1.5), dropping dramatically to a minimum between 35 d after full bloom (0.42-0.45), before returning gradually to the initial level during the last growth phase. The total chlorophyll concentration increased during early development. On a per-fruit basis, chlorophyll content rose strongly until 35



d after full bloom and then remained constant. Anthocyanins, low in immature fruits, showed a very rapid increase during ripening.

Metabolically, immature green fruits have a high capacity to support their own growth and carbon requirement through photosynthesis and dark respiration. Photosynthesis may be supported by CO<sub>2</sub> fixation via PEPcarboxylase, leading to the formation of oxaloacetate (OAA) and subsequently malate. Citric acid accumulates strongly in both immature and ripening fruits. A close correlation was found between citrate content and water content per fruit ( $R_2=0.97$ ). The large amount of citric acid suggests a high metabolic priority. The period of maximum sugar minimum concentration, spanning approximately 30-45 d from full bloom, may reflect a period of critical internal competition for resources.

## 13 1<sup>st</sup> RM for L15-16 - Advances in Fruit Aroma Volatile Research

Fruits produce a range of volatile compounds that form their characteristic aromas and contribute to flavor, which comprises sweetness, acidity, or bitterness (perception in mouth) and odor. Fruit volatile compounds are mainly comprised of esters, alcohols, aldehydes, ketones, lactones, terpenoids, and apocarotenoids. Aroma is a complex mixture whose composition is specific to species and often variety. Volatile esters often represent the major contribution in apple (*Malus domestica* Borkh.) and peach (*Prunus persica* L.). Many C<sub>10</sub> monoterpenes and C<sub>15</sub> sesquiterpenes compose the most abundant group, sometimes determining the characteristic aroma, such as the terpenoids S-linalool, limonene, valencene, and  $\beta$ -pinene in strawberry, koubo, and citrus. The volatile profiles of fruit are complex and vary depending on the cultivar, ripeness, pre- and postharvest environmental conditions. Volatiles are classified as primary (present in intact fruit tissue) or secondary (produced upon tissue disruption). The proportion of glycosidically bound volatiles is usually greater than that of free volatiles, making them an important potential source of flavor compounds [11].

Volatile profiles are highly species-specific. For example, more than 300 volatile molecules are reported in apples, with esters being the most abundant. Hexyl acetate, hexyl 2-methyl butanoate, hexyl hexanoate, hexyl butanoate, 2-methylbutyl acetate, and butyl acetate are prominent in 'Pink Lady' apples throughout maturation. In strawberry, the furanones 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanol) and its methyl derivative (mesifurane) are dominating aroma compounds. Esters, which are the most important group, cover 90% of the total volatiles in ripe strawberry fruit. Banana fruity top notes are primarily volatile esters, such as isoamyl acetate and isobutyl acetate. Terpenes, such as D,L-limonene and valencene, are the major class of compounds in citrus. Peach aroma is defined by C<sub>6</sub> aldehydes and alcohols (green-note) and lactones and esters (fruity aromas).

Factors affecting volatile composition include genetic makeup, maturity, environmental conditions, postharvest handling, and storage. Maturity is critical; immature fruits produce low quantities of volatiles and lose the capability of volatile production more readily during storage than mature fruit. C<sub>6</sub> aldehydes and alcohols are major contributors to the flavor of immature fruits, but their levels decrease drastically during ripening as furanone and ester production increases. Postharvest handling significantly influences volatiles. Storage temperature is fundamental. Storage under Controlled Atmosphere (CA) conditions (low O<sub>2</sub> and high CO<sub>2</sub>) alters production, reducing the capacity to synthesize ethylene and aroma volatiles.

Volatile aroma compounds are biosynthesized via several pathways starting from lipids (fatty acids), amino acids, terpenoids, and carotenoids.

1. Fatty Acids Pathway: Fatty acids are major precursors for C<sub>1</sub> to C<sub>20</sub> straight-chain compounds. The  $\beta$ -

oxidation pathway forms volatiles in intact fruit, providing alcohols and acyl CoAs for ester formation by removing C<sub>2</sub> units. The Lipoxygenase (LOX) pathway produces saturated and unsaturated volatile C<sub>6</sub> and C<sub>9</sub> aldehydes and alcohols, typically when tissue is disrupted.

2. Amino Acid Pathway: Amino acids, such as alanine, valine, leucine, and isoleucine, are direct precursors. They undergo deamination or transamination to form  $\alpha$ -keto acid, followed by reductions, oxidations, and/or esterifications to form aldehydes, acids, alcohols, and esters.
3. Terpenoids Pathway: Terpenoids are derived from the C<sub>5</sub> precursor isopentenyl diphosphate (IPP) and its isomer DMAPP. C<sub>10</sub> monoterpenes originate from geranyl diphosphate (GPP), formed in plastids via the MEP pathway. Terpene Synthases/Cyclases (TPSs) convert these precursors into diverse cyclic and acyclic compounds.
4. Carotenoid Pathway: Apocarotenoid derivatives are produced by the oxidative cleavage of carotenoids, catalyzed by Carotenoid Cleavage Dioxygenases (CCDs). Apocarotenoid volatiles are synthesized only at the latest stage of ripening.

## 14 2<sup>nd</sup> RM for L15-16 - Advances in Fruit Aroma Volatile Research

The formation of volatile aroma compounds is influenced by the ripening process of the fruit, including the effects of harvest date and storage conditions. A negative relationship exists between the fruit/leaf ratio and the availability of assimilates and sizes, as well as the concentration of total and soluble dry matter and acids. Fruit growth is conceived to be affected by the assimilate level, which depends on the ratio of assimilate producing organs (sources) and utilizing organs (sinks). The great import ability of the fruits means that they will dominate over other organs in the competition for assimilates when they are out of the early growth stages [12].

One-year-old apple trees of *Malus domestica* cv. Jonagored were divided into three fruit/leaf ratio groups: Low (130 fruit:leaf DM 10<sup>-3</sup> kg/tree), Cropping median (172), and High (381). The study established that at a low fruit/leaf ratio, larger apples were produced, accompanied by higher concentrations of total dry matter, soluble solids, titrateable acids, and a lower firmness value, compared to apples from trees with a high fruit/leaf ratio. Fruit firmness decreased during the ripening period, and apples from trees with a high fruit/leaf ratio were softer than apples from trees with the low ratio at harvest.

Headspace Gas Chromatography (GC) analysis showed that aroma compounds consisted of approx. 20% esters, 71% alcohols, and 6% C<sub>6</sub> aldehydes. The total concentration of aroma compounds increased during the ripening period and was most pronounced at the lowest fruit/leaf ratio. Esters and alcohols were dominant, while C<sub>6</sub> aldehydes showed no significant difference between the fruit/leaf ratios. The higher aroma concentration associated with low fruit/leaf ratios is supported by the theory that greater availability of assimilates favors the accumulation of precursors for the synthesis of aroma compounds.

Ethylene concentration was measured during a 3-week ripening period after cold storage. The ethylene concentration was between 45 and 83 ppm on the first day and peaked five days later (15 November). The ethylene concentration was most pronounced at the lowest fruit/leaf ratio during the ripening period and was lowest in fruits from trees with a low fruit/leaf ratio during the last part of the ripening period. The greater availability of assimilates at a low fruit/leaf ratio favors accumulation of substrates for synthesis of aroma compounds, larger pools of sugars and organic acids, and greater levels of aroma compound precursors like acetyl CoA and fatty acids, which are transformed into the esters and alcohols found in the study. High fruit load strongly inhibits vegetative growth in young trees, reducing leaf growth to half and shoot growth to one-third to half.

## 15 3<sup>rd</sup> RM for L15-16 - Advances in Fruit Aroma Volatile Research

Fruit growth is an important yield determinant, with at least 90% of the fruit's dry matter originating from assimilates formed in leaves via photosynthesis. In pome and stone fruit, the sugar-alcohol sorbitol is mainly formed and serves as the transport agent, while sucrose is the transport agent in most other species, including fruit bushes and strawberries. Approximately 21% of the dry matter produced in leaves in autumn is stored as reserves (starch, sorbitol, and sugar), with a significant share (18%) stored in the roots. Storage material supplies 50-65% of the building material for flower buds and young shoot tips in the very early growth stages. The great import ability of fruits means they dominate competition for assimilates when they are out of the early growth stages. For example, a fruit in July-August consumes approx. 80% of the assimilates formed in the leaves on the same spur. High fruit load strongly inhibits vegetative growth, potentially reducing leaf growth to half and shoot growth to one-third to half in young apple trees. The effect is more powerful further from the fruit, coinciding temporally with the main growth period of organs like roots (September-October). This strong sink activity causes a greater photosynthetic intensity and more rapid transport out of leaves in fruiting plants.

Biological variation is substantial, and even within the same fruit cultivar, flavor and aroma can vary significantly. This variation affects uniformity and categorization strategies. Apples from three cultivars ('Elshof', 'Holsteiner Cox', and 'Ingrid Marie') were analyzed individually for sugar, acid, and aroma compounds, having been picked at maturity from four zones on the trees (top, bottom, east, west). Using Principal Component Analysis on the relative peak areas of 59 tentatively identified aroma compounds, the three cultivars were completely separated. For 'Ingrid Marie' apples, effects of position could not be elucidated due to inadequate juice yield.

A discriminant Partial Least Squares regression analysis revealed distinct differences based on position for 'Elshof' apples, separating apples from the top and bottom parts of the trees. The level of sugar (soluble solids) was highest in apples from the top (13.4% sugar) and lowest in apples from the bottom (12.3% sugar). This positional effect relates to differences in quality and ripening levels, likely associated with light exposure affecting photosynthetic activity. High soluble solids are generally associated with high production of esters. For 'Elshof', compounds high in the top included 2-Methylbutyl acetate (2400a) and 2-Phenylethyl acetate (2.7a), while Hexanal (1300a) and 2-Butanol (5.6a) were high in the bottom. For 'Holsteiner Cox', apples from the west side separated well, characterized by lower levels of specific compounds, including 4-Methyl-5-vinylthiazole (2.2b), Anethole (1.0b), and 3-Hexenal (16b). Differences between top and bottom were most evident in 'Elshof'.

The magnitude of variations in concentrations of characteristic odorants (e.g., 2-Methylbutyl acetate, pentyl acetate) is substantial, indicating that position on the tree is likely to affect sensory quality. It is concluded that when apples are sampled for categorization and characterization, it is extremely important to sample from all parts of the trees or clearly define the position from which the fruits are sampled. Data also indicates the importance of good light exposure on fruits to ensure high quality.

## 16 1<sup>st</sup> RM for L17+18 - Sustainable production systems for organic apple production

Organic apples often sold for fresh consumption must meet high quality requirements, obtaining the right size and being undamaged without important infections of pest and diseases. The organism most often causing damage

on the skin of apples is apple scab, which creates brown or black spots. This disease causes a big reduction in yield and quality in organic production. The yield reduction for growers not using copper for control was 86% compared to conventional production, depending on the cultivar and the year. Copper is an effective fungicide used in organic apple production in some European countries, but it has not been permitted in Denmark for the last 10 years, and the European Union wants to reject it from the list of permitted pesticides [13].

To improve quality and yield, research has been carried out on cultivars with low susceptibility, using combinations of fertilisation, rootstocks, and planting distance to prevent or reduce apple scab infections. Control has involved using the warning program RIMpro to predict severe ascospore discharge periods. The most important prevention method is to use genetic resistant apple cultivars or cultivars with less susceptibility. Small, open, rather slow-growing apple trees reduce infection possibilities. The resistance to apple scab was broken down in most cultivars, but 'Florina' was still fully resistant during the experiment, and 'Vanda', 'Retina', and 'Redfree' were less infected.

A high level of available nitrogen in the soil causes increasing growth and an extended growth season, which gives better infection possibilities for apple scab. Increased nitrogen also causes a decrease in the content of phenols in plant tissue. The effect of cover crops was investigated, comparing a permanent grass mixture (1), a clover grass mixture (2), and an annual cover crop (3). Fruits produced on trees managed with a permanent grass alleyway (1) had a lower nitrogen supply and obtained the best skin coloration. A lower nitrogen supply, especially during fruit development, resulted in more red fruits. Apple scab infections were more numerous on apples grown in the annual cover crop (3), which gave the largest nitrogen supply. Fruits from the grass alleyways (1) had the highest percentage of marketable fruits. Although the gross yield was bigger from trees grown in the annual cover crop (3), the marketable fruit crop was at the same level due to the higher percentage of disease infections.

Early end of vegetative growth and development of end buds in autumn are very important to reduce the possibility of late infection in autumn and thereby reduce the risk of spring infections from conidia wintering in woody parts. End bud development of 51 rootstocks showed that rootstocks with high winter hardiness also had the earliest end bud development. MM106, a rootstock with poor winter hardiness and the latest end bud development of the 51 rootstocks, should not be recommended for organic apple production. The weak rootstocks M9 and the Russian rootstock B9 are potentially suitable due to:

1. Early end bud development.
2. Weak growth (M9).
3. High winter hardiness (B9).

The fruit quality recorded consists of both outer quality (fruit size, fruit color, and damages caused by pest and diseases on the fruit skin) and inner quality (firmness and content of sugar and starch).

## 17 2<sup>nd</sup> RM for L17+18 - Fruit quality in organic and conventional farming: advantages and limitations

Fruit quality is essential for nutrition and human health, requiring urgent attention in current agricultural practices. Modern agriculture must address the challenges of sustainability, crop quality, and yield, corresponding to United Nations Sustainable Development Goals (SDGs). Conventional agriculture, which accounts for a huge part of global plant production, utilizes an average of 1.81 kg of pesticides and 28.48 kg of nitrogen per hectare. Fruit quality is a complex, multifactorial concept assessed by consumers based on organoleptic traits (appearance, aroma, flavor, and texture), maturity stage, nutritional value, and presence of harmful chemical compounds. Fruits

contain essential bioactive compounds, including vitamins (E, C, B group), essential mineral nutrients (K, Ca, Mg, P, S, Fe, Zn, Mn), flavonoids, isoprenoids, and anthocyanins. Immature fruit is characterized by excessive firmness, poor aroma, low sucrose, high starch, and green color [14].

Organic farming is a more sustainable production model than conventional agriculture. It can provide higher quality in some fruit crops due to the absence of synthetic fertilizers and pesticides, enhanced pollination, and reduced protection treatments, which boosts antioxidant compound production. A meta-analysis of 71 studies published over the past 5 years (2019-2023) showed that organic farming improved overall fruit quality in 38 (53.5%) cases, whereas only 6 (8.4%) showed negative effects. Effects appear largely species-specific, with grape berries, peppers, and apples benefiting most.

Organic farming inherently contributes to high-quality fruit through several properties: biologically active soil, management practices such as compost and green manure, enhanced pollination, low/moderate biotic stress, and the absence of harmful compounds derived from fertilizers and pesticides. Total pesticide content in organic farming soils is 70-90% lower than in conventional soils, and nitrate and nitrite residues are usually lower in organically grown crops. Long-term improved soil health is a key principle of organic farming. The low/moderate biotic stress inherent to organic systems, coupled with the absence of synthetic pesticides, leads to a controlled exposure to stress factors. This pathogen exposure can trigger the synthesis of plant defense metabolites, such as phenolics, terpenoids, and flavonoids. Salicylic acid and jasmonates are phytohormones involved in activating phenylpropanoid metabolism in response to biotic stress, enhancing compounds valuable for human nutrition. Furthermore, organic farms enhance pollination success.

However, organic farming is not without potential risks. The use of compost and organic fertilizers, such as manure, means a significant input of microorganisms, potentially increasing the occurrence of pathogenic bacteria like *Escherichia coli*, yeasts, molds, and *Bacillus cereus* in organically grown vegetables. Nonmicrobial contaminants, such as heavy metals (Cu and Zn), can accumulate from sources like municipal sewage sludge or animal manures. Copper-containing plant protection products used in organic farming can also contribute to heavy metal excess. Despite these risks, organic farms generally perform much better in terms of pesticide residues. Fruit quality is a multifactorial concept affected by factors such as stress intensity, pruning and crop load, genetic traits, and rootstocks in both conventional and organic models. The integration of all parameters is essential for achieving high quality and productivity in sustainable farming models.

## 18 RM for L19 - 9 Light Relations

Visible light in the 400-700 nm waveband is the driving factor of biomass production via photosynthesis. Although the production of dry matter in apple correlates to the amount of visible light intercepted by trees, dry-matter production does not automatically translate into increased yield of marketable fruit. Light plays a twofold role: influencing processes leading to the production of large quantities of high-quality fruit, and supplying energy stored in chemical form (carbohydrates). The quantity of light plants use is defined as Photosynthetic Photon Flux (PPF) or Photosynthetically Active Radiation (PAR). Overhead sunny conditions yield a PPF of around  $2000 \mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ . Diffuse radiation can penetrate the canopy virtually in every direction, resulting in higher light levels in inner tree-canopy positions. Apple leaves absorb approximately 80% of incoming visible radiation [15].

Orchard management aims to maximize light interception, with efficient orchards intercepting 60-90% of available radiation, yielding high marketable production (up to  $120\text{-}140 \text{ t ha}^{-1} \text{ year}^{-1}$ ). Orchard productivity is often higher when light interception is greater than 50% of available light. Total tree net  $\text{CO}_2$  exchange (NCE) is found to be greater for fruiting trees than for non-fruiting trees when expressed per tree leaf area. Fruiting reduces light

interception due to lower leaf area, but stimulates the photosynthetic process, making the foliage more efficient. Yield ( $t \cdot ha^{-1}$ ) is strongly correlated with whole-canopy Leaf Area Index (LAI) ( $R^2=0.774$ ).

Apple leaves develop distinct morphological and physiological traits reflecting their light environment. 'Sun' leaves (exterior, well-illuminated) exhibit greater maximum photosynthetic capacity than 'shade' leaves (interior, low PPF). Shade leaves are typically denser, possessing more layers of cells, and higher nitrogen content. Shading spur leaves in the previous season was found to reduce the leaf photosynthetic rates.

The partitioning of carbon between different sinks (vegetative vs. reproductive) is complex. Primary spur leaves are vital early in the season, supplying carbohydrates for fruit set and cell division. They are the only carbon source until developing shoot leaves become net exporters (around 10-15 unfolded leaves). Early shading delays the transition of shoot carbohydrates to export. Heavy shading (70%) applied 5 weeks after full bloom (AFB) drastically reduced fruit growth and increased fruit drop. Low PPFs reduce flower-bud differentiation, fruit set, fruit size, soluble-solids concentration, and firmness.

Red fruit color formation (anthocyanin synthesis) is light-dependent, requiring light intensity and quality. This process is stimulated by UV-B radiation (<320 nm) and enhanced synergistically by red light. Fruits grown under canopy shade show increased transpiration and shrivelling during storage.

Controlling light levels in the orchard improves quality. Pruning is used to maintain adequate light penetration. The optimum training system for maximizing light distribution is the slender spindle. Reflective materials (Reflectants), such as aluminum or tar-paper, increase light availability in the lower and inner parts of the canopy. Reflective ground cover applied 2-3 months before harvest improves skin color (via UV region effects), increases fruit size, and boosts soluble-solids concentration. Bagging fruits 4-6 weeks AFB, followed by bag removal, also enhances red color formation.

## 19 RM for L20+22 - Planting and training systems, pruning and fruiting control

Woody plants possess high morphological plasticity and strong adaptability. Agronomic and pruning techniques are utilized to steer the "compensatory-adaptive" growth of the tree to obtain an equilibrium between vegetative and productive organs, and between these organs and the root system. Pruning is a powerful technique to control tree shape and size, and to improve fruit yield and quality. Modern intensive orchards utilize small trees and management methods aiming to begin production as early as possible, to facilitate mechanization, and to limit costs and labor. The essential goal of modern pruning is to obtain as early a canopy development as possible and achieve a balanced and independent (natural) growth. Maximizing orchard profitability is prioritized, even at the cost of shortening the life cycle, which usually lasts about ten to fifteen years. Pruning accelerates development, shapes the scaffold, and aims to quickly overcome the initial unproductive phase to obtain high and consistent yields. Young tree pruning is done concurrently with training to obtain a desired shape. Mature tree pruning adjusts crop load and fruit quality, maintains tree shape and size, and delays natural aging [16].

Pruning cuts are classified as either a thinning cut, which is the removal, at the base, of an entire shoot or branch, or a heading back cut, which is a partial removal of the distal part of a branch or limb to shorten it. Heading back cuts on two or more years old branches typically aim:

1. to bring the branch back to its natural position (return back cut).
2. to restrain the development of the branch (diversion cut).

Long pruning leaves the apical part of the branch intact and is almost always rich, promoting the reproductive

phase. Short pruning systematically shortens shoots or branches, creating a spur or shortened shoot, resulting in a strong reduction of buds ("poor pruning"), which produces fewer, but more vigorous shoots. Pear and apple trees tolerate winter cuts better than *Prunus* species.

The architecture of the tree consists of the framework of branches formed according to a training system. Tree size reduction is achieved using dwarfing rootstocks, competition between trees, modulating root growth, or stimulating fruiting to create sinks for photosynthates at the expense of vegetative and root growth. Training systems are classified based on architecture:

1. Volume shapes: Require well-defined, balanced, three-dimensional framework structures, requiring 3 to 5 years to complete (e.g., open vase).
2. Hedgerow systems: Create a thin continuous solid hedge (row of trees), easier to manage and adaptable to mechanical operations, requiring low-vigor rootstocks (e.g., palmette).

Green pruning is a spring or summer practice. If topping (cutting the terminal part of the shoot) is done prematurely, it produces new shoots; if done late (when growth slows), it temporarily favors shoot growth arrest and can promote flower bud formation. Shoot twisting, performed on vigorous shoots (>40-50 cm long), partially breaks the shoot to temporarily block growth, promoting the induction of reproductive buds. Pruning in adult trees pursues general objectives including adjusting fruit load, preserving canopy shape, removing diseased parts, maintaining balance between canopy and root system, and improving fruit quality (size, color, sensory quality).

Modern medium-high density orchards (2,000-3,500 trees ha<sup>-1</sup> for apples/pears) frequently use the Slender Spindle (spindlebush), a semi-free volume shape maintaining a central axis and 4 to 8 primary branches in a spiral, requiring dwarfing rootstocks. The Superspindel is an extremely simplified, almost columnar framework, devoid of branches, planted very thickly (30 to 60 cm on the row). The Regular palmette with oblique branches is a fixed hedgerow shape suitable for medium and medium-high density. In apple cultivation, the most common volumetric shape is similar to the spindle (Spindel busch). In general, the rows in apple orchards are laid out in a North-South alignment to promote a balanced development on both sides of trees.

## 20 RM for L21 - Production of Fruit Juices and Effects of Processing on Quality

Fruit juice is defined as the fermentable but unfermented juice obtained from fruit by mechanical processes, characterized by the color, aroma and taste characteristic of the fruit of origin. The juice must come from the endocarp for citrus fruits. Fruit juice also includes product made from concentrated fruit juice by restoring extracted water and flavor using aromatic substances obtained from concentration of the juice or juice of the same species. Fruit juice from certain fruits destined for concentration may also be obtained through diffusion processes. Apple juice may not be added sugar and must have a refractive dry matter content not less than that of an aqueous solution containing 100g sucrose per 1000g of solution. Fruit nectars are fermentable but unfermented products obtained by adding water and sugar or honey to fruit juice, concentrated fruit juice, or puree. Black currant nectar, for instance, must contain 8 g/L acid, calculated as tartaric acid, and must contain 25% juice [17].

Most apples used for juice production are industrial apples, which are sorted from table apples. Sugar and acid content are probably the most important sensory parameters in apple juice, with a sugar/acid ratio of approx. 15 perceived as optimal by a taste panel. Ripeness significantly affects quality; aroma content rises sharply with increasing ripeness while acidity and total content of phenols decrease. Total phenols give a bitter-astringent "taste". Black currants and sour cherries are major berry crops used for juice, valued for their high content of

sugar, acidity, ascorbic acid, anthocyanin, and aroma compounds. Mechanical harvest and high temperatures expose berries to rapid enzymatic and microbiological changes; studies show that unless sour cherries are cooled, deterioration happens quickly (2-3 days), causing degradation of color, acid, and sugar, and the formation of ethanol and acetic acid.

In juice extraction, apples are washed to remove debris and pesticide residue. Berries are either not washed or sprinkled gently. Fruit must be cut into pieces ( $0.2$  to  $1\text{ cm}^3$ ) before pressing. For berries (especially black and red currant) and ripe apples, pasteurization ( $80$ - $90^\circ\text{C}$ ) or enzymatic treatment using pectin degrading enzymes is often implemented on the fruit mass (maische) to improve juice yield and inactivate enzymes/microbes. Pectin degrades, increasing juice yield and increasing methanol content (e.g., from  $30$ - $100\text{ mg/L}$  to  $300$ - $400\text{ mg/L}$  in enzyme maische), but may reduce esters and aldehydes. Press yield estimates are generally  $65$ - $85\%$  on a weight basis. Alternatives to pressing include extraction with hot water, allowed if the juice (extract) is subsequently concentrated.

Juice clarification is necessary as most apple and berry juices are sold as clear juice. Turbidity is caused by colloidal particles, including protein-phenol aggregates and pectin-coated particles. Centrifugation and filtration alone are insufficient. Clearing agents are used to aggregate particles for sedimentation:

1. Pectin splitting enzymes are used to modify pectin-protein particles.
2. Gelatine is active against phenolic compounds, forming hydrogen-bonded aggregates.
3. Bentonite can form aggregates with protein, removing excess gelatine.

Heat treatment (pasteurization) is necessary to make juice storable due to enzymatic activities and microorganisms. Because fruit juices have a low pH, a relatively mild treatment is sufficient. The necessary time-temperature treatment is highly pH-dependent; apple juice requires only 10 seconds pasteurization at  $90^\circ\text{C}$ . Rapid warming and cooling via HTST treatment avoids heat damage.

Processing causes changes in juice components. Anthocyanin content in black currant decreases significantly, with losses often occurring during the "Warming" step and pressing/clarification. Aromatic compounds like  $\alpha$ -pinene and ethyl butanoate also show a huge drop from berries to clear juice, with losses spread across processing steps, suggesting evaporation or loss to the press cake. Most produced juice is stored and transported as concentrates, reducing storage capacity to  $1/4$ - $1/6$ . Concentration is typically achieved by evaporation combined with distillation of the aromatics fraction.

## 21 RM L23 - Using Water Stress to Control Vegetative Growth and Productivity of Temperate Fruit Trees

Although extensive evidence shows the negative impact of water stress on fruit yields, moderate water stress can reduce vegetative growth, which is often excessive, while simultaneously maximizing fruit size and quality. The long life cycle of fruit trees provides an opportunity for applying Regulated Deficit Irrigation (RDI). The RDI concept involves reducing water availability during specific growth phases when vegetative growth is highly active but fruit growth is less susceptible. The application of moderate water stress reduces vegetative growth primarily by inducing lower leaf water potential and reduced carbon exchange (photosynthesis). This reduction in overall  $\text{CO}_2$  exchange is an early plant response to water stress [18].

Fruit growth in fruit trees follows a double S-shaped pattern, notably in species like peach, apricot, and cherry, characterized by three developmental stages. Vegetative growth (shoots and root system) begins before and continues after the fruit growth period. RDI programs are ideally timed to exploit specific periods of fruit development.



These periods include:

1. The first critical period after fruit set, where stress could reduce total yield and fruit size potential.
2. The second period, which occurs during the lag phase (stage II of peach growth), separating the fruit growth periods that are driven by cell division and cell expansion, respectively.

Stress applied during the lag phase (Stage II) effectively suppresses shoot growth with only a minor impact on fruit growth, but a major impact on vegetative growth. This is because fruit growth and cell enlargement occur rapidly before and after the lag phase. Furthermore, postharvest water stress, applied after harvest (e.g., July to September in California), is used to suppress excessive vegetative growth. In nectarine, RDI applied from mid-June to mid-October achieved substantial savings of water without negatively affecting fruit size or quality, and postharvest RDI resulted in an increase in fruit soluble solids. In peach RDI experiments, a subsequent work demonstrated substantial water savings without productivity loss. However, RDI in pear resulted in reduced total yield, by 19% to 29% greater than control, and reduced fruit size.

The physiological mechanism allowing RDI success is believed to involve the fruit's ability to adjust its turgor (cell size) by internal solute accumulation through osmotic adjustment. This is critical for cell expansion during the last phase of fruit growth. The degree, duration, and timing of stress are vital factors affecting RDI outcome. Trees in a stressed condition may be put under stress earlier in the season because they are already acclimated. Generally, moderate water stress reduces the vegetative growth while providing beneficial effects, often reducing the shoot/root ratio.

## 22 1<sup>st</sup> RM for L24+25 - Leaf absorption of nutrients / Mineral nutrition

High hydrostatic pressures can be generated, leading to guttation (bleeding), typically 0.2-0.3 MPa in leafless birch, but limited to 0.1-0.2 MPa in leaves. Bleeding stops when leaves develop due to assimilation and water consumption, resulting in the transport of organic and inorganic compounds. Guttation occurs via exudation from hydathodes on leaf margins and tips, commonly observed in the cool temperate zone. Pruning of roots or shoots is suggested to reduce bleeding.

The practice of foliar absorption involves applying mineral nutrients as aqueous sprays or suspensions to the leaves to supplement soil application. Leaves possess the capability to absorb mineral nutrients, although less readily than roots. The leaf epidermis is covered by the cuticle, a non-cellular lipoidal barrier that limits the uptake of water-soluble nutrients. The absorption process involves two main steps: diffusion across the cuticle and absorption by the underlying living cells. Nutrient uptake is a slow process, typically absorbing only 5-30% of applied substances over several days. Uptake is dependent on formulation, pH, and cuticle status. No major difference exists in absorption rates between the upper and lower leaf surfaces. For red-skinned apples, absorption over 32 hours included 91.4% for urea, 6.4% for Fe-EDDHA, and 35.1% for MnSO<sub>4</sub>. Surfactants can increase absorption, especially for less surface-active compounds. Applying solutions when leaves are highly turgid or under cool, cloudy, wet conditions enhances uptake. Rapid evaporation under dry conditions decreases uptake.

Foliar application is practical for rapid nutrient supply, especially to correct deficiencies of Mn or Zn, and to supply N in spring to support early growth. Pome and stone fruit often receive foliar nutrients 3-4 times per season. Foliar N application in spring can reduce pre-harvest fruit drop in apples. Table 5-2 illustrates that 'Cox's Orange Pippin' apple trees subject to full standard soil fertilization (FF) combined with full fertilization (FN) achieved the highest fruit weight (175 g), while P fertilization at 5% (P-5%) resulted in the lowest fruit weight (27 g).

Mineral elements are essential for plants, playing roles in osmoregulation, membrane permeability, and enzyme systems. Elements are classified based on their mobility, which determines where deficiency symptoms first appear.

Mobile elements (N,P,K,Mg,S) are easily translocated from older leaves to growing points, causing deficiency symptoms to appear in older leaves. Less mobile elements (Ca,B,Mo) move slowly, resulting in deficiency symptoms appearing in younger tissue, such as shoot tips and fruitlets.

Nitrogen (N) deficiency reduces concentration in plant parts, leading to lighter green or purple coloration. High N supply enhances shoot growth and extends the growth season, delaying autumn senescence. Spring application of N promotes rapid shoot growth but may reduce flower differentiation. Calcium (Ca) is mainly transported through the xylem. Reduced Ca content can lead to reduced ovule development and lower flower quality, decreasing fruiting ability.

## **23 2<sup>nd</sup> RM for L24+25 - Yield Components and Fruit Development in 'Golden Delicious' Apple as Affected by the Timing of Nitrogen Supply**

The Nitrogen (N) supply of 'Golden Delicious' apples was manipulated during the growing season by altering the N concentration in nutrient solutions or by urea sprays. Terminal shoot growth increased with the duration of "high" N supply, especially with early summer application, exhibiting a degree of correlation with the N concentration of the leaves. Blossom density decreased only at continuously low N supply when evaluated on the basis of the previous year's crop level. Fruit set was reduced when N in spur leaves, immediately after bloom, dropped below 2.8-3.0%, which occurred with continuously low N, or with high N supply only in the early summer. Fruit growth at a fixed fruit/leaf ratio increased with N supply and growth variations were seen between years. The N supply during the early part of the fruit growth period was the more important factor. The yellow colouring of the fruit at certain fruit/leaf ratios was greater at low N levels or when the N supply was low during the latter part of the fruit growth period. Effects on fruit quality and yield components were small or experimentally inconsistent where the N-deficient treatments were excluded [19].

The experiment utilized two-year-old 'Golden Delicious'/MM 104 trees in plastic pots, later transferred to 304 pots in the spring of 1970. A similar two-year-old 'Golden Delicious'/M 7a (a virus-tested selection of M 7, not identical to the EMLA clone) was planted in 15-l pots in the spring of 1971 and transferred to 304 pots in the spring of 1973. The experiment involved 12 replicated treatments, comprising combinations of N supply through the roots and urea spraying on the tops (Fig. 1). Cropping was not undertaken until 1972. N concentration in the leaves was affected distinctly by the N supply. N concentration remained low with the "low" N application alone (Treatment 9). "High" N supply in the roots in autumn and by urea spraying in spring (Treatment 7) gave high spring values and also prevented definite deficiency for the remainder of the season. The potassium (K) content of the leaves was highest with continuous "high" N supply (Table I). The concentrations of phosphorus (P) and N were high at "low" N application (Treatment 9) and decreased with the duration of high N supply (Table I).

Regarding Vegetative growth, total dry matter production during the experiment period generally decreased, as the duration of "high" N supply was reduced, falling to about half of the maximum value at "low" N (Treatment 9) (Table II). Terminal shoot growth was particularly affected by the N supply and correlated well with the duration of "high" N supply (Fig. 3). Vigorous growth after additional N in June-July alone (Treatment 8) was partially

due to reduced fruit set.

Blossom density was negatively correlated between flower density and fruit/leaf ratio of the preceding growing season. Low N application (Treatment 9) (perhaps only half) was required to obtain a lower fruit/leaf ratio, compared to other treatments, to maintain a certain blossom density. Fruit set was dependent on the N status of the trees, indicated by leaf values immediately after bloom. N concentration of spur leaves should be 2.8-3.0% or more to ensure proper fruit set. Fruit set was low in Treatment 9 in all 3 years, and with “high” N during June-July (Treatment 8) in 2 of the years. N applied late autumn and in spring only (Treatment 7) conversely achieved adequate fruit set even when N level was comparatively low later in the season.

Fruit growth increased inter alia on fruit/leaf ratio and thus on fruit size. A tendency for more rapid fruit growth existed with increasing duration of “high” N supply. Minimum fruit growth was found at “low” N throughout the season (Treatment 9).

Fruit development (colour) shows that the degree of yellow colouring decreases with increasing fruit/leaf ratio of the tree, which also reduces average fruit size. “Low” N throughout the season (Treatment 9) yielded fruits of a stronger yellow colour. Continuous “high” N (Treatments 1, 2) resulted in greener fruits. Additional N supply applied late autumn and spring alone (Treatment 7) gave more yellow fruits than other treatments. N availability in early to mid-summer may favour vegetative growth at the expense of fruit, whereas in cropping trees, N status must be established in late autumn and spring to ensure proper fruit set. Leaf values below 1.8-2.1% N have been proposed to ensure good fruit quality.

## 24 RM for L25+32 - Pre-harvest factors influencing the quality of berries

Quality of berries is a difficult concept to describe objectively. The concept of quality in berries can be defined as a set of agronomic/commercial, organoleptic, and nutritional qualities. Agronomic/commercial quality comprises characters belonging to the adaptation of the plant to specific cultivation, such as fruit size, plant yield, harvesting speed, and resistance to pests and diseases. Organoleptic quality includes characteristics generally related to attributes recognizable through the five senses of the consumer, such as fruit color, shape, acidity and sweetness, combined with flavor and aroma determined by volatile compounds. Nutritional quality is the “hidden” quality present in berry fruits, comprising all macro- and micro-nutrients, vitamins, and bioactive compounds. Recently, the nutritional value, intended as the content of bioactive compounds with healthy effects, has gained increasing priority in breeding programs [20].

Pre-harvest factors influencing berry quality include genetic factors, environmental factors, and agronomic factors. Genetic factors account for variability among different species and cultivars. The genetic background is the most important factor affecting fruit quality, particularly sensorial and nutritional quality. Environmental factors influence fruit quality through adaptability to different climatic conditions, such as latitude, soil conditions, production cycle, and light exposition. Agronomic factors are related to cultivation systems (open field or protected or soilless), fertilization, water stress and salinity, and fruit harvest.

Plant yield is the amount of fruits with commercial value harvested for each production cycle. Fruit size is certainly one of the most important traits in new genotype selection. The fruit size is evaluated through the implementation of some different parameters:

1. Fruit Weight: indicates the average weight of a representative sample of fruits and refers to the dimension of the fruit;

2. Fruit Length: indicates the average size on the longitudinal region of a representative sample of fruits;

3. Fruit Diameter: indicates the average size around the equatorial region of a representative sample of fruits.

Fruit color is a primary organoleptic trait, determined by pigments (anthocyanin, phenols, etc.) accumulated in the fruit skin cells. Fruit color is determined by the genotype but is also highly influenced by environmental conditions and maturation stage. Firmness indicates the resistance of the fruit to mechanical damages and is a priority for facilitating harvesting, transportation, and post-harvest management. Sweetness is indicated as Total Soluble Solids (TSS), expressed as °Brix, and acidity is expressed as Total Acidity (TA). The sweet-acid balance is measured through the Sugar/Acid (S/A) ratio. An acceptable strawberry flavor requires a minimum TSS of 7% and a maximum TA of 0.8%. Flavor is determined by VOCs (volatile organic compounds).

Nutritional quality relates to the high content of phytochemicals, primarily phenolic molecules, dietary fibers, vitamins (A, C, E, and B complex), and essential macro- and micronutrients (phosphorus, potassium, calcium, magnesium, iron, manganese, copper, sodium, and aluminum). Anthocyanins, a subgroup of flavonoids, act as powerful antioxidants. Ellagic acid is a powerful healthy compound found in different berry species, representing 51% of the total phenolic compounds in berries.

The genetic background defines the capacity of a fruit to accumulate bioactive compounds. Wild species, such as *F. vesca*, often have higher levels of nutritional attributes (e.g., mean sugar concentrations twice or three times higher, higher total polyphenols) than cultivated species, despite potential loss of traits like fruit size and firmness. Plant age can influence fruit quality; higher sugar concentrations and yield were found in the second year of cultivation in *F. x ananassa* fruits.

Environmental factors such as growing location influence nutritional quality; for example, strawberries in northern Europe showed higher dry matter, total acidity, and soluble solids content compared to southern Europe. Light exposure stimulates flavonoid biosynthesis, leading to elevated anthocyanins and flavonols content. Temperature also affects quality; high temperatures negatively affected black currant fruit quality by suppressing anthocyanins biosynthesis. Lower temperatures (e.g., 12°C) increased levels of flavonols and hydroxycinnamic acids in bilberry compared to 18°C. Agronomic practices influence phytochemical content. Organically grown strawberries possess higher levels of total phenolics, ellagic acid, and flavonols than conventional strawberries. Stress factors like minimum salinity or reduced water can induce a stress response that promotes phytochemical production, such as phenols. Deficit irrigation can influence fruit phenol content. Fruit harvest maturity is critical; while fully ripened berries maximize anthocyanins and aroma, picking at earlier stages, like white tip or three-quarter color in strawberries, maintains fruit firmness and phenolic/flavonoid concentration for longer storage.

## 25 RM for L26+27 - Unravelling genetic diversity and cultivar parentage in the Danish apple gene bank collection

Characterization of apple germplasm is important for conservation management and breeding strategies. Genotyping of plant genetic resources, such as the genus *Malus*, is crucial for gene banks to ensure preservation of maximum genetic diversity, as collections risk losing identity or accumulating duplicates. The largest collection of Danish apple (*Malus domestica* Borkh.) cultivars is maintained at the Pomatum (University of Copenhagen, Denmark). This study genotyped a set of 448 *Malus domestica* accessions, primarily of local Danish origin, using 15 microsatellite markers. Ploidy levels were determined by flow cytometry. The study also included a reference set of cultivars, a private nursery collection (Assens), and a small set of *Malus sieversii*, *Malus sylvestris* and

small-fruited, ornamental *Malus* cultivars. Plant material was collected as young leaves from vigorously growing shoots at the Pomatum's gene bank collection. The ploidy level was determined using flow cytometer BD FACSAria™ Illu counting 5000 events and staining with 4',6-diamidino-2-phenylindole (DAPI) solution. For SSR genotyping, a set of 15 SSR markers was used. Parentage analysis for diploid samples was performed with ML-Relate, while parentage analysis for triploid samples was performed with Colony version 2.0.5.9 after converting microsatellite data to binary data [21].

The microsatellite markers amplified 17-30 alleles per loci with an average degree of heterozygosity at 0.78. For the 448 *M. domestica* cultivars, 12 to 26 alleles per loci were amplified, with a mean of 19. The observed heterozygosity ( $H_o$ ) was 0.78 and expected heterozygosity ( $H_e$ ) was 0.81. We found 42 unique (private) alleles. The proportion of genotypes with unique alleles was larger in the Assens collection (19%) than in the Danish collection and reference collection (7% and 8%, respectively).

Of the 393 accessions whose ploidy levels were determined, 17% were triploids. In the Danish cultivar collection, 22% triploids were identified. We identified 104 (23%) duplicate genotypes among all 448 *M. domestica* accessions, including colour sports. The Danish apple gene bank collection contained 10% duplicate accessions, excluding the Assens collection duplicates. Duplicate genotypes were divided into subclones, such as phenotypically distinctive colour mutants that markers were unable to separate, or duplicates with name synonyms. The highest number of identical genotypes was found for Gravensteiner (15 subclones), followed by Almindelig Pigeon, Filipa, and Cox's Orange (all with 6 subclones).

The SSR data allowed for studying putative relatedness such as parent-offspring relationships. We could infer first-degree relationships for many cultivars with previously unknown parentages. The cultivars Cox's Orange and 'Pigeonnet blanc d'hiver' have the highest number of putative offspring relatives. The cultivar Ingrid Marie was confirmed to be an offspring of Cox's Orange, and Cox's Pomona was unambiguously identified as the other parent, resulting in the hybridization event Cox's Orange × Cox's Pomona.

STRUCTURE analysis provided no evidence for a genetic structure but allowed us to present a putative genetic assembly that was consistent with both PCA analysis and parental affiliation. A putative assembly of five genetic clusters was shown, though no more than 21% of genotypes had strong assignment ( $>0.80$ ) to a specific group. The lack of genetic structure is supported by the Bayesian model-based approach and delta K values in a very low and tight range ( $\Delta K < 1.5$ ). The largest genetic differentiation was found between groups 2 and 5 ( $F_{st}=0.056$ ). Group 5 (derivatives of Cox's Orange) is the genetically most distinctive group and the least diverse (allelic richness 9.60), while groups 1 and 4 are the most diverse groups (allelic richness 14.73 and 12.20, respectively). The PCA plot indicates a cluster of Cox's Orange and first-degree relatives and no distinction between Danish cultivars and the reference collection.

The Danish cultivar collection contains many unique accessions and considerable genetic diversity, making it a valuable resource within the European apple germplasm. The fingerprints can be used for cultivar identification and future management of apple genetic resources.

## 26 RM for L29 - The effect of source-sink status on flowering and the performance of everbearing strawberry cultivars

Everbearing strawberry cultivars offer the potential to extend the growing season and boost productivity through recurring flowering. Cultivation of everbearing cultivars is high-cost and intensive, necessitating high fruit yields for economic viability. A major challenge is the great fluctuations in the cropping pattern, which typically involves an initial flush of berries, followed by a lag-period with a significant decrease in production, and a subsequent second flush of berries. This study aimed to elucidate the effect of the source-sink status of the plant on the flowering pattern and performance of the everbearing cultivars 'Favori' and 'Murano'. It was hypothesized that the level of continuous floral initiation and flower development is dependent on the source-sink status of the plant, and manipulating this balance could potentially be used as a tool to gain an optimal or desired harvest profile and a more stable production.

The methodology included Flower Mapping to determine the starting generative potential for the first flush. For the first flush, 'Favori' showed a greater potential with an average of 13,2 flower trusses per plant, while 'Murano' had 8,2. Based on this, 80 plants of each cultivar were divided into sections of 20 plants to be exposed to four source-sink treatments based on the removal of flower trusses. The treatments were defined by Growing Degree Hours (GDH) thresholds and allowed maximum Leaf Area Index (LAI) of 3. Flowers emerging until 3.500 GDH hours were removed for all treatments except the control to establish good vegetative growth. The treatments applied until 16.500 GDH were:

1. Control: No trusses removed until 3500 GDH; All trusses allowed 3500-16500 GDH.
2. Treatment 1: All trusses removed until 3500 GDH; All trusses allowed 3500-16500 GDH.
3. Treatment 2: All trusses removed until 3500 GDH; 6 trusses allowed 3500-16500 GDH.
4. Treatment 3: All trusses removed until 3500 GDH; 3 trusses allowed 3500-16500 GDH.

Runners were removed upon the emergence of the first node. Leaf management was applied by keeping the total leaf area below a LAI of 3 to maintain a uniform source capacity. Harvesting defined three phases: First Flush (FF), Lag-Phase (LP), and Second Flush (SF). Quality parameters included individual sugars and organic acids, pH, total acidity, and Brix measurements (% soluble solids).

Results show that removal of flower trusses belonging to the first flush (F2, F3, M2, M3) has marketable and significant effects on yield and yield parameters. The general trend is that increased removal of trusses belonging to the first flush results in a yield increase. Since the number of berries per plant is similar across the treatments (excluding the control), the yield increase is attributed to increased berry weight. This sink relief provided yield compensation in the form of increased berry weight during the first flush. There is a contrasting clear and general trend of increased yields as the response to increased removal of flowers during the lag-phase and second flush. New initiation of flowers is supported as the likely explanation for the similar number of berries despite initial removal. For Favori, removing only very early trusses emerging before 3.500 GDH (F1) seemingly had the greatest influence on both yields and average berry weight compared to the control (FC).

Regarding quality, the increased flower truss removal during the first flush yielded berries with an increasingly higher sugar content for both cultivars. This suggests that during this first flush, the berry development and dry matter accumulation are strictly source-limited, and sink relief promotes development in the remaining fruit sinks. The redistribution of yield to the lag-phase and second flush had no negative impact on assimilate partitioning in those phases for the flower truss removal treatments. No trends were observed in the change of concentrations of

organic acids, titratable acidity, or pH.

Findings confirm that continuous flower initiation is resource limited. Altering source-sink status through early truss removal effectively manipulates flowering and fruiting patterns, leading to a more balanced and stable cropping with increased yields during the normal slowdown periods. Relieving internal competition enhances dry matter partitioning, increasing sugar concentrations in remaining fruits and improving crop quality.

## 27 RM for L30 - The European Blueberry (*Vaccinium myrtillus* L.) and the Potential for Cultivation. A Review

The European blueberry (EB), also known as bilberry (*Vaccinium myrtillus*), is an important wild berry in Northern Europe, highly demanded by the processing industry due to its delicious taste and high dietary value. EB is a deciduous woody dwarf shrub typically found in spruce- and pine-dominated heath forests of medium fertility. It grows on better-drained acid soils. No efforts towards the domestication of EB have been made; it is still harvested in forest fields without cultivation. The successful domestication of the sweet lowbush blueberry (*V. angustifolium*), which shares similar growth and morphological characteristics, suggests opportunities to increase the highly variable yearly EB yield (potential close to 2 tons per hectare) through practices such as fertilization, irrigation, cutting trees, and weed control [22].

The genus *Vaccinium* belongs to the family Ericaceae. EB belongs to the section *Myrtillus*, while American blueberries belong to the section *Cyanococcus*. EB is a shrub varying in size from approximately 5 to 90 cm, with a chromosome number of  $2n=24$ . Unlike American blueberries, which produce an inflorescence containing a cluster of berries, the EB grows one (rarely two) flower from a leaf axil bud. EB growth pattern is rhizomatous, forming open colonies. Rhizomes can be up to 5.5 m long, and one single clone may occupy several square meters. Aerial shoots, or ramets, typically live for less than 6 years, although ages up to 34 years have been recorded.

EB flowers are born singly (rarely in pairs) in the axils of leaves on one-year-old twigs, which are located on aerial shoots at least three years old. Flower initials develop the year before flowering and overwinter in a dormant bud. A biannual flowering pattern, where EB flowered heavily every second year, has been observed. EB is obligately insect pollinated. The rate of selfing through geitonogamy is significant due to the extent of large EB clones. Cross-pollinated flowers matured approximately four times as many seeds and aborted fewer seeds than self-pollinated flowers, suggesting pollen limitation and inbreeding depression at the seed stage. Berry fresh weight is positively related to the total weight of seeds and the number of seeds.

The most significant characteristic of blueberries is their high content of beneficial nutrients and bioactive phytochemicals. EB has blackish fruit flesh, distinguishing it from American blueberries, which have whitish flesh. EB berries are characterized by 15 anthocyanins and higher levels of total anthocyanins, phenols, and antioxidants compared to highbush varieties. *V. myrtillus* shows a relatively higher degree of vacuolization at full maturation, making them more suitable for local markets rather than long-term storage or transport.

Regarding enhancement of fruit yield by management, mineral nutrition, especially when water is sufficient, increased fruit yield. Fertilization with 150 kg ha<sup>-1</sup> of ammonium nitrate generally increased fruit yield in the first years after implementation. EB is a calcifuge plant and its growth is reliant on the symbiosis with ericoid mycorrhiza for nutrient availability, especially in nutrient poor environments. EB growth is markedly dependent on the P-level in the soils. Optimal conditions for EB include high humidity and minimal shade. Climate has a decisive impact, with issues noted including lethal injury during mild winters in the absence of snow cover, and frost during bloom, which can be rate limiting. Extreme winter warming reduced flower production by more than

80%. Clear-cutting severely reduces vegetative growth, shoot survival, ground cover, and fruit production. When attempting domestication on farmland, adjusting soil pH, often by adding sulphur or organic matter of low pH, may be necessary. Vegetative propagation for homogeneous plant material currently has a low success-rate.

## 28 1<sup>st</sup> RM for L31 - Bioactive Compounds in Cranberries and their Biological Properties

Cranberries are healthy fruit that contribute color, flavor, nutritional value, and functionality. The North American cranberry (*Vaccinium macrocarpon*) is one of only three fruits native to America. Public interest has been rising due to potential health benefits linked to numerous phytochemicals present in the fruit: the anthocyanins, the flavonols, the flavan-3-ols, the proanthocyanidins, and the phenolic acid derivatives. The presence of these phytochemicals appears to be responsible for the cranberry property of preventing many diseases and infections, including cardiovascular diseases, various cancers, and infections involving the urinary tract, dental health, and *Helicobacter pylori*-induced stomach ulcers and cancers. The predominant bioactive compounds found in cranberries are the flavonols, the flavan-3-ols, the anthocyanins, the tannins (ellagitannins and proanthocyanidins), and the phenolic acid derivatives.

Cranberries and cranberry products are known for their elevated concentration in total polyphenols. On a serving size basis for all cranberry products, the order of total polyphenols is: frozen > 100% juice > dried > cocktail > sauce > jellied sauce. More specifically, cranberries are rich in anthocyanins, in tannins (ellagitannins and proanthocyanidins), and have significant concentrations of the flavonoids—flavonols and flavan-3-ols.

The cranberry is one of the leading fruit sources of flavonols on a weight basis, with an average content of 20–30 mg/100 g fresh fruit weight. Approximately 75% of the cranberry flavonols consist of quercetin glycosides (quercetin 3-O-galactoside). Flavan-3-ol content averages 7 mg/100 g for a ripe fruit at harvest. Cranberries have a high content of oligomeric and polymeric pigments, referred to as condensed non-hydrolyzable tannins or proanthocyanidins. The proanthocyanidin content of raw cranberries can average 410 mg/100 g fresh fruit weight. Cranberry proanthocyanidins tend to occur as tetramers to decamers, often with A-type linkages between epicatechin units. Phenolic acids, including derivatives of hydroxycinnamic acid (HCA) and hydroxybenzoic acid (HBA), contribute to the characteristic and unique flavor of berries.

Regarding biological properties, the ability of American cranberry juice (*Vaccinium macrocarpon*) to protect the urinary tract from adherence of uropathogenic bacteria such as *Escherichia coli* is well-known. The French Food Safety Authority issued a health claim stating that "cranberry proanthocyanidins, in a daily dose of 36 mg, help reducing the adhesion of certain *E. coli* bacteria to the urinary tract". Cranberry antimicrobial activity was also demonstrated toward other illness-causing pathogenic bacteria, including *Helicobacter pylori*, *Salmonella*, *Staphylococcus aureus*, and *Campylobacter*. The polymeric tannins, and in particular, proanthocyanidins consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, seem to be the protecting element against pathogenic bacteria.

Considerable in vitro evidence exists, showing that cranberry phenolics are powerful antioxidants. Cranberry phenolics exhibit free radical-scavenging properties against superoxide radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radicals ( $\cdot OH$ ), and singlet oxygen ( $^1O_2$ ). Analysis found that the anthocyanins contributed 54.2% of the antioxidant activity of cranberries, whereas the flavonols contributed 34.6% of the observed antioxidant activity.

The compounds also exhibit antimutagen and anticarcinogen properties. Cranberry phenolic compounds modulate cell signalling pathways, potentially interfering with tumor development. Total cranberry extract (200  $\mu g/mL$ )



inhibited the proliferation of human oral, colon, and prostate tumor cells in vitro. The tumor inhibition by cranberry is likely to involve synergistic activities between the cranberry major phytochemicals—the anthocyanins, the flavonols, and the proanthocyanidins.

## 29 2<sup>nd</sup> RM for L31 - Antioxidant Polyphenols from Tart Cherries (*Prunus cerasus*)

The Montmorency (*Prunus cerasus*) variety of cherries constitutes >95% of tart cherry cultivations in Michigan and the United States. However, Balaton tart cherry (*P. cerasus*) is a new cultivar being planted commercially, which is regarded as a better variety due to higher anthocyanin contents. Flavonoids, a group of widely distributed polyphenolic compounds, have been reported to act as antioxidants in biological systems, sometimes having activity similar to that of  $\alpha$ -tocopherol, vitamin E. This study described the isolation, identification, and efficacy of antioxidant polyphenolic compounds from Balaton and Montmorency tart cherries. Pitted and frozen Montmorency and Balaton tart cherries were obtained from commercial growers in Traverse City, MI [23].

Montmorency and Balaton tart cherries were lyophilized and sequentially extracted with hexane, ethyl acetate, and methanol. The extraction of 200 g of lyophilized Balaton tart cherries yielded crude extracts of 0.42, 1.49, and 116.3 g, respectively. Methanolic extracts of dried Balaton and Montmorency tart cherries inhibited lipid peroxidation induced by  $\text{Fe}^{2+}$  at 25 ppm concentrations. The percent inhibitions of hexane, EtOAc, and MeOH extracts of Balaton tart cherry were 9.9, 28.3, and 13.6%, respectively. Further partitioning of the methanol extract with EtOAc yielded Fraction I, which inhibited lipid peroxidation by 76% at 25 ppm. Fraction I from Balaton cherries contained the most active antioxidant compounds, which were purified by medium-pressure liquid chromatography (MPLC), preparative silica gel TLC, and HPLC.

Purification of Fraction I afforded eight polyphenolic compounds, characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR experiments. The purification yielded:

1. 5,7,4'-trihydroxyflavanone (naringenin)
2. 5,7,4'-trihydroxyisoflavone (genistein)
3. chlorogenic acid
4. 5,7,3',4'-tetrahydroxy-flavonol-3-rhamnoside (quercetin 3-rhamnoside)
5. 5,7,4'-trihydroxyflavonol 3-rutinoside (identified as kaempferol 6''-O- $\alpha$ -L-rhamnopyranosyl- $\beta$ -D-glucopyranoside)
6. 5,7,4'-trihydroxy-3'-methoxyflavonol-3-rutinoside (assigned as rhamnazin 6''-O- $\alpha$ -L-rhamnopyranosyl- $\beta$ -D-glucopyranoside)
7. 5,7,4'-trihydroxyisoflavone-7-glucoside (genistein 7-glucoside)
8. 6,7-dimethoxy-5,8,4'-trihydroxyflavone

Compounds 6 and 8 are reported for the first time from tart cherries. Antioxidant assays revealed that 6,7-dimethoxy-5,8,4'-trihydroxyflavone (8) is the most active at 10  $\mu\text{M}$  concentrations, followed by quercetin 3-rhamnoside (4), genistein (2), chlorogenic acid (3), naringenin (1), and genistin (7). The superior antioxidant activity of compound 8, despite lacking a 3-hydroxyl group and having only one hydroxyl group on the B ring, was probably due to the hydroxyl and two methoxy groups in ring A. The inhibitory effect of flavonoids on  $\text{Fe}^{2+}$  lipid peroxidation is due to their ability to chelate  $\text{Fe}^{2+}$  and their free radical scavenging activities. When assayed independently at the concentrations present in a 25 ppm mixture, compound 8 showed 110% inhibition and compound 3 showed 76.7% inhibition of  $\text{Fe}^{2+}$ -induced lipid peroxidation. The mixture of compounds 1-8 gave 89.6% inhibition at 25 ppm, but the sum of the antioxidant activities of the individual compounds was higher than

that of the mixture, suggesting some purified compounds are more effective inhibitors of lipid peroxidation when tested alone. Compounds 8 and 3 were identified as the most active components in the mixture and probably in Fraction I.

## 30 3<sup>rd</sup> RM for L31 - Antioxidant Polyphenols from Tart Cherries (*Prunus cerasus*)

An overwhelming body of research has firmly established that the dietary intake of berry fruits has a positive and profound impact on human health, performance, and disease. Commonly consumed berry fruits in North America include blackberry (*Rubus* spp.), black raspberry (*Rubus occidentalis*), blueberry (*Vaccinium corymbosum*), cranberry (*Vaccinium macrocarpon*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria × ananassa*). Other popularly consumed berry fruits worldwide include arctic bramble (*Rubus arcticus*), bilberries (*Vaccinium myrtillus*), black currant (*Ribes nigrum*), and lingonberries (*Vaccinium vitis-idaea*). The International Berry Health Benefits Symposium, a series of biennial conferences initiated in 2005, was organized to investigate the latest scientific research related to berry consumption and human health [24].

The various biological properties of berry fruits have been largely related to their high levels and wide diversity of phenolic-type phytochemicals. Both lipophilic (minor) and hydrophilic (major) phytochemicals are found in berries, but the latter class has been largely implicated in the bioactivities. Berry phenolics include flavonoids (anthocyanins, flavonols, and flavanols), tannins [condensed tannins (proanthocyanidins) and hydrolyzable tannins (ellagitannins and gallotannins)], stilbenoids, and phenolic acids.

Among berry phenolics, anthocyanins (pigments that account for attractive colors) are best studied and possess a wide range of bioactivities, including antioxidant, anticancer, and anti-inflammatory properties. The specific class of tannins present varies considerably among berries: blueberries and cranberries contain predominantly proanthocyanidins, whereas blackberries, black raspberries, red raspberries, and strawberries contain predominantly ellagitannins. For example, the bacterial antiadhesive properties observed for the cranberry are accounted for by its oligomeric proanthocyanidins, which possess an A-type structural linkage. Anthocyanins were previously reported to cross the blood-brain barrier of aged rats and localize in various brain regions, important for learning and memory.

Although berry phenolics are best known for their ability to act as antioxidants, the biological activities exerted *in vivo* extend beyond antioxidation. Berry phytochemicals regulate the activities of metabolizing enzymes; modulate nuclear receptors, gene expression, and subcellular signaling pathways; and repair DNA oxidative damage. It is noted that the complementary, additive, and/or synergistic effects resulting from multiple phytochemicals are believed to be responsible for their wide range of observed biological properties, rather than these effects being due to a single constituent alone.

Symposium discussions covered recent research on identifying berry phytochemicals, elucidating cellular and molecular mechanisms of actions, and assessing the bioavailability, metabolism, and tissue distribution of berry phenolics. Oral presentations focused on selected chronic human diseases that show promise in being positively affected by berry consumption, including heart health and cardiovascular disease, neurodegenerative and other diseases of aging, obesity, and certain human cancers, such as esophageal and gastrointestinal cancers. Discussions also addressed the effects of berry consumption on symptoms of the metabolic syndrome and on human performance enhancement.

Future research should emphasize the interdisciplinary cross-fertilization of basic and clinical sciences to cul-

minate in translational research. Focus on nutrigenomics (effects of nutrients on the genome, proteome, and metabolome) and nutrigenetics (effects of genetic variation on the interaction between diet and disease) is essential. Future studies must focus on the metabolomics of berry phenolics, evaluating whether metabolites (such as glucuronidated, sulfated, and methylated derivatives) formed in vivo accumulate within target tissues and act as "pro-drugs". Evaluating whether biological effects are enhanced by the complex interactions of multiple components within the food matrix is also necessary.

## 31 RM for L32 - Deficit Irrigation as a Strategy to Save Water: Physiology and Potential Application to Horticulture

Water is an increasingly scarce resource globally, and irrigated agriculture accounts for about two-thirds of the total fresh water diverted to human uses. Improving crop water-use efficiency (WUE), expressed as the ratio of harvested yield to water use, is a major concern. Deficit irrigation (DI) strategies have emerged as complementary approaches to increase WUE, deliberately allowing crops to sustain some degree of water deficit with only marginal yield decrease and significant reduction of irrigation water. The two main deficit irrigation strategies, based on crop physiological knowledge, are regulated deficit irrigation (RDI) and partial rootzone drying (PRD) [25].

The foremost principle of RDI is that plant sensitivity to water stress is not constant during the growth cycle. In RDI, water is supplied to maintain plant water status within certain limits of deficit during phases where fruit growth is least sensitive to water reductions, potentially benefiting WUE, increasing water savings, and improving harvest quality. PRD involves exposing roots to alternate drying and wetting cycles, enabling plants to grow with reduced stomatal conductance but without overt signs of water stress. PRD operates based on root-to-shoot chemical signaling, where dehydration promotes the synthesis of hormonal signals, such as abscisic acid (ABA), in the drying roots. ABA travels to the leaves via the transpiration stream, reducing stomatal conductance, decreasing water loss, and increasing WUE.

Stomatal regulation is a central process in determining WUE. Stomatal closure is among the earliest plant responses to drought. Stomata are sensitive to environmental signals (light, humidity, temperature, CO<sub>2</sub>) and physiological signals (phytohormones, calcium). Stomatal closure responds earlier to soil water content than to leaf turgor. Xylem sap pH becomes more alkaline in response to soil drying, leading to an accumulation of ABA in the leaf apoplast adjacent to guard cells, inducing stomatal closure.

Deficit irrigation has proven successful for several horticultural crops, particularly fruit crops. Grapevine (*Vitis vinifera* L.) is well adapted to deficit irrigation. PRD and conventional DI (irrigation at 50% ET<sub>c</sub>) promoted intrinsic WUE (A/g<sub>s</sub> ratio) and long-term WUE ( $\delta^{13}\text{C}$  increase) compared to full irrigated grapevines (FI, 100% ET<sub>c</sub>). PRD also reduced vegetative vigor, potentially improving fruit quality due to better light exposure. In deciduous fruit trees, DI strategies mainly aim to reduce vegetative growth with minor changes to fruit development. DI and PRD in apple saved 45% to 75% water, of ten without affecting yield or fruit size, and advance ripening, increased flesh firmness, and increased total soluble solids (TSS). RDI in peach generally increased WUE and reduced vegetative growth without negative yield effects. Incitrus, DI(60–66% of control) increased TSS and titrable acidity (TA), though fruit diameter reduced by about 10%. For soft fruits, PRD (up to 50% reduction) in raspberries did not reduce yields and resulted in higher WUE. However, in strawberries, DI and PRD (at 60% ET<sub>c</sub>) decreased fresh berry yield and individual berry weight, while saving about 40% of irrigation water. Vegetable crops gen-

erally tend not to cope as well as fruit crops. For tomato, DI can improve quality (e.g., higher soluble sugars and color intensity) but may result in pronounced yield loss (e.g., 60%), attributed to flower abortion. However, PRD for processing tomato from fruit set until harvest resulted in significantly higher TSS and saved up to 25% water. In potato, PRD (50-70% ETc) maintained tuber yields and increased irrigation water use efficiency by 60%.

Deficit irrigation strategies also show commercial potential for ornamentals by reducing excessive growth and water consumption by 50% to 90%. Future research must focus on the combination of deficit irrigation with practices like mulching or protected cultivation, and the use of rootstocks adapted to water stress, to further improve WUE and minimize yield or quality losses.

## 32 RM for L33 - CHAPTER 8 Vitamins, aroma compounds and colors

The synthesis of aroma compounds and colors is almost exclusively linked to the progression of maturation. Vitamin C comprises ascorbic acid and its oxygenated form, dehydro-ascorbic acid, with ascorbic acid especially found in fruits. The content of Vitamin C varies widely with the fruit species and may have significant differences between varieties. The developmental stage and level of light exposure are probably the factors with the strongest effect on Vitamin C content. The majority of Vitamin C appears to have been formed when maturation begins. During ripening, the content may remain almost unchanged (strawberries and blackcurrants), decrease (blackcurrant and sour cherry), or increase (apple). During storage and postharvest maturation of apples, there is a clear decrease in Vitamin C content. In both apple and sour cherries, a higher concentration has been found in small compared to larger fruits within a tree, which is explained by the small fruits being behind in development. The light has an influence on Vitamin C content. Illuminated fruits or fruit tissues have a slightly higher content than in shade in both apple and sour cherries, and the content is higher on the south than on the north side of the tree. The colored parts of an apple have a higher content than the uncolored parts. The content is highest in the peel. Impacts found from nitrogen supply, pruning and rootstock on Vitamin C content are explained as indirect effects through the effects on the illumination of the fruits or degree of development. Ascorbic acid content in apples, averaged across five varieties (mg / 100 g fresh weight), demonstrates this concentration gradient:

1. Peel (epidermis) 70
2. Chlorophyllous cell layer under peel 21
3. Outer portion of fruit meat 11
4. Inner part of fruit meat 6
5. Core house (without cores) 5

During maturation, major color changes occur: green changes to more yellow shades, and red colors are formed in many varieties. Young fruits contain chlorophyll (a and b) in the (green) chloroplasts. The yellow-reddish colorants in the fruit are due to carotenoids, found in chloroplasts and chromoplasts. Carotenoids are built up of isoprene units ( $\text{CH}=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$ ) and are often divided into carotenes (hydrocarbons,  $\text{C}_{40}\text{H}_{56}$ ) and xanthophylls (oxygenated substances derived from carotenes).  $\beta$ -carotene is the most common carotene, and cryptoxanthene, zeaxanthin, antheraxanthin and violaxanthin are common xanthophylls. When fruits become yellow during maturation, it is primarily due to chlorophyll decomposition. The green color of chlorophyll covers the yellow carotenoids, making the fruit appear more yellow, even if the carotenoid content does not rise or possibly falls.

The red-blue dyes are anthocyanins, which are water soluble and found in the cell sap. They behave as indicators from reddish shades in acid through colorless to blue at  $\text{pH} > \text{approx.} 6$ . Anthocyanins chemically belong to flavonoids, which typically occur in conjunction with a sugar moiety, i.e., as glycosides. The aglycone is called

anthocyanidin; Cyanidin is a common anthocyanidin. Along with galactose, cyanidin forms idaein, which is common in apples.

For apples, the development of both yellow and red color is enhanced by increasing leaf / fruit ratio (e.g., 10 leaves per fruit yield 23% red color, while 75 leaves per fruit yield 58% red color) and increasing source activity, similarly to sugar content. Nitrogen inhibits the development of both yellow and red color. Light has a decisive and specific effect on the formation of over color (red) on apples, likely activating enzymes, including phenylalanine ammonia lyase (PAL), which participates in the anthocyanin synthesis. Good light conditions in the outer parts of the tree increase color formation significantly. Temperature affects color development. A common rule suggests red color development is promoted by sunny days and cold nights in the fall. Studies indicate that early in the ripening process, color formation is promoted by light and low temperature. However, later, during maturation, color formation gradually happens best in light and at relatively high temperatures. Sugar accumulation seems to be a necessary prerequisite for anthocyanin formation. In other fruit species, color development is also primarily related to the maturation process. Light does not have the same decisive significance for anthocyanin formation as in apples, as it occurs in many other fruits even in shadow, though color formation may be slower or later.

## **33 1<sup>st</sup> RM for L34 - Using the Streif Index as a Final Harvest Window for Controlled-atmosphere Storage of Apples**

A final harvest window (FHW), expressed as Streif Index coefficients, was developed for identifying maximum fruit quality for strains of 'McIntosh', 'Cortland', and 'Jonagold' apples (*Malus × domestica* Borkh.) following 8 months of controlled-atmosphere (CA) storage. Determining an optimal harvest period is critical for growers to maximize poststorage fruit quality and minimize losses. The Streif Index is comprised of three maturity measurements: firmness, percentage soluble solids concentration (SSC), and starch conversion. The Index has been successfully used to estimate the time of optimum harvest for various apple cultivars in Germany, the Netherlands, Hungary, and Poland. Previous research utilized the Streif Index to identify a single optimum harvest date; this study sought to use the Streif Index to delimit a harvest window—a period of a few days—that more realistically parallels fruit maturation and assesses when to conclude harvest to exclude over-mature fruit from long-term storage [26].

Experimental data were collected in the Annapolis Valley of Nova Scotia over three consecutive years (1995-97) on five apple strains: Redmax, Marshall, and Summerland 'McIntosh'; Redcort 'Cortland'; and Wilmuta 'Jonagold'. Samples were collected during nine preharvest intervals (twice per week) and four weekly harvest periods. Starch-to-sugar conversion was evaluated on a 1 (100% starch) to 9 (0% starch) scale using the McIntosh Starch Test Guide. Firmness (N) was assessed using a Ballauf penetrometer with an 11-mm-diameter tip, and juice SSC was determined with a hand-held refractometer. Titratable acidity (TA) was expressed as malic acid equivalents ( $\text{g} \cdot \text{L}^{-1}$ ).

The Streif Index coefficient was calculated as the quotient of  $[\text{firmness}/(\text{SSC} \times \text{starch index})]$ . The relationship between the Streif Index (dependent variable) and day of year (independent variable) was derived by negative first-order linear regression equations. Apples from the four harvest periods were stored in standard CA storage (SCA) for 8 months at 3.5°C (McIntosh and Cortland) or 0°C (Jonagold), with 2.5 kPa O<sub>2</sub> and 4.5 kPa CO<sub>2</sub>, followed by a 7-day shelf-life test at 0°C and 5 days at 20°C. Poststorage quality data—including firmness, SSC, TA, percentage of healthy fruit, and sensory data—were categorized and additively combined to produce an overall fruit quality

rating scale. The final harvest (i.e., day of year) was identified as that which directly preceded at least a 10% drop in the poststorage fruit quality rating compared with the first harvest rating.

The FHW was calculated as the 3-year final harvest mean, with boundaries determined by the standard deviation of the mean, and expressed as Streif Index coefficients via the regression equations. The lower to upper FHW boundaries were determined as:

1. 4.18 to 5.34 for Redmax
2. 4.12 to 5.46 for Marshall 'McIntosh'
3. 4.51 to 5.68 for Summerland 'McIntosh'
4. 5.23 to 5.99 for Redcort 'Cortland'
5. 1.38 to 2.34 for Wilmuta 'Jonagold'

The coefficients of simple determination ( $r^2$ ) for the negative first-order regression models ranged from 0.71 to 0.88. The significantly lower Index values for 'Jonagold' result from the pattern of starch-to-sugar conversion, as cultivars that show rapid declines in starch content tend to display lower Streif Index values at optimal maturity. The Streif Index appears not to be strongly affected by year-to-year or orchard-to-orchard differences within a geographical region of similar climate. The practical utility of the Streif Index method lies in the ease with which apple fruit maturity at harvest can be evaluated for its suitability for long-term CA storage. For long-term CA storage (e.g., 8 months), maximum poststorage fruit quality will be obtained when harvest is concluded within the specified FHW.

## 34 2<sup>nd</sup> RM for L34 - Fruit Ripening and Postharvest

Fruit thinning is required to avoid excessive fruit load that inhibits flower differentiation, potentially causing alternate bearing. Mechanical thinning, such as using the Darwin machine, or chemical thinners amplify natural abscission. In apple, thinning must be completed within 30 days after full bloom, when the 'king' fruit reaches a maximum cross diameter of 15 mm. The optimal leaf/fruit ratio in apple may range between 20 and 40.

Fruit development follows two main models: a single sigmoid curve typical of pome fruit, or a double sigmoid curve characteristic of drupe and berry. In peach, fruit growth is divided into four developmental stages (S1, S2, S3, S4). S1 involves cell division/expansion, while S3 involves only expansion. The duration of the cell division phase (S1) significantly impacts the final fruit density, which affects storability and nutritional value. S2 in drupes is characterized by pit hardening. Hormones such as auxins, cytokinins, and gibberellins control the cell cycle during the cell division stage. Auxins, cytokinins, and gibberellins are associated with high levels during the cell division stage, while auxin increases again at S3, linking to both exponential growth phases. Ethylene regulates cell expansion processes.

Physiological fruit drop is a self-regulatory mechanism. Abscission induction in apple fruitlets is linked to correlative inhibitions between shoot and fruits, which trigger a nutritional stress, the appearance of ROS, isoprene emissions, and an increase of ABA levels in the fruit cortex. AZ activation (iii) is likely caused by a decrease of Polar Auxin Transport (PAT) from the fruit, enhancing ethylene sensitivity. AZ activation leads to cell separation via the hydrolytic digestion of middle lamella and cell wall, mediated by abscission-specific polygalacturonase and cellulase genes.

Assimilate transport and storage regulate sink strength and competition among plant organs. Sucrose is the main translocated carbohydrate, but species like apple, peach, and cherry (Rosaceae) also transport sorbitol. Phloem unloading can be symplastic (following a concentration gradient) or apoplastic (requiring specific membrane transporters). In grape berry, the phloem unloading shifts from symplastic (early development) to apoplastic (progres-

sion of growth/ripening), characterized by the hydrolysis of sucrose into glucose and fructose.

Ripening is a complex physiological syndrome. Fleshy fruits are categorized as climacteric (e.g., apple, peach) or non-climacteric (e.g., grape, strawberry). Climacteric fruits show an increase in respiration and ethylene biosynthesis. Ethylene concentrations as low as  $0.1\text{--}1.0\ \mu\text{l l}^{-1}$  can activate ethylene-dependent processes in climacteric fruits. Ripening involves: (i) pulp structural changes (softening) due to cell wall degradation (pectins, hemicellulose, cellulose), (ii) taste changes (sugar increase, organic acid decrease), and (iii) color changes (chlorophyll degradation, carotenoid and/or anthocyanin accumulation). Anthocyanin biosynthesis is promoted by pronounced day and night temperature fluctuations associated with high light intensities during ripening. Starch represents the main form of sugar accumulation in pome fruits (2 to 4% FW at commercial harvest), which is converted to soluble sugars post-harvest.

Harvest criteria traditionally rely on destructive indexes like flesh firmness (penetrometer), soluble solids content (refractometer), and starch content (Lugol's solution). Non-destructive techniques, such as Near-Infrared Spectroscopy (NIR), offer rapid, field-applicable analysis. The simplified NIR device provides the IAD (absorbance difference) index, which correlates with ripening progression, including ethylene biosynthesis, allowing for the establishment of homogeneous classes of fruit at harvest.

Postharvest quality management aims to limit deterioration, primarily by slowing metabolic activity, especially respiration. Storage protocols use low temperature and Controlled Atmosphere (CA), which controls  $\text{O}_2$ ,  $\text{CO}_2$ , and ethylene concentrations. For long-term CA storage (e.g., 8 months for apples), the Streif Index, calculated as  $[\text{firmness}/(\text{SSC} \times \text{starch index})]$ , can be used to delimit a final harvest window (FWH). The use of 1-methylcyclopropene (1-MCP), an ethylene antagonist, is effective in reducing ethylene production and respiration rates in many climacteric fruits.

## 35 1<sup>st</sup> Supplementary Reading - MU Guide: Budding

Budding is a method of grafting in which the scion (upper portion of the graft) is a single bud rather than a piece of stem or twig. This technique is frequently used for producing fruit trees, roses, and ornamental trees and shrubs to multiply a variety that cannot be produced from seed. Budding may also be used for topworking stone fruits, such as cherry, plum, and peach, which cannot be easily grafted with cleft or whip grafts. Budding, particularly "T" budding, is faster than other grafting techniques and typically results in a high percentage of successful unions; experienced budders may achieve 90 to 100% take. Budding also forms a stronger union than those made with other grafting techniques. Because only a single bud is inserted, the technique allows for the production of numerous new plants even when scion wood of a new variety is scarce. Budding is well adapted to plant shoots from 1/4 to 1 inch in diameter.

"T" budding should be performed when the bark of the stock slips (easily separates from the wood) and buds are fully developed. Most budding occurs from late July to early September (fall budding), where buds normally remain dormant until the following spring. Fall budding is the most common technique for producing fruit trees, although spring budding (March and April) and June budding are also possible. Young plants selected for the understock must have new, vigorous growth, and shoots on the lower 6 inches of the trunk should be removed to provide a smooth working surface 2 to 3 inches above the ground.

The budstick is usually a twig from the current season's growth of the desirable variety, characterized by average vigor, health, and plump, well-developed buds. Buds on the center of the twig are generally superior. Leaves must be clipped off immediately, allowing approximately 1/2 inch of the leaf-stalk to remain as a handle.

To prepare the bud for insertion, a sharp knife is required. The bud is cut from the budstick starting 1/2 to 3/4 inch below the base of the bud, making a smooth, slicing cut upward extending 1/2 to 3/4 inch above the bud. It is critical to cut the wood (shield) attached to the bud straight, as a curve leads to poor contact. Beginners are advised to leave the wood on the bud piece. The most common cut made in the understock for budding is the T cut. The steps for the T cut and insertion are:

1. Select a smooth, branch-free location.
2. Make a vertical cut about 1 1/2 inches long, parallel with the grain of the wood, drawing the knife upward.
3. Make a cross cut at the top of the vertical cut, forming a T, cutting through the bark but not into the wood.
4. Gently lift the bark at the junction of the T cut if the bark is not slipping properly.
5. Slide the base of the bud shield into the vertical slit at the top of the T cut until the top of the shield is even with or below the cross cut.

Prompt insertion is vital to prevent the bud or understock from drying. After insertion, the bud should be wrapped tightly, typically with rubber budding strips, starting above the bud and wrapping downward to keep the bud from being pushed out. Wrapping should cover the horizontal cut but never cover the bud itself.

The bud should be checked 7 to 10 days after setting; if the bud and surrounding bark appear fresh, a union has formed. The understock should not be cut back until the bud union is completed. For fall budding, the stock is cut off near the cross of the T in spring, after the bud starts swelling. Any shoots developing from the rootstock must be removed immediately.

Alternative methods include Chip Budding and Patch Budding. Chip budding involves inserting the bud shield into a small notch on the understock, where it is wrapped tightly with plastic film to conserve moisture. Patch Budding is useful for thick-barked trees, such as walnuts and pecans. Patch budding requires the bark of both the understock and the budstick to be slipping easily, and the removed patch of bark containing the bud must be the exact size of the patch cut in the understock. Double-bladed knives are often used for making the parallel horizontal cuts, usually about an inch apart. The patch must fit snugly on all four sides. Immediately after insertion, the patch must be wrapped, ensuring all four cuts are covered but the bud itself is left exposed. Tension should be released about 10 days after budding by making a single vertical cut on the back side of the understock.

## 36 2<sup>nd</sup> Supplementary Reading - Grafting

Grafting is defined as the act of joining two plants together. The upper part of the graft is the scion, which becomes the top of the plant, while the lower portion is the understock (or rootstock), which provides the root system or part of the trunk. A piece of plant grafted between the scion and understock is known as an interstem (or interstock). Grafting is employed because some cultivars do not come true from seeds or are difficult/impossible to reproduce from cuttings. It is also a method for topworking large trees, using a root system better adapted to soil or climate, or producing dwarf plants via special understocks or interstems. Grafting is not a means of developing new varieties, nor does it intermingle the genetic structure of two different plants; any resulting different shoot types are the result of a chimera, a type of mutation.

For a good graft union, the cambium (a single cell layer between the wood and bark) of the scion must line up as closely as possible with the cambium of the stock. Only plants closely related botanically generally form a good graft union, and the stock and scion must be compatible. Plants of the same genus and species can usually be grafted. Plants of different genera are less successfully grafted, for example, quince, genus *Cydonia*, may be used as a dwarfing rootstock for pear, genus *Pyrus*. Plants of different families cannot be successfully grafted for woody plants.



Most grafting is done in late winter or early spring before new growth begins, after the chance of severe cold has passed but well before hot weather. Scion wood must always be dormant. Necessary materials include a sharp knife, grafting wax (hand or brush) to prevent drying, and materials like grafting tape or rubber budding strips for binding. Half-inch nails are used for veneer, bridge, and inarching grafts.

Grafting techniques are determined by the size relationship between the scion and understock. Whip Graft: This graft is suitable for stock and scions of similar diameter, preferably between 1/4 and 1/2 inch. The strongest graft results from a whip-and-tongue system. Both stock and scion require a smooth sloping cut (1 to 2 1/2 inches long). The tongue is formed by a downward cut about 1/2 inch long made one-third down from the tip of the slanting cut, as close to parallel with the grain as possible. The union must be wrapped and waxed.

Cleft Graft: This is commonly used to topwork trees on branches not more than 2 1/2 inches in diameter. The stock must be sawed off at a right angle and split through the center about 2 inches deep. The scion is 1-year-old wood, about 1/4 inch in diameter, containing two or three buds. Preparing the scion involves:

1. Starting below the lowest bud and making a long, smooth cut toward the base (1 to 1 1/2 inches long).
2. Turning the scion and making a second smooth cut of the same length so that the side containing the lowest bud is slightly thicker.

The scion is inserted with the thicker side outward so the cambiums match, often with two scions inserted per slit. All cut surfaces must be waxed thoroughly.

Bark Graft: This technique is performed when the bark slips, usually in early spring. The stock is cut at a right angle, and a slit or two slits are made in the bark. The dormant scion (4 to 5 inches long) is cut to form a shoulder and a long, smooth cut 1 1/2 to 2 inches long. The scion is pushed under the bark until the shoulder rests on the stub.

A common reason for graft failure is that the cambiums were not meeting properly. Other causes include incompatibility, incorrect timing, unhealthy or non-dormant scions, improper waxing, or girdling because tape was not cut or released in time.

# Chapter 3

## Exam Questions and Answers

This chapter of the course notes compiles the exam questions for the course held in February 2026, along with their respective answers prepared by me. The purpose of this section is twofold: firstly, to provide a reflective exercise that consolidates understanding of the course material; and secondly, to document my comprehension of the course topics as assessed through the exam questions.

To ensure citation accuracy and academic transparency, NotebookLM has been employed as the primary generative AI platform. Its use has focused on verifying that all citations accurately reference the uploaded course materials and lecture slides provided by the professors. Beyond citation control, this section also represents an ongoing exploration of prompt engineering - refining interaction design to optimise AI output quality, precision, and academic reliability. Through this approach, the work aims to maintain a high academic standard while enhancing clarity, structure, and depth in written responses.

There are a total of 17 questions in the exam, each comprising between three and five sub-questions. The numbering of the sections in this chapter corresponds directly to the numbering of the exam questions, ensuring a clear and consistent structure throughout. Questions 1-9 address aspects related to crop physiology, while questions 10-17 focus on fruit quality, maturity, and usability. Each question is presented below, followed by its respective sub-questions and answers.

## **Questions within: Crop Physiology aspects**

# **Chapter 4**

## **Individual Report**

The individual report submitted for the exam is included in the following pages. It has been inserted as a full PDF document to preserve formatting and layout - unfortunately, this means that the links, references, and citations may not be fully functional.



# **Aroma Development and Volatile Composition in Mango**

**A Theoretical Report on the Biochemical Pathways, Ripening Physiology, and Quality Determinants in mango.**

**Lucas Daniel Paz Zuleta, TZS159**

MSc students at the University of Copenhagen

Last compiled: 30-10-2025

[Link to Git repository](#)

# Preface

This written report has been prepared as part of the course NPLK14014U - Fruit and Berry Crop Physiology and Quality at the University of Copenhagen. The course explores the physiological and biochemical factors determining fruit development, yield, and quality in relation to fresh consumption and processing. Emphasis is placed on how genetic, environmental, and management factors influence internal and external fruit quality, including the formation of secondary metabolites and sensory attributes such as aroma.

The present project is a theoretical study on the development and composition of aroma in mango, *Mangifera indica* L., The focus is on the biochemical formation of volatile organic compounds (VOCs) and their relationship to fruit ripening and sensory quality. The report integrates physiological and biochemical knowledge to describe how preharvest conditions, cultivar differences, and post-harvest handling affect aroma expression and the perception of fruit quality.

The assignment was carried out by Lucas Daniel Paz Zuleta (TZS159), MSc student at the University of Copenhagen, as an individual written report fulfilling the course requirements for NPLK14014U - Fruit and Berry Crop Physiology and Quality.

# Summary

Mango (*Mangifera indica* L.) is renowned for its distinctive aroma. This characteristic results from a complex mixture of volatile organic compounds (VOCs) that are developed during the fruits ripening and maturation process. These VOCs include terpenes, esters, aldehydes, alcohols, and lactones, which in combination contribute to the mangoes unique sensory qualities and the overall aroma profile. The primary volatile compounds are biosynthesised through three main metabolic pathways: The terpenoid pathway, which produces terpenes. The fatty acid derivative pathway, that forms aldehydes and esters. And the amino acid derivative pathway, where branched chain volatiles are generated. Some key enzymes involved in these pathways are Alcohol Dehydrogenase (ADH), Terpene Synthases (TPSs), and Lipoxygenase (LOX), which during ripening and maturation plays a crucial role in the formation and balance of the aroma profile. Genetic factors indicates a significant influence on the aroma profile across mango cultivars. New World varieties are generally terpene rich, wheareas Old World cultivars are rich in esters and alcohols. Environmental conditions, as well as post-harvest treatments, has a significant impact on the volatile composition. Ethylene driven ripening enhances aroma formation, while chilling stress will suppress monoterpenes and increase C<sub>6</sub>/C<sub>9</sub> aldehydes. Processing methods, e.g. drying, can lead to significant losses of volatiles, although it has been shown to preserve lactones and esters when osmotic dehydration is applied as a pre-treatment. Analytical characterisation of volatile compounds in mango relies on solvent-free extraction methods, such as Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS). Sensory tools like GC-Olfactometry are also used to identify odour-active compounds.

Integrating biochemical, environmental, and analytical perspectives provides a foundation for optimizing cultivar selection, post-harvest handling, and good processing practices to obtain a consumer desirable aroma quality in mangoes.

**Signature**

Copenhagen, 30-10-2025



Lucas Daniel Paz Zuleta (TZS159)

30-10-2025

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# 1 Introduction

Fruits are complex and heterogeneous structures that comprise a wide variety of metabolites that serve as precursors to volatile organic compounds (VOCs), such as carbohydrates, fatty acids, and pigments [1]. The mango fruit is no exception of this complexity and have in recent years been the focus of many studies, studying the activity of volatiles in the fruit [2].

## 1.1 Background

Mango is a tropical fruit which belongs to the Anacardiaceae family and is scientifically known as *Mangifera* [2]. It is popularly characterised as a sweet, juicy, aromatic fruit with a low fibre flesh [3]. It is primarily cultivated in tropical and subtropical regions, where it is of significant economic importance [3]. The annual tropical production of mango is over 46 million tons and is thereby the most produced tropical fruit after banana [3, 4]. The perishable nature and susceptibility to post-harvest losses and diseases pose challenges for the mango industry, restricting the production and potential [3].

## 1.2 Focus on Mango

The mango is an important fruit crop worldwide, valued for its high nutritional content, with significant levels of fibre, vitamin C and  $\beta$ -carotene [4]. However, beyond its nutritional benefits, the mango is particularly renowned for its distinctive aroma, which plays a crucial role in consumer preference and marketability [5] as an indicator of quality and freshness [3]. A study by Badar et al. (2016) found that aroma is one of the most important quality attributes that influence consumer preference and acceptance of mango fruit [5]. The main aroma contributors in mango are the VOCs; aldehydes, alcohols, esters and ketones [6]. Among these, 3-carene, limonene,  $\beta$ -pinene, acetaldehyde, ethanol and hexanal [6]. Understanding the origin and behaviour of these volatiles is therefore essential for improving fruit quality, post-harvest handling, and processing applications.

## 1.3 Aim and Scope

The aim of this report is to explore the formation, composition, and significance of VOCs in mango fruits. The main focus is on how these compounds contribute to the fruit's aroma and overall quality, both in terms of maintaining freshness and enhancing consumer appeal. The scope includes an overview of the chemical classes and key VOCs identified in mango, the metabolic- and enzymatic pathways involved in their biosynthesis, and the influence of pre- and post-harvest conditions on their abundance. Finally, the report outlines the methods commonly applied for the extraction and identification of VOCs and their relevance for assessing fruit quality and consumer perception.

This approach connects biochemical aroma formation with physiological and environmental factors, providing a deeper understanding of mango fruit quality from farm to table.

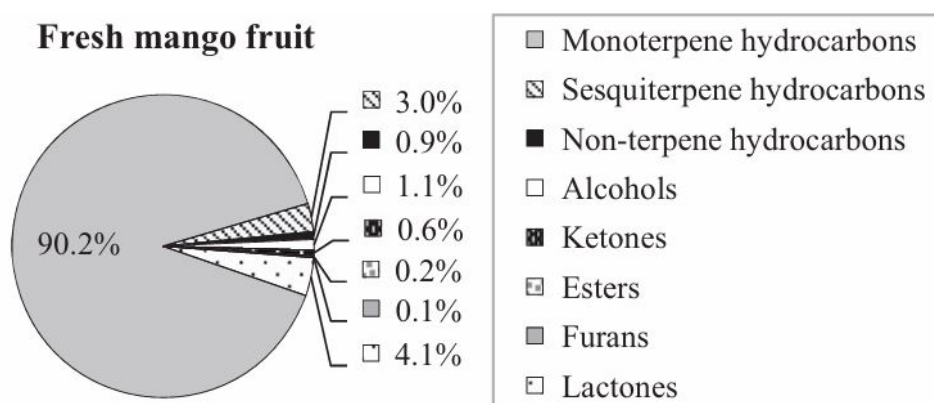
# 2 Aroma Composition in Mango

The complexity of aromas in mango is due to the variety of VOCs that are present in the fruit's matrix. These compounds arise from different biochemical pathways and contribute to the overall sensory experience of the fruit [3]. These pathways are most prominently manifested during the ripening processes of the fruit. Also visual and textural changes occur during ripening, which further influence the perception of aroma [7, 3].

## 2.1 Chemical Classes of Mango Aroma Compounds

The volatile composition in fresh mango fruits has been extensively studied, revealing a diverse array with several hundred identified volatile compounds, occurring in free form in the fruit [4]. By calculating the flavour dilution factor (FD) of the volatile compounds using gas chromatography-olfactometry (GC-O) analysis, it has been possible to identify the key aroma compounds for the odour-active fraction [4].





**Figure 1.1:** Distribution [%] of chemical classes of volatile compounds in fresh mango. Adapted from Bonneau et al. (2016) [4].

Mango aroma is primarily composed of several chemical classes. The most dominant classes is monoterpene hydrocarbons, which account for 90.2% of the total volatile compounds in fresh mango, as illustrated in Figure 1.1. Other significant classes include lactones (4.1%) and sesquiterpene hydrocarbons (3.0%) [4].

## 2.2 Key Aroma Compounds in Mango

In this subsection, the key aroma compounds identified from fresh mango fruits in the study by Bonneau et al. (2016) are discussed [4].

### 2.2.1 Terpenes and Terpenoids

Terpenes represent the largest class of mango aroma compounds, derived from isoprene units via the terpenoid biosynthetic pathway [8]. They include both monoterpenes and sesquiterpenes, which together account for the majority of mango volatiles [4]. Terpenes and terpenoids are found in many different natural sources, including fruits, plants, animals, microbes, and fungi. The terpenes belong to the largest class of secondary metabolites in nature and consist of five connected carbon atoms, known as isoprene units. These carbon units can be assembled in thousands of ways [9]. The terpenoids are further subcualified into five sub-groups based on the number of isoprene units they contain: monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), and triterpenes (C30) [9].

**Monoterpene hydrocarbons** A total of 11 monoterpene hydrocarbons were identified as key aroma compounds in fresh mango [4]. The most significant ones include:  $\alpha$ -phellandrene,  $\gamma$ -terpinene,  $\delta$ -3-carnene,  $\beta$ -myrcene,  $\alpha$ -terpinene, limonene,  $\beta$ -phellandrene, and  $\alpha$ -terpineolene.

Monoterpenes are the smallest molecules in the isoprenoid family with conserved hydrocarbons [8]. They share the formula  $C_{10}H_{16}$  and over 400 different chemical structures has been classified as such [8]. The key monoterpene hydrocarbons identified in mangos are reported to have significant impact on the overall odorants [4].

**Sesquiterpene hydrocarbons** Compared to monoterpenes, sesquiterpenes are larger molecules with the formula  $C_{15}H_{24}$ . In mango fruit, the study by Bonneau et al. (2016) identified four key sesquiterpene hydrocarbons, including:  $\alpha$ -gurjunene,  $\alpha$ -copaene,  $\beta$ -caryophyllene, and  $\alpha$ -caryophyllene [4].

### 2.2.2 Alcohols and Aromatic Alcohols

Alcohols and aromatic alcohols are, like terpenes and terpenoids, major contributors to the key aroma of mango, though they differ in chemical structure and biosynthetic origin. A study by Singh et al. (2010) highlighted the role of alcohol dehydrogenase (ADH) in the enzymatic reduction of aldehydes leading to the formation of these compounds [10].

A total of nine alcohols were identified as volatile compounds in *Kent* fresh mango [4]. Among these compounds, 1-butanol was quantified as one of the key aroma contributors. Other identified alcohols include 3-methyl-1-butanol, 2-decanol, and 1-hexanol. Many compounds, such as 2-methyl-1-propanol did not appear in the fresh *Kent* mango, but increased significantly during drying [4].

### 2.2.3 Aldehydes and Aromatic Aldehydes

Aldehydes are volatile compounds primarily formed through the oxidation of unsaturated fatty acids, such as  $\alpha$ -linolenic acid, via the lipoxygenase (LOX)-hydroperoxide lyase (HPL) pathway. These reactions generate C<sub>6</sub> and C<sub>9</sub> aldehydes, often referred to as green leaf volatiles, which are associated with fresh and grassy notes in the mango fruit aroma [11]. In mango, this pathway becomes particularly active under chilling stress, leading to increased levels of compounds such as 1-hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenal before visible quality loss occurs.

In the fresh *Kent* mango, a total of one aldehyde, hexanal, was identified as key aroma compounds [4]. While not detectable in the fresh fruit, the study did identify a total of six aldehydes post-drying, include hexanal, (*E,E*)-2,4-heptadienal, nonanal, and (*E,Z*)-2,4-heptadienal [4].

### 2.2.4 Lactones

Lactones are VOCs that quantitatively represents a smaller fraction of the mango aroma profile compared to terpenes and terpenoids. They are nonetheless important contributors to the overall sensory experience of the fruit, and make up two times the quantitative amount of alcohols, ketones, esters, and furans combined [12, 4]. Lactones are cyclic esters formed through the intramolecular esterification of hydroxy acids. In mango, they are primarily derived from the oxidation and subsequent cyclization of fatty acids during the ripening process [13].

A total of five lactones were identified as volatile compounds in fresh mango, in the study by Bonneau et al. [4]. Among these compounds, the most significant constituent in the fresh fruit was  $\gamma$ -butyrolactone. Other lactones detected includes:  $\alpha$ -methyl- $\gamma$ -butyrolactone,  $\gamma$ -hexalactone,  $\delta$ -hexalactone, and  $\delta$ -octalacetone [4].

### 2.2.5 Minor Volatile Compounds: Ketones, Esters, and Furans

Ketones, esters, and furans are present in much smaller amounts compared to terpenes and terpenoids, but still contribute to the complexity of mango aroma [4]. Ketones are important volatile compounds that can be identified in exotic fruits such as mango, e.g. 1-octen-3-one, which provides a mushroom aroma [7]. Esters are associated with fruity and sweet nuances, whereas the furans provide the mango fruit with mild caramel-like notes. Despite low abundance of these three classes of compounds, they may enhance the overall balance of aroma perception in fresh mango [13].

## 2.3 Variation in Aroma Profiles Among Mango Varieties

The volatile composition of mango (*Mangifera indica* L.) varies substantially among cultivars, reflecting genetic differences and environmental influences on fruit metabolism and ripening [7]. Comparative analyses of multiple mango varieties have shown that the abundance and composition of key aroma compounds differ significantly across cultivars, leading to distinct sensory profiles [7, 6].

A recent study by Tandel et al. [14] investigated sixteen Indian mango cultivars, revealing notable differences in their volatile profiles. Cultivars such as *Alphonso*, *Kesar*, and *Ratna* were characterised by high levels of terpenes, whereas *Amrapali* was dominant in esters [14]. Within the terpene class, *allo-ocimene* and  $\beta$ -ocimene were frequently dominant, while the presence of butanoic acid derivatives contributed to the sweeter aroma of ester-rich cultivars [14].

El Hadi et al. [13] further distinguished the aroma profiles of mango cultivars according to their geographical origin. New World varieties, such as *Haden*, *Irwin*, and *Tommy Atkins*, were typically dominated by terpene hydrocarbons—especially  $\delta$ -3-carene—whereas Old World cultivars contained higher proportions of esters, alcohols, and ketones, resulting in sweeter and fruitier profiles [13].

As discussed by Tandel et al. [14], previous studies comparing Chinese cultivars *Tainong* and *Jinmang* found high levels of  $\beta$ -ocimene, myrcene, and  $\alpha$ -terpinolene, which significantly contributed to their aroma profiles. These results aligned with Xie et al. [15], who also reported terpenes as a dominant volatile class in *Jinmang*. In a separate comparison of *Tainong* and *Hongyu*, Xie et al. [15] observed that *Tainong* had higher levels of terpenes and aldehydes, producing a grassy aroma, while *Hongyu* exhibited higher ester content, leading to a more fruity and sweet aroma.

## 3 Biochemical Pathways of Aroma Compound Formation

VOCs are low-molecular-weight molecules with functional groups such as alcohols, esters, ketones, aldehydes, and terpenes [7, 9]. These compounds are synthesised through metabolic and enzymatic reactions during maturation and ripening, and significantly impacts the fruits aroma profile [7]. VOC profiling is therefore an important indicator of fruits quality, cultivar differentiation, and ripeness [7].

The study of VOCs is known as *volatilomics*, which investigates the biosynthesis and metabolic pathways of aroma compounds [7]. The biosynthesis of VOCs in fruits is based on three major pathways: the amino acid derivative pathway, the fatty acid derivative pathway, and the terpenoid pathway [13]. These pathways will be described in detail below. Once the basic molecular forms are synthesised, enzymatic reactions, such as hydroxylation, acylation, methylation, oxidation-reduction, and ring closure, enhance volatility and molecular diversity [13].

### 3.1 Enzymes Involved in Aroma Biosynthesis

The diversity of VOCs in mango arises from enzymatic reactions that modify basic molecular structures, e.g. hydroxylation, acylation, methylation, oxidation-reduction, and ring closure [13]. ADH plays a key role in converting aldehydes to alcohols and supplies substrates for ester synthesis. Mangoes expresses multiple ADH isoforms (MiADH1-3) that are differentially regulated during ripening [10]. Other enzymes which contributes to the aroma formation includes methionine  $\gamma$ -lyase and 3-ketoacyl-CoA thiolase B, which are active in amino-acid- and fatty-acid-derived pathways [3]. In the fatty acid pathway, LOX and HPL cooperates with ADH to produce  $C_6$  and  $C_9$  volatiles, whereas terpene synthases (TPSs) catalyses the cyclisation of prenyl diphosphate precursors in the terpenoid pathway [13].

### 3.2 Amino Acid Derivative Pathway

The Amino Acid Derivative Pathway is responsible for synthesizing the fruits characterised flavour and aroma compounds [13]. Amino acids, such as alanine, valine, leucine, isoleucine, phenylalanine, and aspartic acid, serves as direct precursors for these pathways, forming a variety of compounds including alcohols, carbonyls, acids, and esters [13]. The biosynthesis typically starts with a deamination or transamination reaction that produces the corresponding  $\alpha$ -keto acid [13]. Subsequent steps follows, including decarboxylation, reduction, oxidation, and/or esterification, to produce the volatile compounds [13], e.g. branched chain volatiles, which often are derived from leucine, isoleucine, and valine [3].

### 3.3 Fatty Acid Derivative Pathway

The fatty acid derivative pathway is a major source of aroma volatiles in fruits. The pathway produces straight-chain alcohols, aldehydes, ketones, esters, and lactones [13]. There are mainly three processes involved in the fatty acid derivative pathway:  $\alpha$ -oxidation,  $\beta$ -oxidation, and the LOX pathway [13, 14]. The  $\beta$ -oxidation process shortens fatty acids by removing  $C_2$  units (acetyl-CoA), forming precursors for ester synthesis [13]. The LOX pathway converts polyunsaturated fatty acids such as linoleic and linolenic acid into  $C_6$  and  $C_9$  aldehydes and alcohols via HPL and ADH [13]. Compounds like hexanal and nonanal are formed by linolenic and oleic acid degradation, and hydroxylated intermediates can undergo spontaneous lactonization to form lactones such as  $\gamma$ -butyrolactone [4, 12].

### 3.4 Terpenoid Pathway (MEP and MVA Pathways)

Terpenoids represent the largest class of plant secondary metabolites, the two primary groups are monoterpenes and sesquiterpenes [2, 6, 8]. They all share the  $C_5H_8$  isoprenoid unit and are derived from the universal precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), and are synthesised by two distinct routes: the cytosolic mevalonic acid (MVA) pathway, and the plastidial methylerythritol-4-phosphate (MEP) pathway [8, 13]. MVA starts from acetyl-CoA, whereas MEP uses pyruvate and glyceraldehyde-3-phosphate (G3P) as precursors [8]. These pathways happen simultaneously in different cellular compartments, enabling an exchange of intermediates [8]. Subsequently, IPP and DMAPP are joined together by prenyltransferases which produces geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP) [8, 13]. TPSs then catalyses the cyclisation into different terpene skeletons that defines the mango aroma [8, 13].

## 4 Environmental and Genetic Factors Influencing Aroma Production

The aroma profile of fruit is complex and determined by a combination of both genetics and environmental factors [13, 14]. The composition and concentration of VOCs are specific to the fruit species and across cultivars [13, 14]. Variations among mango cultivars, for example, demonstrate that genetic background defines the abundance of specific volatile classes (e.g., some are monoterpene-dominant, while others has high levels of esters, fatty acids, or sesquiterpenes) [14].

### 4.1 Pre-harvest Factors

Pre-harvest conditions significantly influence the final volatile composition [13]. Environmental conditions such as climate (temperature and humidity) and geographic region (sunlight, precipitation, and fertilisation) can modulate VOC quantity in plants [7, 14]. These factors affect crop growth and aroma quality, including flavour and texture [13].

### 4.2 Post-harvest Factors

The stage of maturity and ripening is a critical post-harvest factor since VOCs are released during the maturation processes [7, 13]. In climacteric fruits like mango, ripening is associated with an increase in ethylene production and subsequent synthesis of aromatic volatiles [3]. In mango, the expression of key aroma genes, such as ADH, is regulated by hormones like ethylene and Absciscic Acid (ABA) at the initial stages of ripening [10]. Furthermore, storage temperature has a significant impact on the volatile profile [13]. Storing mangoes at sub-optimal temperatures (e.g. 5°C) induces chilling stress, which results in a reduced formation of desirable aroma compounds such as monoterpenes, while it simultaneously increases  $C_6$  and  $C_9$  aldehydes [11].

### 4.3 Processing Implications on Aroma Retention

Processing methods, particularly drying, results in a significant loss of aroma compounds [4]. A study by Bonneau et al. [4] on drying *Kent* mango fruit showed a total volatile loss of approximately 58.8% [4, 14]. This decline is primarily attributed to evaporation and thermal degradation of compounds such as monoterpenes, sesquiterpenes, and lactones [4]. However, pretreatment techniques like osmotic dehydration (DO) has shown to help decrease these losses and aid in the conservation of volatile compounds [6]. It was proposed that sugar impregnation blocked the exit of internal gases during subsequent drying [6]. Some compounds, such as hexanal and nonanal, showed a post-drying increase possibly due to the degradation of unsaturated fatty acids [4].

## 5 Analytical Techniques for Aroma Compound Identification

### 5.1 Extraction and Analysis Methods

The most common technique for sampling volatile compounds in fruits is Solid Phase Microextraction (SPME) [7]. SPME is highly favored because it is solvent-free, fast, sensitive, and requires minimal sample manipulation by reliably collecting analytes from the headspace (HS) [7, 1]. The technique typically utilizes adsorbent fibers composed of materials such as DVB, CAR, PDMS, and PA to capture the volatile compounds [7]. When extracted, the Gas Chromatography coupled with Mass Spectrometry (GC-MS) is used, which separates molecules and identifies them via mass detection [7]. Other used methods include Liquid-Liquid Extraction (LLE) for non-polar VOCs [7], Solvent Assisted Flavour Evaporation (SAFE) for preparing aroma extracts of the original product [7], and Single Drop Microextraction (SDME) for miniaturized analysis [1].

### 5.2 Quantification and Sensory Evaluation

Sensory analysis is a crucial aspect. Odour and aroma are quality attributes directly linked to consumer acceptance [7]. To quantify the sensory impact, the Odor Activity Value (OAV) estimates odor potency by calculating the ratio between a compound's concentration and its odor detection threshold [7, 4]. Similarly, Aroma Extract Dilution Analysis (AEDA) can rank the perception of compounds which are important to the overall aroma via the flavor dilution (FD) factor [7, 4]. Specialized techniques combines analysis with human perception, such as GC-Olfactometry (GC-O), which routes the column effluent to a "sniffing port" for sensory evaluation by a trained panel [7]. Furthermore, Multivariate Data Analysis, such as Multiple Correspondence Analysis (MCA), is used to explore statistical correlations between molecules, aromas, and fruits [7, 15].

## 6 Comparative Perspective

Mango aroma varies significantly across cultivars, geography, and processing, indicating both genetic and environmental influences on volatile biosynthesis [13, 14]. Indian cultivars showed differences like *Alphonso*, *Kesar*, and *Ratna* which often are terpene-dominant, while other cultivars exhibited different major components, such as *Neelum* which had high levels of sesquiterpenes (74.80%) [14]. Geographical factors also showed significant variations. New World varieties (*Haden* and *Tommy Atkins*) were dominated by terpene hydrocarbons, while Old World mangoes contained higher proportions of esters, alcohols, and ketones [13]. Similarly, analysis confirmed that *Tainong* expressed grassy notes due to high terpene and aldehyde content, while *Hongyu* was rich in esters and were perceived as fruity and sweet [15].

Ripening and post-harvest handling significantly impacts the aroma profiles. During maturation, ethylene activation enhances the synthesis of aromatic volatiles, including terpenes and esters derived from pathways involving ADH [10]. Conversely, chilling stress (e.g., storage at 5°C) compromises aroma quality by suppressing monoterpenes and increasing the concentration of stress-related C<sub>6</sub> and C<sub>9</sub> aldehydes and alcohols [11]. Drying *Kent* mangoes greatly reduces the total volatile content, mainly through the loss of terpenoids, but compounds like lactones and esters may be retained or enriched, altering the sensory balance [4]. Techniques like osmotic dehydration has been proposed to help mitigate these losses [6].

The variation observed in aroma studies is often attributed to the analytical choices, as the volatile profile is highly sensitive to the method used [13]. Techniques such as SPME, SDME, and SAFE capture different classes of compounds from the fruit headspace [7, 4]. Crucially, GC-O, often utilizing flavor dilution analysis, identifies compounds based on their potent sensory impact, such as a high OAV, rather than only their chemical abundance recorded by GC-MS [7, 4].

## 7 Conclusion

Mango aroma complexity stems from the interaction of hundreds of VOCs, predominantly monoterpene hydrocarbons, which originate primarily from three core biosynthetic routes: the Terpenoid pathway, the Fatty Acid Derivative pathway, and the Amino Acid Derivative pathway.

The specific aroma profile is highly influenced by the cultivars genetic background, leading to significant differences between terpene-dominant New World cultivars and ester/alcohol-rich Old World varieties. This inherent chemistry is critically influenced by environmental and post-harvest factors. Ripening promotes the synthesis of aromatic volatiles, while stresses, such as cold storage (5°C), lead to less formation of monoterpenes and an increase in both  $C_6$  and  $C_9$  aldehydes from the oxidation of  $\alpha$ -linolenic acid. Furthermore, processing methods like drying, causes significant losses of volatile compounds, approximately 58.8%, although pre-treatment strategies like osmotic dehydration can mitigate the aroma decrease.

Accurate characterization of the aroma profiles relies on analytical tools, primarily SPME extraction coupled with GC-MS, complemented by sensory techniques like GC-O and OAV to identify the few odor-active compounds that define consumer perception. Comprehensive understanding of these biochemical and environmental factors are essential for the mango industry to optimize post-harvest handling and select cultivars that guarantee specific, consumer-desirable aroma traits. Future research should aim to combine volatilomics with genetic data, thereby linking the key synthesis enzymes like ADH, TPSs, and LOX to cultivar-specific aroma formation.

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# **Chapter A**

## **Appendix**

### **1 Appendix 1 - GAI declaration**



## UCPH's AI declaration

### Declaration of using generative AI tools

☒ I/we have used generative AI as an aid/tool (please tick)

☐ I/we have **NOT** used generative AI as an aid/tool (please tick)

*If generative AI is permitted in the exam, but you haven't used it in your exam paper, you just need to tick the box stating that you have not used GAI. You don't have to fill in the rest.*

### List which GAI tools you have used and include the link to the platform (if possible):

GitHub Co-pilot (No link since its integrated in Visual Studio Code)

ChatGPT (<https://chatgpt.com/>)

NotebookLM (<https://notebooklm.google.com/>)

### Describe how generative AI has been used in the exam paper:

- 1) GitHub Co-pilot was used for the following:
  - a. Layout setup and administration of project folder
  - b. Code trouble shooting
  - c. Ideas for how to overcome coding challenges
- 2) ChatGPT was used as a sparring partner in the following steps:
  - a. Help with structuring sections and respective subsections for a good reading flow
  - b. Creation of summaries and elaboration of terminology and biochemical pathways
  - c. Finding peer-reviewed papers on specific topics, when only pay-per-view content was found
- 3) NotebookLM was used as a sparring partner in the following:
  - a. To administer sources
  - b. Cross-check if citations have been sufficiently given
  - c. Elaboration of sections within the source

# Chapter 5

## Abbreviations and Explanations

Topic	Abb.	Description
Leaching	n.a.	<i>leaching refers to the process by which substances, such as ions, minerals, or nutrients, are removed or lost from the soil. This often occurs due to water penetrating the soil and displacing these substances</i>

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