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Phenology and effect of climate on apple cultivars (*Malus domestica* Borkh.) in Norway

Rodmar Rivero Casique

PHENOLOGY AND EFFECT OF CLIMATE ON APPLE CULTIVARS (*Malus domestica* Borkh.) IN NORWAY

Submitted by:

BSc. Rodmar Rivero Casique

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Department of Plant Sciences

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i. Abstract

Successful fruit growing is dependent on a good control of flower-bud formation (FBF), knowledge on the timing of developmental processes in the tree, and how these are related to each other in the Norwegian climate and in different cultivars. In the current study, FBF was investigated in relation to climate and developmental processes such as growth cessation, anthesis, fruit ripening and leaf abscission in 14 early, middle and late flowering apple cultivars at Ås in Norway. In addition, the effect of the local climate at Landvik, Ås, Ullensvang, Kapp and Stjørdal on FBF and development of young trees of 'Aroma' and 'Gravenstein' was studied. The onset of FBF differed between shoot types and cultivars. It started first in spurs of actively growing trees, approximately 8 weeks after full bloom. In extension shoots, FBF occurred after growth cessation, approximately 10 weeks after full bloom, and extended throughout the autumn. Large variations were found in the proportion of buds that became floral between sampling dates, shoot types and cultivars, and this reflects the lack of synchrony of the FBF process. The onset of FBF was somehow related to leaf senescence, harvesting, and fruit ripening time in some cultivars, however, these relationships were unclear and may be artificial. The local climate at the five locations studied, especially accumulated temperatures of 15°C and low precipitation during July, accounted for over 70% of the variation in the proportion of flower buds formed. Flower buds from the northernmost location (Stjørdal) were in a less developed stage compared to buds from the southernmost location (Landvik). Despite the slightly higher temperatures during July and August at Landvik, the proportion of flower buds formed was higher in trees from Ås and Kapp, and this may indicate that other factors rather than air temperature were involved (e.g. gardener practices and placement of trees).

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1. Introduction

Flowering in deciduous fruit trees has been an interesting subject for both growers and scientists for many years. For fruit growers, the importance of flowering lies in the fact that flowers are a prerequisite for the formation of the crop (Tromp et al. 2005). The amount and quality of flowers are central factors determining the size of the crop, thus representing the potential yield in the orchard. For scientists, the interest for this subject lies not only in its economic importance, but also in its complexity and significance as a crucial step of the reproductive cycle of the tree and as a model for other woody species in the *Rosaceae*, e.g. pears (Buban & Faust 1982). Moreover, the study of flowering is also interesting in connection to climate and climate change, for the breeding of new cultivars adapted to different climatic regions, to understand the mechanisms of biennial bearing and for modelling the risk of attack by pests, such as the apple fruit moth, *Argyresthia conjugella*, the major pest of apple in Norway (Kobro et al. 2003).

As for most deciduous fruit trees, apple trees have a reproductive cycle in which they shift from vegetative to generative (floral) growth (Hanke et al. 2007). Generative growth involves the formation of flower buds, which is divided into the processes of floral induction, initiation and differentiation of the different organs in the flower cluster (Dadpour et al. 2011; Hanke et al. 2007; Tromp et al. 2005). A manifold of internal and external cues promote the activation of floral induction genes involved in floral growth. This leads to cytochemical, histological and morphological changes in the shoot apical meristem, such as the appearance of floral primordia and later development of floral organs (floral initiation and differentiation) (Buban & Faust 1982; Hanke et al. 2007).

Despite the importance of flowering for apple production and research, most of the studies in the literature have focused on solving practical problems and on later stages of flower and fruit development (Tromp et al. 2005). Little attention has been paid to the understanding of the different stages of the process, their timing under specific environmental conditions and their relationship to other developmental processes in the tree, such as anthesis, vegetative growth and fruit maturation (Foster et al. 2003; Koutinas et al. 2010; Tromp 2005b). Verheij (1996) suggested three main reasons for this. First, the various internal and external factors triggering the process of flower-bud formation (FBF) complicate its study. Second, the process extends over a long period of time (approximately one year from floral induction to anthesis), in which environmental conditions vary greatly and

interactions with other developmental processes occur. Third, the fact that just a fraction of the total buds in the tree develops into flower buds, makes the study practically challenging.

Several studies dealing with FBF have been conducted in many countries where apple production is of economic importance (Abbott 1984; Dadpour et al. 2011; Foster et al. 2003; Fulford 1965; Fulford 1966a; Fulford 1966b; Hirst & Ferree 1995; Hoover et al. 2004; Koutinas et al. 2010; McArtney et al. 2001; Tromp 1984). However, such studies are restricted to a different climate and cultivars unsuited for Nordic growing conditions. Little information is available on the process of FBF and development, and its relationship to other stages in the annual growth cycle in cultivars in Norway (Skogerbø 1987).

This knowledge represents valuable traits for cultivars adapted to the Nordic climate, in terms of genetic resources. Furthermore, a better understanding of these traits may provide useful background information for future studies on breeding and selection of new cultivars, and for the timing of horticultural practices intended to improve flowering, achieve regular yields and thus, a profitable apple production (Bangerth 2005).

The lack of knowledge mentioned above motivated the current study, in which the main objective was to investigate the process of FBF in relation to climate and other developmental processes in apple cultivars grown in Norway. The specific objectives were, firstly, to determine the time of growth cessation and floral initiation and their relationship to the time of flowering, ripening of the fruits and leaf abscission in early, middle and late flowering apple cultivars. Secondly, to illustrate the morphological changes occurring at the shoot apex during floral differentiation by means of scanning electron microscopy (SEM). Finally, in a parallel experiment, the effect of climatic conditions in 2013 on growth and FBF in the apple cultivars Aroma and Gravenstein, placed at different geographical locations across Norway, was studied.

2. Literature Review

2.1. The apple tree (*Malus domestica* Borkh.)

Cultivated apples are a result of extensive ancient hybridization of various species of the genus *Malus* Mill., a member of the *Rosaceae* Juss., subfamily *Pomoideae* (pome fruits) (Jackson 2003; Webster 2005a). Over hundred botanical names have been published for the cultivated apple (Qian et al. 2010), however, *Malus domestica* Borkh. is the most commonly used name, especially in the horticultural sciences (Qian et al. 2010; Webster 2005a). Some morphological characteristics shared by apple cultivars in the world are: woolly pubescence on young stems and on the abaxial surface of the leaves, dull green leaves, elliptic-ovate in shape, with irregularly saw toothed margins, woolly pubescence on flower stalks and calyx, and pome fruits indented at the base with persistent calyx (Webster 2005a). The basic chromosome number for cultivated apples is 17 (Jackson 2003).

2.2. Cultivation and commercial use

Apples are among the oldest and most important fruit crops in the world (Harris et al. 2002; Jackson 2003). They have been cultivated since ancient times, in fact, archeological studies have shown that they were cultivated already in 1000 BC (Juniper et al. 1996). Apple cultivation is more extensive in the northern hemisphere, but it has also spread to the southern hemisphere, including tropical regions (Jackson 2003; Qian et al. 2010). According to the Food and Agriculture Organization of the United Nations (FAO), apple production worldwide has increased considerably in the last ten years (FAO 2014). Since 1992, the amount of apples produced globally has almost doubled, from approximately 50 million tons in 1992 to 80 million tons in 2012 (Fig. 1). Currently, these following countries are the leading apple producers in the world: China, United States of America (USA), Turkey, Poland and India.

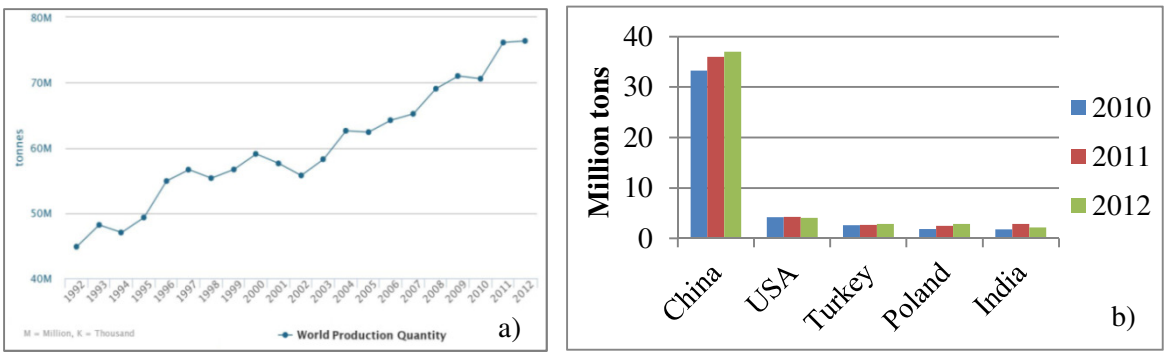

Figure 1. *a) World total apple production in the period 1992-2012. b) Total production of the top five apple producing countries in the world in 2010-2012 (FAO 2014).*

In Norway, apples have been cultivated since Christianization times (around 1000 AD), when missionaries from apple- producing areas in Europe settled down and implemented its cultivation in the country (Stedje & Skard 1939). Nowadays, most of the apple production is concentrated in southern Norway and the most important counties are Hordaland, Telemark, Buskerud, Vestfold, Sogn and Fjordane and Rogaland (Fig. 2) (SSB 2014b). The total cultivated area by 2010 was 14 277 decares (daa), with a total production of 11.5 thousand tons apples (SSB 2014b).

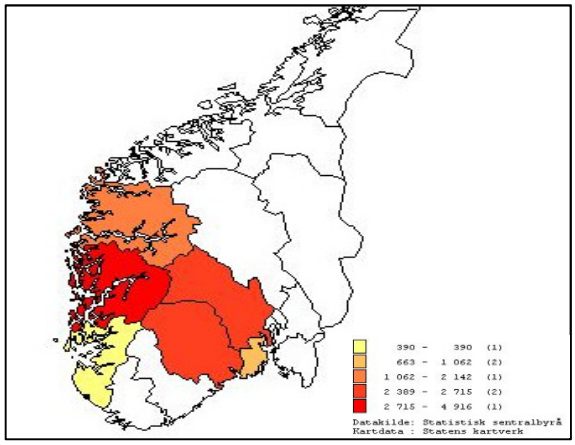

***Figure 2**. Apple production area (acres) in southern Norway (SSB 2014a).*

Regarding commercial use, apples are used for fresh consumption and processing. A high proportion of the apple production globally is used for the fresh market, locally and for export. The robustness of the fruits provides short and long term storage, and make them suitable for long distance transportation (Webster 2005a). In addition, a variety of processed products are made out of apples, e.g. sauces, pastry and cakes, non-alcoholic juices, alcoholic ciders and even apple chips (Jackson 2003).

2.3 Cultivars

A large number of cultivars, together with wild species, are maintained in living collections as genetic resources for breeding (Harris et al. 2002). Over ten thousand cultivars have been selected in the last centuries, but just a small fraction of these are currently used in commercial production (Jackson, 2003). Worldwide, the major commercial cultivars are ‘Delicious’ (golden and red variants), ‘Gala’, ‘Granny Smith’, ‘Fuji’, ‘Jonagold’ group, ‘Idared’, ‘Champion’ and ‘Elstar’ (Data from 2009 by Lauri et al. (1995)). 'Braeburn', 'Elstar', 'Fuji', ‘Golden and Red Delicious', 'Granny Smith' and 'Pink Lady' are relevant cultivars for fruit import to Norway (SNL 2014).

Over 200 apple cultivars have been reported grown in Norway (Asdal 2013), and the most relevant for production are 'Discovery', 'Summerred', 'Gravenstein', 'Aroma' and their red variants (Måge 2003, 2010). These are grouped based upon fruit maturation and harvesting time. However, they also differ in other morphological and quality traits, e.g. time of flowering, amount and distribution of flowers, and appearance and taste of the fruits.

2.4. Morphology of shoots and buds

Nowadays, apple cultivars are almost exclusively compound trees consisting of a scion grafted onto a rootstock (Jackson 2003; Webster 2005a). Rootstocks are used to propagate apple scions that cannot be propagated by sexual (e.g. seeds, due to genetic variation) or asexual means (e.g. cuttings, due to the low rooting capacity of the cuttings). In addition, rootstocks are used to avoid juvenility, to control vegetative growth, to promote flower- bud formation, to improve cropping efficiency and quality of the fruits, and in some cases to provide winter hardiness (Hanke et al. 2007; Wertheim & Webster 2005).

The scion is the productive part of the tree and bears three different types of shoots; extension, non-extension and bourse shoots (Fig. 3). Extension shoots are long (> 20 cm) and indeterminate in growth, often referred to as one-year-old shoots. Non-extension shoots are shorter, determinate in growth and terminate in flower buds (regularly after the second year) (Webster 2005b). These shoots are variable in length, and based upon it they can be classified as brindles (10-20 cm), dards (5-10 cm) and spurs (very short shoots). Finally, bourse shoots originate on the axil of vegetative primordia in spurs and may become extension shoots or remain short as bourse shoots (Foster et al. 2003; Jackson 2003; Webster 2005b).

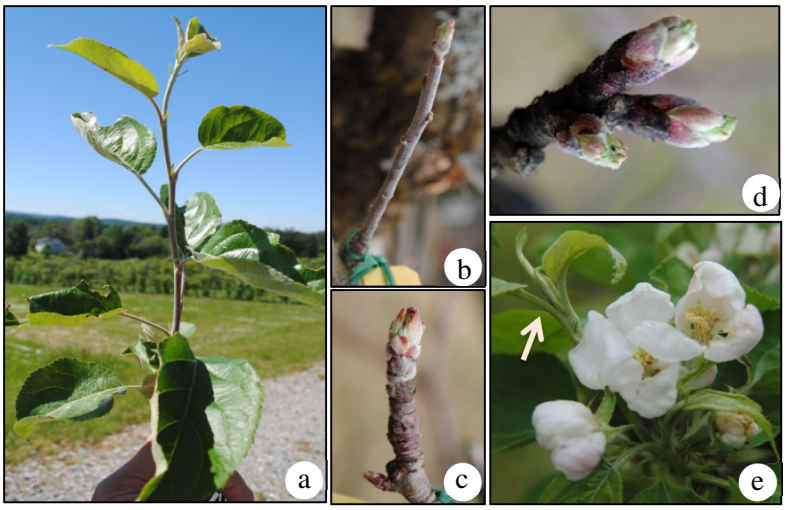

***Figure 3**. Types of shoots on apple trees. a) Extension shoot on one-year-old 'Aroma'; b) brindle on 'Prins'; c) dard on 'Vista Bella'; d) spurs on 'Elstar'; e) bourse shoot emerging from a spur on 'Julyred' (Photos by R. Rivero).*

Regardless of type, all shoots emerge from buds, which in apple trees have the potential to produce both leaf and flower primordia. If a bud produces leaf primordia only, it is considered a vegetative bud. This type of bud is common on extension shoots (both terminally and axillary) before growth stops in mid- to late summer (Abbott 1984). On the contrary, if a bud produces flowers in addition to leaf primordia, it is considered a mixed (flower) bud. Flower buds are found terminally on all types of non-extension shoots (i.e. brindles, dards and spurs) and terminally or axillary on extension shoots after vegetative growth has stopped (Jackson 2003; Tromp 2005b). These consist of a compressed axis in which leaf and flower appendages are inserted in spiral sequence (Jackson 2003). In general, the number of appendages is 21, with some exceptions (Tromp 2005b). For instance, 'Cox Orange Pippin' have an appendage number of 20, while on 'Golden Delicious' the number is 16 (Jackson 2003). Fig. 4 shows the different appendages found in mixed apple buds.

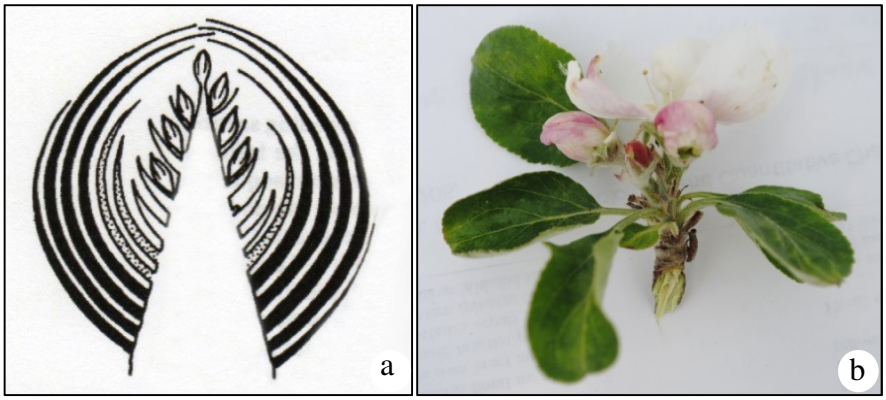

***Figure 4**. Mixed buds on apple trees. a) Schematic longitudinal section showing (from bottom to top) 9 buds scales (bold lines), 3 transitional leaves (stippled), 6 true leaves (outlined), 3 bracts (lines) and 7 flowers (Abbott 1970); b) spur bud ('Gravenstein') after bud-break, showing (from top to bottom) 1 open king flower, 3 lateral flowers, 5 true leaves, remains of bracts/stipules, a small transition leaf and remains of bud scales (Photo by R. Rivero).*

2.5. Annual growth cycle

Apple trees, as deciduous fruit species, are adapted to temperate regions in which they overcome large seasonal changes in air temperature. Such adaptation is a result of an annual growth cycle in which all developmental processes in the tree are finely tuned with the annual course of the growing conditions (Hänninen & Kramer 2007). The growth cycle of an apple tree last approximately one year and includes all developmental events occurring normally every year, from budburst, flowering, extension growth, fruit set and development, to extension growth cessation, flower-bud formation, leaf abscission and winter dormancy. All these processes are interrelated and synchronized with the growth season.

Early in the spring, a high proportion of buds in the tree emerge from dormancy, as a response to chilling temperatures during the preceding winter (Webster 2005b). These buds are ready to develop when the air temperature rises above a certain level (Faust 1989). It is important to point out that both the amount of chilling required to break dormancy and the threshold temperature for bud-break are variable between cultivars, and generally, in cultivars with a low chilling requirement, bud-break occurs at lower temperatures (Faust 1989; Jackson 2003; Wertheim & Schmidt 2005).

The buds opening in spring may have flower primordia (generative buds differentiated the previous season) or just leaf primordia. Those with flower primordia normally open first and develop flower clusters that pass through a series of phenological stages[1]. This is the case of terminal buds on extension and non-extension shoots and some lateral buds on extension shoots (Abbott 1984). On the contrary, buds with only leaves open later and produce a rosette of leaves with a "naked bud" in the center (a bud without bud scales), or grow out to form a new shoot (Abbott 1984; Webster 2005b). Rosettes of leaves with a "naked bud" continue to develop throughout the season and may produce bud scales and flower primordia (generative resting buds) or just bud scales and leaf primordia (vegetative resting buds). New shoots continue to extend during the summer, leading and suppressing growth on axillary buds. By the end of the summer, extension growth stops in these shoots, and is followed by the formation of a terminal resting bud (Webster 2005b). From this point onwards, flower buds may be formed in terminal or axillary buds within the current year's extension shoot.

It is also important to point out that shoot growth and formation of flower buds are processes that occur in parallel to fruit development. Consequently, a strong competition for immediate available resources and hormonal inhibition takes place between these developmental processes during summer (Tromp 2005b). It has been reported that the presence of fruit has a negative effect on shoot growth (Jackson 2003), mainly due to the strong sink effect of fruits (Webster 2005b). Inhibition of flower-bud formation by fruits has also been reported and explained as a result of the inhibitory effect that hormones from developing seeds impose on this process (Jackson 2003; Tromp 2005b).

During the autumn, after extension growth has stopped, the development of flower buds continues and may extend throughout the winter when temperatures are high enough

[1]According to Chapman & Catlin (1976) the phenological stages of development of flower buds in apples are green tip, half- inch green, tight cluster, pink and full bloom. These stages are practically significant on the control of spring frost, pest and diseases (Wertheim & Schmidt 2005).

(Tromp 2005b). In spurs, all floral parts are differentiated before winter (Buban & Faust 1982; Jackson 2003; Tromp 2005b). In extension shoots, differentiation of floral parts also continues throughout the winter and some buds may have all parts differentiated by the end of the winter, while some others may continue differentiation early in the spring (Hanke et al. 2007).

As autumn progresses, temperatures and day length decreases, triggering a series of physiological changes in the trees that lead to leaf abscission and development of the maximum dormancy (Abbott 1984; Jackson 2003; Webster 2005b). Changes such as chlorophyll degradation, which causes discoloring of leaves, remobilization of leaf components to the woody parts of the tree, followed by degradation of cell walls causes leaves to shed (Faust 1989). By the time of leaf abscission, the tree is in a state of rest in which bud-break does not occur even if environmental conditions are conducive to do so (Jackson 2003). From this point onwards, exposure to chilling temperatures reduces gradually the depth of the rest/dormant period to a point in which accumulated temperatures above a certain threshold will lead to bud-break the following spring (Jackson 2003; Webster 2005b). During the spring, buds formed in the previous season will grow and, depending on their nature, will have only leaves or leaves and flowers. Buds with flowers complete their development, open and pass through various phenological stages, and at this point, a one-year cycle on the life of the apple tree is completed.

2.6. Flowering

Flowering in apple trees includes the formation of flower buds, which comprises the processes of floral induction, initiation, differentiation, and anthesis (Hanke et al. 2007). In this section, the focus will be on cytochemical, histological and morphological, changes in the buds during floral induction, initiation and differentiation

2.6.1. The flower-bud formation (FBF) process

Floral induction is the first stage in the FBF process and implies the transition of the apical meristem from vegetative to floral development (Buban & Faust 1982; Fulford 1965; Hanke et al. 2007). At this stage, there are no visible morphological changes. Instead, extrinsic and intrinsic signals induce genetic changes in the cells of the apical meristem, such as the expression of genes involved in the inception of flower primordia (Buban & Faust 1982; Dadpour et al. 2008; Verheij 1996). The physiological changes involved are complex

and the specific time at which this process occurs is still unclear (Hanke et al. 2007; Tromp 2005b).

Because of the complexity of this process, which arises from the fact that no morphological changes can be detected, in addition to the many intrinsic and extrinsic factors involved, it is difficult to determine the exact time at which floral induction occurs (Hanke et al. 2007). Tromp (1972), based on studies where inhibitors of floral induction were used at different times after full bloom, proposed that the sensitive period for floral induction in spurs of 'Cox Orange Pippin' in The Netherlands, occurs during a relatively short period right after bloom. Buban and Faust (1982) carried out studies on FBF using spurs of 'Jonathan' in Hungary, and proposed that floral induction occurs three to six weeks after full bloom. Other experiments showed that flower induction takes place during the vegetative phase of the bud, a less clear period that extends from the time of bud-break until the shoot apex starts to broad and the amount of leaf primordia exceeds 12 (Hanke et al. 2007).

Faust (1989) stated that in order for induction to occur, a vegetative bud must be fully developed, which means that it must have a certain number of appendages. This statement is based on extensive defoliation studies carried out by Fulford (1965; 1966a; 1966b), who determined that the critical number of appendages in spurs of 'Miller's seedling' and 'Laxton's Superb' was 20. In addition, Fulford (1965; 1966a; 1966b) found that the rate of production of new primordia, also called plastochron, cannot exceed 7 days, in order to reach the critical amount of nodes early enough in the growing season, and thus assure flower primordia production. These findings are also supported by Abbott (1977) and Luckwill (1974).

The critical number of appendages is variable between cultivars (Tromp 2005b), e.g. 21 for 'Cox Orange Pippin' (Abbott 1977), 19 for 'Miller's seedling' and 'Laxton's Superb' (Fulford 1966b), 16 for 'Golden Delicious' (Luckwill 1974), 18 for 'Summerred' (Zhu et al. 1997) and 15 for 'Granny Smith' (Costes 2003). It has also been reported that considerable variation is found within a cultivar. Zhu et al. (1997) reported higher appendage number in spurs buds than in lateral and terminal buds of 'Summerred'. Verheij (1996) found large differences in appendage number within the same type of shoots and between different bud positions in 'Cox Orange Pippin' and 'Jonagold'. In this study, it was concluded that such results did not support the suggestion of a critical number of nodes for the formation of flower buds.

Once the apical meristem has been induced to floral development, it undergoes a series of cytochemical and histological changes, all part of the floral initiation process (Hanke et al. 2007). At this stage, DNA and RNA synthesis increases and the content of nucleohistones in the meristems decreases (Fig. 5a,b,c) (Buban & Faust 1982; Faust 1989). Hanke et al. (2007) pointed out, based on studies from Schmidt (1978) and Schmidt & Egerer (1990), that the increment in DNA and RNA content in spur apices is more prominent two times during the growing season. First, between full bloom and early summer (during floral induction in spurs) and second, in mid- summer (presumably during flower differentiation in spurs).

Based on histological observations in the apical meristem of spurs of 'Jonatan', Buban (1981) described three main cytological changes during floral initiation. First, mitotic activity increases in the whole meristem and rearrangement of cells takes place. The central meristem (Z_3) relocates under the 'subdermatogen' (part of Z_1) and causes the 'accessory tunica layer' (Z_2) to disappear (Fig. 5d,e) (Skogerbø 1987). Consequently, the now 'committed' meristem starts its morphological transformation that leads to the inception of a flower cluster (Koutinas et al. 2010).

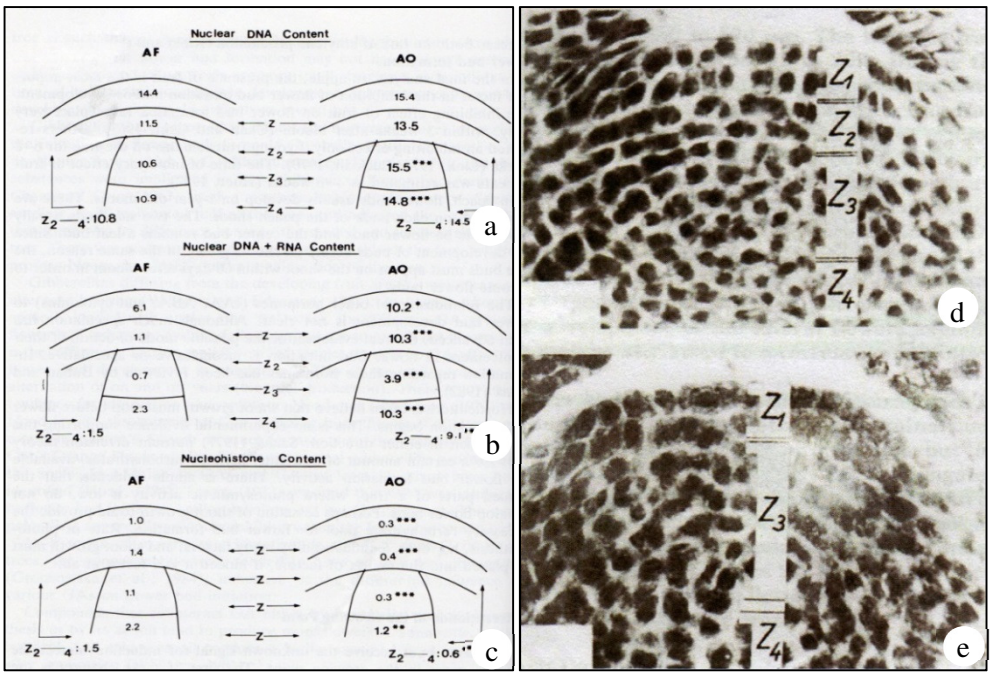

Figure 5. *a), b), c) DNA, RNA and nucleohistone content in the apex of spurs bearing fruits (AF) and without fruits (AO); d) histological structure of the apex of spurs in vegetative stage; e) restructuring of the apex' structure at floral initiation. The different zones of the meristem, using tunica-corpus terminology are Z_1: dermatogen and subdermatogen, Z_2: accessory tunica layer, Z_3: central meristem, Z_4: pith meristem. Partially modified from Buban and Faust (1982).*

The specific morphological changes that mark the differentiation of flowers differ between studies. For some authors, floral differentiation starts with the broadening of the apex from a flat to a prominent convex shape (Abbott 1977; Hirst & Ferree 1995; Hoover et al. 2004; Luckwill 1974; Skogerbø 1987). Similarly, Buban & Faust (1982), Hanke et al. (2007), Tromp (2005b) and Skogerbø (1987) considered the morphological changes leading to the doming of the apex as part of the differentiation process. Foster et al. (2003) proposed the broadening of the apex, alone, as the first morphological sign of transition to floral development. Their study was restricted to measurements of meristem diameter without taking into consideration cellular division patterns. Dadpour et al. (2011) investigated the first signs of floral initiation by studying architectural and cellular patterns in the shoot apical meristem. These authors proposed that for spurs of 'Golden Delicious', the first sign of floral development was the broadening of the meristem, together with the appearance of a furrow between the latest leaf primordia and the meristem mantel. In the current study, the first sign of floral differentiation was considered to be the doming of the apex.

In domed meristems, the rate of appendage production increases with time, and the appendages produced are bud scales, transition leaves, true leaves and flower primordia (Verheij 1996). The first primordia formed become bud scales and the following primordia become bracts instead of leaves (Pratt 1988). Bud scales are firm and tough in appearance and are the outermost protective structures of the bud (Tromp 2005b). Bracts are variable in shape; the first two to three bracts have a wide lamina and stipules, while subsequent bracts are narrow and devoid of stipules (Foster et al. 2003). From the axillary meristems of these bracts and of the uppermost leaves, lateral flower primordia are differentiated (Abbott 1977; Fulford 1966b; Pratt 1988).

Differentiation of flower primordia starts in the lowest, continues in the terminal and ends in the lateral meristems following an acropetal sequence (from the base of the axis towards the apex). The terminal meristem differentiates first and becomes the 'king flower' (largest and first flower to open the following spring). Differentiation of all flowers starts with the inception of two bractlets and five sepals, followed by five petals, three whorls of stamens (10 + 5 + 5) and ends with the differentiation of carpel primordia (Pratt 1988). The processes of macrosporogenesis (production of macrospores and formation of the embryo sac), and microsporogenesis (production of microspores and formation of pollen sacs) occur during the spring, prior to anthesis (Koutinas et al. 2010).

The differentiation of flower buds is similar in buds from extension shoots and non-extension shoots, but the time in which this process starts and ends, in addition to the rate of differentiation, vary considerably (Tromp 2005b). Zeller (1960) suggested that flower differentiation occurs first in spur buds, which by the onset of winter have all flower parts differentiated, and several weeks to months later in terminal and lateral buds of extension shoots. The main reason for this delay in the differentiation of flower buds in extension shoots is the correlative inhibition exhibited by active apical meristems, which suppresses the floral development of lateral buds. Therefore, FBF starts in these buds after growth has stopped (Jackson 2003).

2.6.2. Factors affecting FBF

Whether a bud remains vegetative or becomes floral (generative) depends on a large number of internal and external factors (Jackson 2003). Environmental conditions, such as light and temperature, plant growth regulators and carbohydrate level have been mentioned as important factors modulating the transition from vegetative to generative growth (Wilkie et al. 2008). Nevertheless, the mechanism of action of such factors and the way they are interconnected are still hypothetical (Tromp 2005b).

The proportion and location of floral buds differs between apple cultivars. Intrinsic characteristics of each cultivar (genetically determined) and the relationship between scion and rootstock may affect the FBF process (Koutinas et al. 2010). Differences between cultivars on the proportion of flower buds has been reported, and are regarded as the main cause in the phenomenon of biennial fruit bearing (Jackson 2003). Jonkers (1979) summarized data from various studies and classified apple cultivars according to their susceptibility to biennial bearing. ‘Elstar’, ‘Golden Delicious’, ‘Lobo’ and ‘Mutsu’ were classified as unsusceptible, ‘Discovery’ and ‘Granny Smith’ as middle susceptible and ‘Gravenstein’, ‘Cox’s Orange Pippin’ and ‘Laxton’s superb’ as strongly susceptible. Ljones (1951) confirmed the strong biennial nature of ‘Gravenstein’ under Nordic climatic conditions.

The relationship between scion and rootstock has also been reported as a regulating factor on FBF, especially on the proportion of flower buds formed (Jackson 2003), the amount of flowers per cluster (Hirst & Ferree 1995), and the time of initiation of flower buds (Koutinas et al. 2010). Hirst & Ferree (1995) found that rootstocks influence flowering indirectly by either reducing or promoting vegetative growth. For instance, dwarfing

rootstocks limit the growth of extension shoots, and as a consequence more spur buds are initiated on scions grafted on this type of rootstocks.

Regarding other internal factors controlling FBF, a series of theories have been proposed over the years, based on experimental research, e.g. C/N theory and hormone theory (Skogerbø 1987). Tromp (2005b) mentioned the work of Klebs (1910), in which it was proposed that FBF was mainly ruled by the ratio of carbohydrates (C) to nitrogen (N) in the cells of the apical meristem. In this theory, FBF is stimulated by a high C/N ratio, which means conditions conducive to carbohydrate accumulation. Further analytical research showed that the C/N ratio alone could not explain variations in flower buds formed in fruit trees.

Nitrogen has been shown to be a promoter of vegetative growth and an antagonist of FBF, nonetheless, its effect may vary, depending on the form of N applied, the time of application, and the nutritional status of the tree (Jackson 2003). Grasmanis and Edwards (1974) found that fertilization with ammonium (NH_4^+), instead of nitrate (NO_3^-), increased the amount of flower buds produced in apple trees, through an indirect increment on the amount of arginine, a precursor of polyamines. In fact, application of polyamines have been shown to increase the amount of flower buds formed (Verheij 1996). Jackson (2003) pointed out that N-deficiency leads to poor leaf development and reduced FBF. Williams (1965) found enhanced FBF on apple trees with suboptimal N supply, as a result of late summer N fertilization. Buban et al. (1978) reported that nitrogen nutrition had significant effects on the content of zeatin, a cytokinin in the xylem sap of apple trees that may have a positive effect on FBF. Despite the different results reported in the literature, it seems that the effect of nutrients, particularly N, is not decisive for formation of flower buds when the trees have an optimal C/N ratio (Hanke et al. 2007; Tromp 2005b). Instead, it appears that nutrients and carbohydrate status of the tree are one of several internal factors controlling FBF (Verheij 1996).

Other studies have shown that the effect of exogenous applications of plant growth regulators (PGRs) may point to endogenous hormonal content as a decisive regulatory factor. Luckwill (1974) proposed that FBF was controlled by the balance between gibberellins (GAs) from developing seeds and active shoots, and cytokinins (CKs) from the roots. According to this theory, floral induction takes place right after flowering, when the ratio GAs/CKs in spur buds is low (Skogerbø 1987). GAs are mostly associated with direct inhibition of FBF, while

CKs are associated with promotion of FBF (Verheij 1996). Consequently, a low content of GAs and higher content of CKs will promote FBF.

Developing seeds and leaves are important GA- producing organs (Faust 1989; Jackson 2003; Luckwill & Silva 1979; Tromp 1972). Both, the exogenous application of GAs to spur buds and the presence of seeded fruits have resulted in a marked inhibition of FBF, thus providing evidence to Luckwill's theory and suggesting that endogenous GAs may have the same effect. Moreover, buds are most responsive to floral induction during a short period after full bloom (Tromp 2005b), a period in which developing seeds and leaves (on extension shoots) are not present, and CK-content from the roots is at its highest (Luckwill & Whyte 1968). Despite this, Verheij (1996) pointed out that the experimental evidence of enhanced flowering under a low GAs/CKs ratio is not conclusive enough to ascribe FBF entirely to hormonal changes.

Since correlations between the amount of GAs and their inhibitory effect on FBF are sometimes unclear (Bangerth 2005b), the effect of other endogenous hormones such as auxins (e.g. Indoleacetic acid, IAA) has been studied. Callejas and Bangerth (1997) proposed that synthesis and polar IAA- transport is increased by high levels of GA (from developing shoots and seeds), and may act as a second messenger in the inhibition of FBF. As a second messenger, IAA does not enter the meristem, instead, it transports the inhibitory signal of FBF, and may also lower the levels of other hormones such as cytokinins (Bangerth 2005a).

The interaction between buds, either from spurs or from extension shoots, and other organs in the tree has also been studied in terms of differentiation of flower buds. For flower buds to differentiate, a sufficient amount of well-developed leaves is needed to provide enough assimilates that maintain meristematic activity (Tromp 2005b; Verheij 1996). This idea is supported by defoliation studies in spurs of different apple cultivars (Fulford 1966b; Jackson 2003). Davis (2002) found that FBF was inhibited by defoliation in early summer (period of floral induction) in spurs of 'Braeburn', 'Golden Delicious', 'Ramey York', and 'Fuji'. This author also pointed out that the time and severity of defoliation were positively correlated with the degree of floral inhibition. The effect of leaves on FBF has been ascribed to their role providing assimilates from the photosynthetic process, as hormone- producing organs and as receptors of environmental signals that may regulate the activity of the meristem (Hanke et al. 2007). Moreover, leaves are important in keeping the flux of floral

inducing substances, such as CKs, from the roots to the aerial parts of the tree, thus ensuring their availability in the bud region (Tromp 2005b; Verheij 1996).

In addition to the direct effect of leaves in spurs, it has been reported that the first visible sign of flower bud differentiation, i.e. the doming of the meristem, coincides with growth cessation on extension shoots (Fulford 1966b; Hanke et al. 2007; Jackson 2003; Luckwill & Silva 1979). Floral differentiation may then be a consequence of the ceased activity of the apical meristem, and the concomitant break of the apical dominance, which allows floral differentiation to occur in lateral meristems of the shoot (Jackson 2003). Despite this, Zhu et al. (1997) found that floral differentiation in lateral buds of 'Summerred' trees started while the shoots were actively growing. This suggests that shoot growth and FBF may be independently controlled (Hanke et al. 2007). Based on the evidence available in the literature, it seems more appropriate to conclude that even if growth cessation may not always be a prerequisite for floral initiation, the fact that apical dominance is removed, enables the formation of flower buds at least in extension shoots (Tromp 2005b).

Crop load has also been reported in the literature and by fruit growers as a factor regulating FBF in apple trees (Davis 2002; Jackson 2003; Tromp 2005b). Depending on the cultivar, heavy cropping one year may translate into reduced flowering the following year (biennial bearing) (Jackson 2003). The cause of this phenomenon was first thought to be the fruits. However, Chan and Cain (1967) studied the effect of seedless fruits on FBF and found that such fruits did not affect the amount of flowers formed the following year in adjacent spur buds (Tromp 2005b). These results have also been validated by the studies of Ebert & Bangerth (1981) and Hoad (1977), thus making clear that hormones from developing seeds, in particular GAs, are responsible for the inhibition of FBF on years with heavy crop load. Further studies, in which bearing spurs were defruited at different periods after full bloom, showed that the strongest inhibition of FBF was 3-6 weeks after full bloom. This period coincides with the time of floral initiation and the maximum levels of GAs from developing seeds. As for the effect of leaves on FBF, further research of cropping variability in time suggests that not only endogenous factors are involved in the phenomenon of biennial bearing, but also exogenous factors such as temperature may explain the variations in the proportion of flower buds formed (Hanke et al. 2007).

FBF in apple trees is also influenced by external factors, such as temperature, photoperiod and water status. The effect of temperature and photoperiod is not as direct as in annual/biennial species (that respond to a specific environmental factor) (Buban & Faust 1982; Tromp et al. 2005). Instead, such effect in apple trees is through the start and break of dormancy, and on vegetative growth and the rate of bud development (Hanke et al. 2007).

Studies dealing with the effect of environmental conditions on FBF are scarce, mainly due to the practical challenges involved in using deciduous fruit trees (Verheij 1996). Most of the attention has been directed towards temperature. Tromp (1976) found a negative effect of increasing temperatures on FBF and flower quality in 'Cox Orange Pippin' under controlled conditions. Verheij (1996) reported that the effect of temperature depended on cultivar and bud position. For instance, high temperatures particularly inhibited FBF in spur buds of 'Jonagold' and enhanced it in 'Cox Orange Pippin'. Despite the incongruences between both studies, these authors concluded that for apples, the optimum temperature for floral initiation was 16°C (Tromp 2005b). Zhu et al. (1997) reported that increasing temperatures from 20 to 27°C, throughout the season and 6-7 weeks after full bloom, enhanced flowering, but at the same time gave some delay and lowered the number of flowers per cluster. Abbott (1984) reported that high temperatures promoted floral initiation and increased the number of flowers per cluster under orchard conditions. Verheij (1996) concluded that increasing temperatures stimulate shoot growth and shortened the plastochron in spur buds under controlled conditions. Nevertheless, the latter may be attributed to changes in intrinsic factors rather than to temperature only.

The effect of temperature on FBF should be interpreted as a balance between positive and negative influences (Jackson 2003; Tromp 1976). For instance, high temperatures enhance the differentiation of flowers directly through increased meristematic activity, but also delay it indirectly because of the enhancement of shoot growth and the antagonism between vegetative growth and FBF.

Light is also an external factor related to FBF, and its effect should be discussed in terms of length (photoperiod) and quantity (intensity). Experimental evidence supporting the effect of photoperiod on flowering in apple is scarce and the most accepted hypothesis is that apple trees are day- neutral plants (Tromp 2005b). Heide & Presterud (2005) have confirmed the lack of photoperiodic regulation of growth cessation and dormancy in apple trees. These authors demonstrated that temperatures below 12°C consistently induced growth cessation

and dormancy in apple rootstocks, regardless photoperiodic conditions. Since FBF is often associated with cessation of growth (Hanke et al. 2007), the lack of photoperiodic regulation may also have important bearings on FBF (Heide, O.M, pers. comm.). Regarding light intensity as an external factor affecting FBF, experiments have demonstrated that shaded trees differentiate less flower buds compared to non-shaded trees (Jackson 2003). Tromp (1984) studied the effect of light intensity on growth and FBF under controlled conditions, and found that high light intensity during a 7 weeks period after bloom led to differentiation of more flower buds. This author ascribed the observed effect to an increased level of carbohydrate substrate, which may have affected the length of the plastochron, and thus led to the formation of more flower buds.

Finally, studies dealing with water supply as an external factor affecting FBF are inconclusive, the effect may vary with circumstances, and in some cases give conflicting results(Jackson 2003). One of the reasons is that most studies have focused on finding solutions to specific practical problems, and not on studying the actual mechanism by which water supply may affect FBF (Tromp 2005b). In general, excessive water supply, which maintains extension growth, is associated with less formation of flower buds (Jackson 2003). Tromp (1984) investigated the effect of high and low relative humidity (RH) on FBF in apple trees, and found that under high RH the plant water- deficit tended to decrease, vegetative growth was stimulated, and less flower buds were formed. These results confirm the idea of antagonism between extension growth and FBF. The effect of water supply may be positive for FBF when fertilization is applied at the same time (fertigation). This effect is mainly based on the rapid availability of nutrients in the bud's tissues (Tromp 2005b).

3. The present investigation

In the present investigation, the process of FBF was studied in relation to climate and developmental processes such as flowering, vegetative growth, growth cessation, leaf abscission and ripening of the fruits in early, middle and late flowering apple cultivars grown in an experimental orchard at Ås, Norway. In addition, the morphological changes occurring at the shoot apex during floral differentiation were identified and illustrated by means of scanning electron microscopy. In parallel, the effect of local climate, especially temperature and precipitation in 2013, on growth and FBF in the apple cultivars Aroma and Gravenstein, placed at different geographical locations across Norway, was studied.

3.1. Materials and methods

Growth and development of apple cultivars in the experimental orchard at Ås

3.1.1. Plant material

Growth measurements and phenological observations were conducted on 14 apple cultivars (*Malus domestica* Borkh.), well established in the experimental orchard (Åsbakken 6) at the Norwegian University of Life Sciences (NMBU) at Ås, Norway (59° 39′N, 10° 47′E, Ås, Akershus, 96 meters above sea level (m.a.s.l.)).

The following cultivars were studied: 'Aroma'*, 'Discovery'*, 'Elstar'*, 'Franskar', 'Gravenstein'*, 'Julyred', 'Lobo', 'Mutsu'*, 'Prins', 'Quinte', 'Summerred'*, 'Sävstaholm', 'Vista Bella' and 'Åkerø'. Table 1 summarizes the most relevant characteristics. Marked (*) cultivars were grafted on M9 rootstocks.

3.1.2. Growth measurements

Vegetative growth was measured as the weekly extension growth (cm) and number of leaves on extension shoots. At the beginning of the growing season, 10 extension (current year's) shoots per cultivar were marked. Well exposed and south facing shoots were selected and followed up until growth cessation late in the summer. Measurements were performed weekly during the period June 25-August 21, 2013.

Table 1. General description of cultivars in the experimental orchard at NMBU at Ås.

Cultivar	Origin, year and parents	General description				References
		Tree	Fruits	Harvest- ripening	Production (1999-2008)	
'Aroma'	Sweden, 1973. 'Ingrid Marie' x 'Filippa'	Diploid, moderately vigorous, productive and bears early	Medium to large, round- oblate to conic, green-yellowish to yellow.	September- early October. November to December	2890 tons. (including red variants) Main cultivar in Norway	(Asdal 2014; Bø et al. 1998)
'Discovery'	UK, 1974. 'Worcester Permain' x 'Beauty of Bath'	Diploid, compact, of weak vigor and its productivity is slightly low	Medium, round- oblate, yellow	Early to mid-September. September to early October	340 tons	(Asdal 2014; Bø et al. 1998; Måge 2003)
'Elstar'	The Netherlands, 1972. 'Golden Delicious' x 'Ingrid Marie'	Diploid, moderately vigorous and bears early	Medium to large (70-80 mm), round and yellow	Early to mid-October. November to January	No data found	(Bø et al. 1998; Jackson 2003; Måge 2003)
'Franskar'	Hardanger, Norway. Unknown year and heritage	Moderately vigorous, with upright growth and bears rather early	Medium, round to round- oblate, and yellow- greenish to white- yellowish	Late August to early September. September to October	Household purposes	(Asdal 2014; Stedje & Skard 1939)
'Gravenstein'	South Jutland, Denmark, 1698. Unknown heritage	Triploid, vigorous, productive and has a tall, wide crown	Large, oblong to oblong- conic, angular, green-yellowish to yellow	Early to mid-September. October to December.	2000 tons (including red variants). Second most important cultivar in Norway	(Måge 2010; Stedje & Skard 1939)
'Julyred'	USA, 1962. 'Petrel' x 'Early McIntosh' and 'Melba' x ('Williams' x 'Starr')	Very vigorous and productive	Medium, round-oblate and yellow-greenish	Mid- August. From harvesting to late August	280 tons. Low scale production in Eastern parts of Norway	(Asdal 2014; Måge 2003)

'Lobo'	Canada, 1910. Free pollination of McIntosh	Diploid, moderate vigor, productive and bears early	Medium to large, round- oblate, yellow- greenish	Late September to early October. October to December	250 tons. Low scale production in Eastern parts of Norway	(Bø et al. 1998; Måge 2003)
'Mutsu'	Japan, 1948. 'Golden Delicious' x 'Indo'	Triploid, very vigorous, productive and stable	Large, green	Early October in USA. 3 months from harvesting	Too late for commercial production in Norway	(Jackson 2003; Måge 2003)
'Prins'	Hardanger, Norway, before 1860. Unknown heritage	Moderately vigorous, has a wide- flat crown and bears early	Medium, round-conical, green-yellowish. It has red variants	Early- to mid-September. September to October	420 tons. Predominant in Western parts of Norway	(Asdal 2013; Måge 2003)
'Quinte'	Canada, 1964. 'Crimson Beauty' x 'Red Melba'	Diploid, moderately vigorous and bears early	Medium, round to ovate and green to white- yellowish	Mid- to late August.	Household purposes	(Asdal 2013; Måge 2003)
'Summerred'	Canada, 1964. Free pollination of 'Summerland'	Diploid, moderately vigorous and bears early	Medium, round to ovate and yellow-greenish to yellow	Mid- to late September. October to December	1430 tons. Third most important cultivar in Norway	(Bø et al. 1998; Måge 2003)
'Sävstaholm'	Sweden, 1830. Seedling propagation	Diploid, moderately vigorous and bears early	Medium, round to ovate. Green- to white- yellowish	Early September September to October	Household purposes	(Asdal 2014; Stedje & Skard 1939)
'Vista Bella'	USA, 1964. 'Melba' x 'Early McIntosh'and 'Julyred'	Diploid, very vigorous and bears early	Small to medium, round to round-oblate and yellow-greenish	Mid- August Late August	Cultivated in the western parts Norway	(Måge 2013)
'Åkerø'	Sweden, 1858. Unknown heritage	Diploid, very vigorous, with upright growth bears late	Medium to large, oblong to oblong-conic and greenish to white- yellowish	Early October November to January	310 tons	(Måge 2003; Stedje & Skard 1939)

3.1.3 Assessment of floral initiation and differentiation

The time of floral initiation and differentiation was assessed in spurs and extension shoots by dissecting buds throughout the growing season. Initiation in spurs was determined by randomly sampling five spurs per cultivar weekly. Spurs from 1-5 trees were sampled during the period July 4-September 11, 2013. Spurs were fixed overnight in glutaraldehyde (1.25%) and paraformaldehyde (2%), and further kept in PIPES buffer 0.05 M at 4°C until dissection. Flower initiation in extension shoots was assessed by weekly sampling three shoots on well exposed, south facing branches on each tree. Cultivars Aroma, Discovery, Elstar, Franskar, Gravenstein and Summerred were chosen. Shoots from 1-5 trees were sampled during the period August 9-September 26, 2013.

3.1.4. Dissection of buds

Buds from spurs and extension shoots were dissected using a binocular microscope (Wild Heerbrugg 50X, Switzerland). Expanded leaves and bud scales were removed to reveal the shoot apex, and the morphological stage of development was determined by using the scale proposed by Foster et al. (2003) (Table 2). In this scale, the author describes 8 stages of development from vegetative to initiation of floral organs, and the two first stages (0 and 1) are defined by means of meristem diameter. In the current study, meristem diameter was not measured, and therefore stages 0 and 1 are considered as stage 1 or vegetative meristem. Further, the first sign to floral commitment was the doming of the apex (stage 2), and the last sign was considered to be the formation of sepals on all floral meristems (stage 7).

Table 2. Developmental stages of the shoot apex in its transition from vegetative to generative. Partially modified from Foster et al. (2003).

Stage	Morphological features	Meristem identity
1	Flat meristem, leaf primordia	Vegetative
2	Domed meristem, first bracts	Inflorescence meristem
3	First visible floral primordia	Inflorescence meristem
4	Bract and bractlets on terminal and lateral meristems	Floral meristem
5	Visible sepals on terminal floral meristem	Floral meristem
6	Visible sepals on proximal lateral floral meristem	Floral meristem
7	Visible sepals on all floral meristems	Floral meristem

3.1.5. Phenological observations

The phenological stages of bud-break in spurs and extension shoots were followed from early spring to full bloom in 2013 and 2014. Dates for the stages 'green tip', 'pink' and full bloom were registered (Fig. 6). Visual assessment was done, and the dates on which more than 80% of the buds were in the same stage were recorded. Green tip is defined as the time in which the fruit bud is broken at the tip and shows about 1-2 mm of leaves (Chapman & Catlin 1976). Pink stage is defined as the time in which the king flower is about to open, pedicels are fully extended and the rest of the flower buds are still closed and show a pink tip (Chapman & Catlin 1976; Rommetveit 1979). Moreover, development of overwintered extension shoots (from 2013) was followed up in spring 2014. Diagrams of each of the 10 shoots were made indicating the total amount of generative and vegetative buds that burst and their position on the shoot.

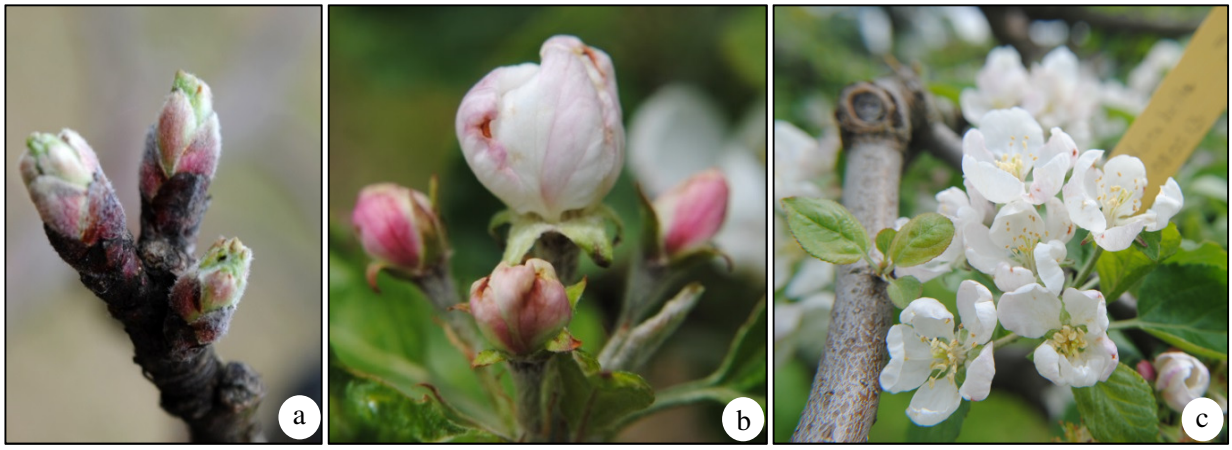

***Figure 6**. Phenological stages of bud-break registered on apple trees. a) Green tip on 'Elstar' (May 8, 2013); b) pink stage on 'Vista Bella' (May 25, 2013); c) full bloom on 'Vista Bella' (May 29, 2013) (Photos by R. Rivero).*

3.1.6. Scanning electron microscopy (SEM)

Terminal buds from spurs of cv. Summerred were collected weekly during the period July 4-September 9, 2013. The buds were fixed overnight in glutaraldehyde (1.25%) and para formaldehyde in 0.05 M PIPES buffer, pH 7.2, and subsequently kept in the same buffer at 4°C. Samples were dissected following the same procedure described in section 3.1.4 of materials and methods, and a minimum of 20 shoot apices per stage were selected. Dehydration was performed using a series of ethanol solutions at 70, 90, 96%, one time, in addition to four times at 100% (10 minutes on each immersion). Once dehydrated, all samples were dried in liquid CO_2 using a critical point dryer (CPD 030, Bal-Tec, Balzers, Lichtenstein), and mounted on stubs using double faced carbon tabs (Agar Scientific, Essex,

U.K.). After drying, samples were checked using a binocular microscope (Wild Heerbrugg 50X, Switzerland) and leaf primordia covering the shoot apex were removed. Once checked, the samples were sputter coated with approximately 500 Å Pt in a SC7640 sputter coater (Quorum Technologies Ltd, Newhaven, U.K.). Dried shoot apices were examined in a Zeiss EVO-50 scanning electron microscope, operated at 20-25 kV (Zeiss, Jena, Germany). Pictures of each developmental stage were taken and descriptions are included.

Effect of geographical location on growth and development of 'Aroma' and 'Gravenstein'

In a parallel experiment, one-year-old 'Aroma' and 'Gravenstein' trees were used to determine the effect of climatic conditions, at five different geographical locations across the country, on growth and FBF. Six trees of each cultivar were placed in locations well exposed to the local climate at the following Bioforsk research stations: Kvithamar (63° 27' N, 10° 57' E, Stjørdal, Nord-Trøndelag, 28 m.a.s.l.), Ullensvang (60° 19′ N, 6° 39′ E, Ullensvang, Hordaland, 13 m.a.s.l.), Apelsvoll (60° 40' N, 10° 51', Kapp, Oppland, 255 m.a.s.l.), NMBU (59° 39′ N, 10° 47′ E, Ås, Akershus, 96 m.a.s.l.), and Landvik (58° 20′N, 8° 31′ E, Grimstad, Aust-Agder, 5 m.a.s.l.) (Fig. 7). Climatic data were received from the nearest meteorological station from all locations and downloaded from Bioforsk's online meteorological service for agriculture (Bioforsk 2014b).

Figure 7*. Location of the five Bioforsk research stations in Norway (partially modified from Bioforsk (2014a)).*

3.1.7. Production of one-year-old trees

A total of 60 one-year-old 'Aroma' and 'Gravenstein' trees, grafted on rootstock M9, were delivered by 'Fjeld hagebruk' to Bioforsk Apelsvoll the last week of April in 2013. On May 3, the trees were re-potted in plastic containers of 7.5 L filled with peat and fastened to bamboo sticks. On May 7, all the lateral branches were removed and the trees were topped to a height of 105-110 cm from the pot's edge. The two last top buds were removed. On May 24, all trees were sprayed against apple scab and fertilized. Each pot was supplied with 44 g of Osmocote™, a controlled-release fertilizer with a release rate of 3-4 months (Scotts UK Ltd., Nottingham, U.K.), containing 14% (w/w) N, 4.2% (w/w) P, 11.6% (w/w) K, in addition to micronutrients. On June 1, all trees were packed and sent to the different research stations, where they stayed well exposed to the local climate. In addition, they were fastened onto 1-2 horizontal wires and watered regularly.

3.1.8. Cold storage and dissections

On November 10, 2013, all the trees were sent to NMBU, Ås, except for trees from Ullensvang, which overwintered at that research station due to infestation with apple leaf-curling midge (*Dasyneura mali*). One tree per cultivar and location were selected randomly for dissection, this to determine the positions in which flower buds had been developed on the shoots. Illustrations indicating length of the shoots, their position within the tree, number of buds per shoot and stage of development of each bud were made. Remaining trees were placed in cold chambers at 0-1°C, 90% RH, from November 21, 2013, until April 30, 2014.

3.1.9. Forcing to flower

The trees were taken out of the cold chambers and forced to flower in open air on April 30, 2014. They were placed in rows of 10 trees each nearby the nurseries at NMBU at Ås (Åsbakken 6) and watered regularly.

3.1.10. Phenological observations

Growth and flowering was assessed on overwintered trees in the period May 29-June 19, 2014. Diagrams were made for each tree, indicating the length of the extension shoots, their position within the tree and the total number of flower buds, vegetative buds and flowers per shoot. Moreover, time of budburst was assessed visually on each tree.

3.1.11. Calculation of growing degree days (GDD)

In order to evaluate the relationship between the different developmental processes in the tree's annual growth cycle and temperature, in terms of accumulation of heat, growing degree days were calculated by the following formula:

$$GDD = \left(\frac{Tmax+Tmin}{2}\right) - Tbase.$$

where, Tmax and Tmin are the mean daily maximum and minimum temperatures, respectively, and Tbase is the threshold temperature below which the specific process studied does not progress (e.g. shoot growth, anthesis, FBF). The Tbase used varied between the processes and the values were chosen according to the optimal temperature ranges reported in the literature for each of them. For instance, for shoot growth the range of Tbase was 10 to 14°C, for anthesis 0 to 4°C and for FBF the range was 15 to 20°C. Accumulated GDD for anthesis were calculated from the date of snowmelt, and for shoot growth and FBF the GDD were calculated from the date of full bloom.

3.1.12. Statistical analysis

All statistical analyses were performed using Minitab software (version 17.1.0, 2013 Minitab Inc.). Data were analyzed using a one- way analysis of variance (ANOVA) for the separation of means with a confidence interval of 95%. Grouping information was obtained by comparing means using Tukey's method. In cases were the grouping information showed overlapping between cultivars, a cluster analysis (by observations) was performed using a hierarchical agglomerative clustering procedure with complete linkage and Euclidean distance. The purpose of this analysis was to divide the whole multivariate dataset of measurements into groups of cultivars that shared similarities. For each analysis, a diagram of relationships (dendrogram) is presented. Due to large unit variations between vegetative parameters, the data were transformed using $\log_{10}$ to perform the cluster analysis.

In cases were relationships between parameters were observed, the strength of such relationships was measured by either Pearson correlation analysis or simple linear relation analysis. Pearson correlation analysis was used for the parameters length of the growing period vs. growth rate and accumulated GDD vs. shoot growth, and simple linear regression analysis was used for the parameters phenological stages vs. accumulated GDD, accumulated GDD and average daily mean precipitation vs. FBF in trees from different locations.

3.2. Results

Growth and development of cultivars in the experimental orchard at NMBU at Ås

3.2.1. Shoot growth

Shoot growth was measured as the length of extension shoots (cm) and the number of new leaves produced from June 25, 2013 (approximately 27 days after average full bloom for all cultivars) until growth cessation. On this date the average shoot length was 24.9 cm and average number of leaves was 13. Fig. 8a shows general growth curves that illustrate average cumulative shoot length and number of leaves for all cultivars throughout the growth period (June, July and August). The curves show a linear increment in both vegetative parameters from the first registration date until approximately July 23. From this date onwards, both shoot length and production of new leaves slowed down. By August 13, no further changes were observed on either of the vegetative parameters, and therefore, this is regarded as the average date for growth cessation in Åsbakken.

Individual growth curves are shown in Fig. 8b,c. The slope of the curves denotes the growth rate, expressed as centimeters (cm) of shoot extension and number of leaves per week (Fig 8b,c, respectively). The point of inflexion on each curve (where no considerable change in shoot growth or production of new leaves was observed) represents the date of growth cessation, and together with the date of full bloom was used to calculate the period of growth. Since no considerable shoot growth occurs before full bloom, the period of vegetative growth is given as the amount of days between full bloom and growth cessation.

Considerable differences in growth rate, growth period and total shoot length were found between cultivars. Table 3 summarizes average growth rates, growth periods and total shoot length for each cultivar. ‘Lobo’, ‘Aroma’, ‘Julyred’ and ‘Sävstaholm’ had the largest shoot growth rates (6.5–11.2 cm/week), while ‘Franskar’, ‘Prins’ and ‘Summerred’ had the smallest rates (≤ 4 cm/week). ‘Vista Bella’, ‘Elstar’, ‘Gravenstein’, ‘Mutsu’ and ‘Quinte’ had middle shoot growth rates (4.5–5.3 cm/week). Interestingly, cultivars with the highest growth rates stopped growth later, approximately by August 21, 2013, resulting in a growth period of about 70.5-76.6 days after full bloom (DAFB) and cultivars with the lowest growth rates stopped growth earlier, already by August 5 (equivalent to 52.6-59.7 DAFB). Cultivars with middle growth rates stopped growth by August, 13 (equivalent to 67-75 DAFB).

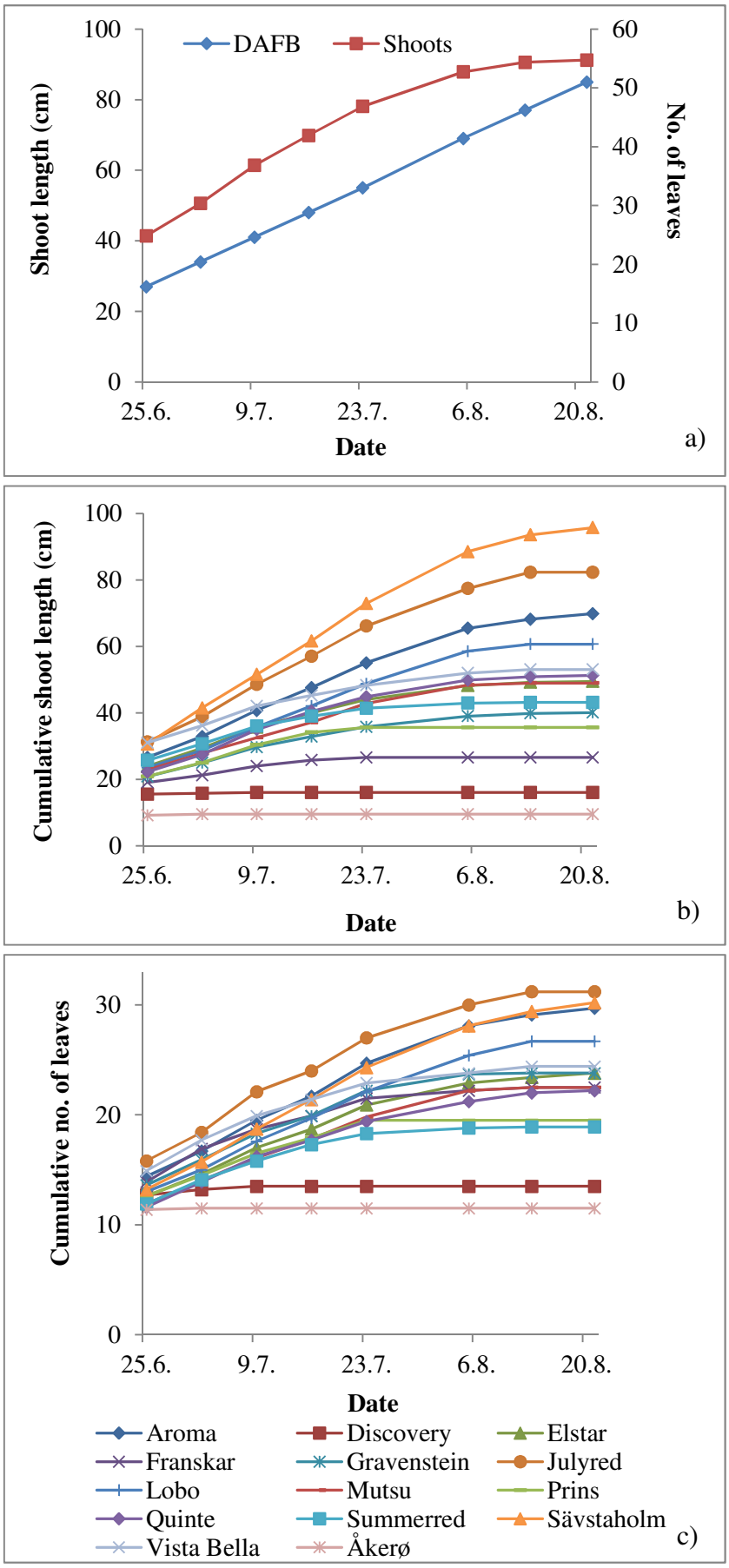

Figure 8. *Growth curves of 14 apple cultivars in Åsbakken in 2013. a) Total average; b) cumulative shoot length; c) cumulative number of leaves.*

It's important to point out that for 'Discovery and 'Åkerø' growth cessation occurred even earlier than for the rest of the cultivars (29.6 and 39 DAFB, respectively), had the lowest growth rates and shortest shoot length of all (0.4 cm/week, 16 and 9.5 cm, respectively). Such results represent outliers and therefore they were not included in further statistical analysis.

Table 3. Average growth rates*, growth period (expressed as days after full bloom (DAFB) to growth cessation) and total shoot length for 14 apple cultivars in 2013 in Åsbakken.

	Growth rates		Growth period	Total shoot length
Cultivar	cm/week	leaves/week	(DAFB)	(cm)
'Sävstaholm'	11.2_{a}	2.9_{a}	75.0_{ab}	95.8_{a}
'Julyred'	8.8_{ab}	2.9_{ab}	72.6_{abc}	82.4_{ab}
'Aroma'	7.0_{bc}	2.5_{abc}	76.6_{a}	69.9_{bc}
'Lobo'	6.5_{bcd}	2.5_{abc}	70.5_{abcd}	60.7_{bcd}
'Quinte'	5.3_{cd}	2.3_{abc}	68.1_{abcd}	51.3_{cde}
'Mutsu'	5.1_{cd}	2.1_{c}	68.6_{abcd}	49.0_{cde}
'Gravenstein'	4.8_{cd}	2.1_{c}	67.4_{abcd}	49.8_{cde}
'Elstar'	4.7_{cd}	2.1_{bc}	68.5_{abcd}	49.5_{cde}
'Vista Bella'	4.5_{cd}	2.2_{abc}	62.0_{bcde}	53.1_{cde}
'Summerred'	3.9_{cd}	1.9_{c}	59.3_{de}	43.2_{de}
'Prins'	3.8_{d}	1.9_{c}	52.6_{e}	35.6_{e}
'Franskar'	3.4_{d}	2.3_{abc}	59.7_{cde}	36.6_{de}
'Åkerø'	0.4	0.1	39.0	9.5
'Discovery'	0.4	0.5	29.6	16.0
Average**	5.8	1.8	66.8	54.7

*The data are means of 10 shoots per cultivar. Mean values within each column followed by a different lower-case letter are significantly different (P ≤0.05). *Growth rates were calculated as the weekly average shoot length and number of leaves during the active growth period. **ANOVA and total averages exclude 'Discovery' and 'Åkerø'.*

As previously mentioned, a relationship between growth rate and length of the growth period was observed. In order to measure the strength of this relationship, a scatterplot was made and a correlation analysis (Pearson correlation) was performed (Fig. 9). This analysis suggests a strong positive correlation between both variables, with correlation coefficients of 0.77 and 0.76 for rate of leaf production and rate of shoot growth, respectively (Fig. 9a). This means that the lower the growth rate, the shorter the period of vegetative growth and vice versa.

Growth was also correlated with temperature (T), in terms of accumulated heat. A strong positive correlation was found between shoot growth and accumulated growing degree days with a base T of 10°C ($GDD_{T = 10°C}$) (r= 0.96). Moreover, weekly mean T during the period of growth (June 25-August 20) ranged from 14°C to 20°C.

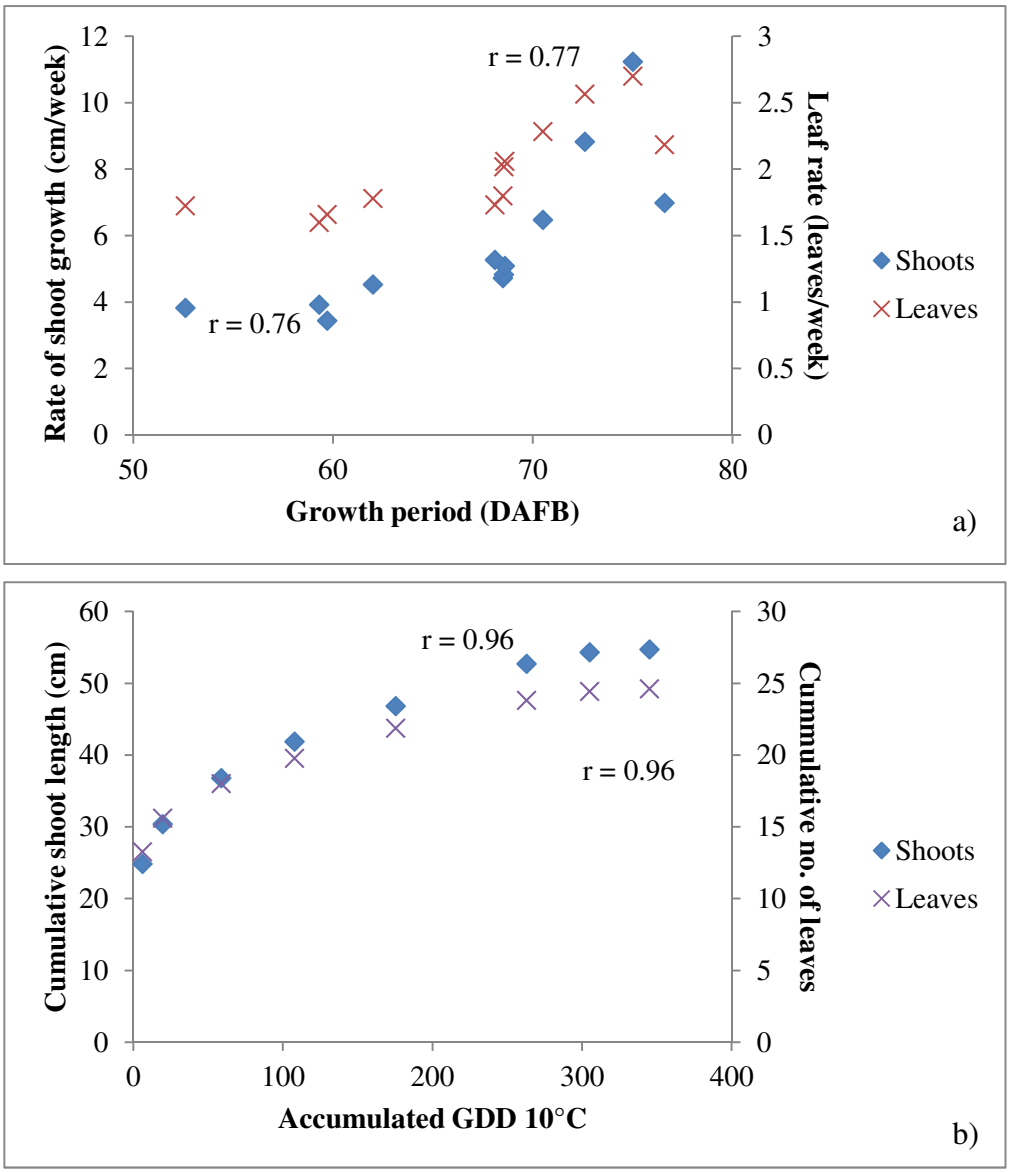

Figure 9. *a) Scatterplot of the mean shoot growth rate (cm/week) and leaf production rate (leaves/week) versus mean growth period (DAFB); b) scatterplot of cumulative shoot length (cm) and cumulative number of leaves versus accumulated* $GDD_{T = 10°C}$. *Pearson correlation coefficients (r) are given in each graph. Data are mean values of all cultivars studied in Åsbakken (except for 'Discovery' and 'Åkerø').*

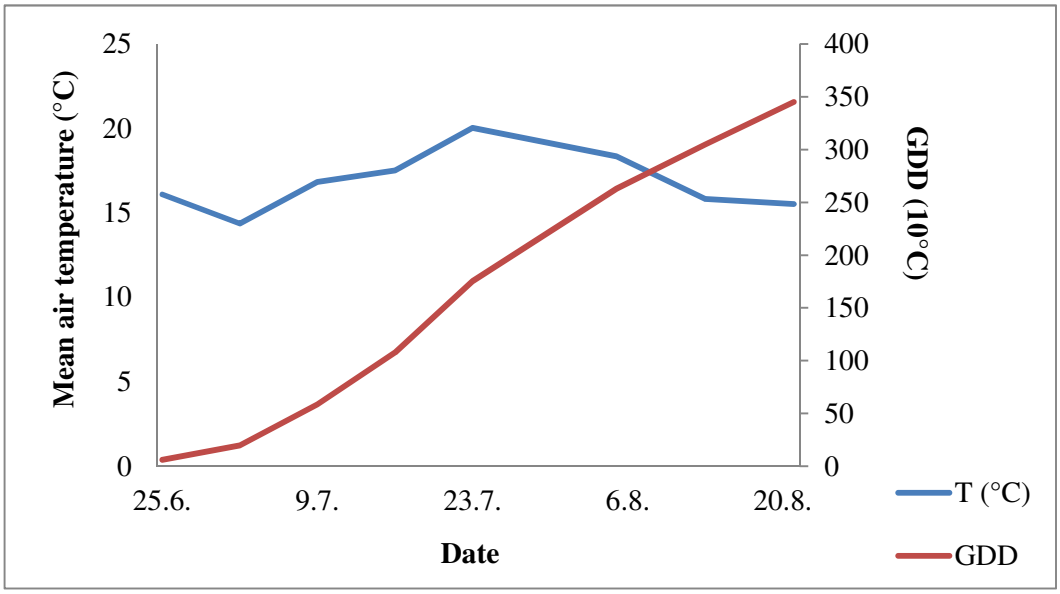

***Figure 10**. Weekly mean temperature and accumulated $GDD_{T = 10°C}$ during the period of shoot growth (measured from June 25 to August 20, 2013).*

An ANOVA was performed to the vegetative growth parameters evaluated, and as shown in Table 3, there were considerable differences between cultivars. The grouping information obtained shows overlapping of many cultivars, thus complicating the task of creating homogeneous groups of cultivars based on the four vegetative variables measured. In order to divide the whole multivariate dataset of measurements into groups of cultivars sharing similarities, a cluster analysis (by observations) was performed.

The diagram of relationships (dendrogram) for the cluster analysis is summarized in Fig. 11. Two main branches are separated at a similarity level of 0.0, meaning highly dissimilar groups. The first branch (from right to left, red branches) contains two sub-clusters: 'Summerred' and 'Prins', and 'Franskar' by itself. These cultivars had the lowest growth rates, growth periods and total shoot length (Table 3). The second branch is further divided into two sub-clusters at a similarity level of 31.53. The first sub-cluster on that branch (from right to left, green branches) contains 'Sävstaholm' and 'Julyred' (with similarity level of 84.04). These cultivars had the largest growth rate, growth periods and total shoot length (Table 3). The remaining cluster includes the rest of the cultivars and is further divided into two sub-clusters at a similarity level of 62.76. As seen in Table 3, cultivars included in these sub-clusters had middle to large growth rates, growth periods and total shoot length.

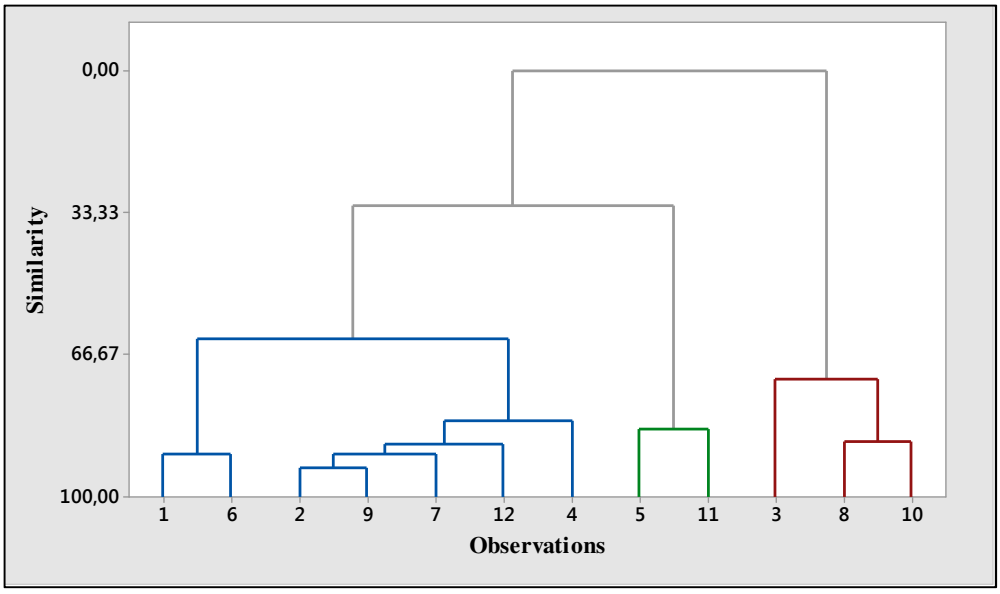

Figure 11. *Diagram of relationships (dendrogram) between 12 apple cultivars in Åsbakken based on transformed vegetative data. Observations on the X- axis correspond to cultivar code numbers: 1= 'Aroma', 2= 'Elstar', 3= 'Franskar', 4= 'Gravenstein', 5= 'Julyred', 6= 'Lobo', 7= 'Mutsu', 8= 'Prins', 9= 'Quinte', 10= 'Summerred', 11= 'Sävstaholm' and 12= 'Vista Bella'. Distance on the Y-axis shows how close clusters are by a similarity level (0 = dissimilar, 100 = identical).*

Based on the shoot growth parameters measured in 2013, 12 of the 14 cultivars studied in Åsbakken can be grouped into 1) cultivars with fast growth rates, long vegetative growth period and large shoot length (e.g. 'Sävstaholm' and 'Julyred'); 2) cultivars with middle growth rates, middle vegetative growth period and middle shoot length (e.g. 'Elstar', 'Quinte', 'Gravenstein', 'Vista Bella' and 'Mutsu'); and 3) cultivars with low growth rates, short vegetative growth period and short shoot length (e.g. 'Franskar', 'Summerred' and 'Prins'). 'Aroma' and 'Lobo' were somewhere in between the middle and fast growing groups.

3.2.2. Developmental stages of the FBF process. Scanning electron microscopy

Based on the examination of buds sampled in 2013 under a scanning electron microscope (SEM), the stages of FBF in apple buds are illustrated in Fig. 12. Such stages represent an artificial denomination of the continuous process of floral morphogenesis. In the present study, since meristem diameter was not measured due to practical reasons, the description of the different stages of floral development focuses mainly on the doming of the shoot apical meristem (SAM), and the appearance of floral meristems, bractlets and sepals.

The different stages of the FBF process are as follows:

Stage 1. At this stage, the SAM was enclosed by bud scales, transitional leaves and the latest leaf primordia. In order to expose the meristem bud scales, transitional leaves and some leaf primordia were removed. The SAM appeared flat, somewhat narrow (compared to subsequent stages) and had all leaf primordia positioned at the same level (nor higher nor lower than others). Fig. 12a shows a vegetative meristem with three leaf primordia and scars of three removed leaves.

Stage 2. At this stage, the SAM was wider and more protuberant than in stage 1, a shape often referred to as 'domed meristem'. Leaf primordia were separated from the central region of the apex and positioned at different heights (i.e. the latest primordium was positioned higher than older primordia). In addition, the first bracts were initiated (seen as protuberances at the flanks of the meristem). Fig. 12b shows a domed meristem with two leaf primordia, scars of removed leaves and two protuberances indicating initiation of bracts.

Stage 3. At this stage, the SAM was even more protuberant than in stage 2, several bracts were formed at the flanks and lateral floral meristems appeared beneath bracts. Fig. 12c shows an inflorescence meristem with removed leaves and six bracts: three with visible floral meristems that appear as a fold of tissue beneath each bract, two bract primordia and one bract without visible floral meristems.

Stage 4. At this stage, floral meristems were larger and had developed bractlets (second-order bracts). Each lateral floral meristem developed two bractlets at their periphery, and depending on the stage of development they appear as either folds of tissue or as elongated protuberances. The terminal floral meristem, which later differentiated into the king flower of the inflorescence, had developed a terminal bract and a bractlet. Fig. 12d shows an inflorescence meristem with removed leaves, six bracts (two of them were removed, lateral floral meristems at the axil of each bract have two bractlets each, and the terminal floral meristem has one bract (terminal bract)) and a protuberance that corresponds to a bractlet.

Stage 5. At this stage, floral meristems were large in size, more or less flat and the bractlets elongated and adopted a lanceolate to elliptical form with acute apices. The terminal floral meristem initiated five sepals. Fig. 12e shows an inflorescence meristem with five lateral floral meristems (four of them with a more or less flat surface and elongated bractlets) and a terminal floral meristem subtended by a bract (terminal bract) and with five sepals of different sizes.

Stage 6. At this stage, lateral floral meristems initiated sepals except for the distal meristem (less developed). The terminal floral meristem initiated petals (seen as rounded protuberances on the inside of the sepals). Fig. 12f shows an inflorescence meristem in which lateral meristems initiated sepals, except for the distal one. The distal lateral meristem seems still elliptical in shape and has only initiated bractlets, while the terminal floral meristem has initiated petals.

Stage 7. At this stage all floral meristems initiated sepals. Fig. 12g,h show inflorescence meristems in which bracts have been removed and all lateral meristems have initiated sepals that are highly pubescent on the inside.

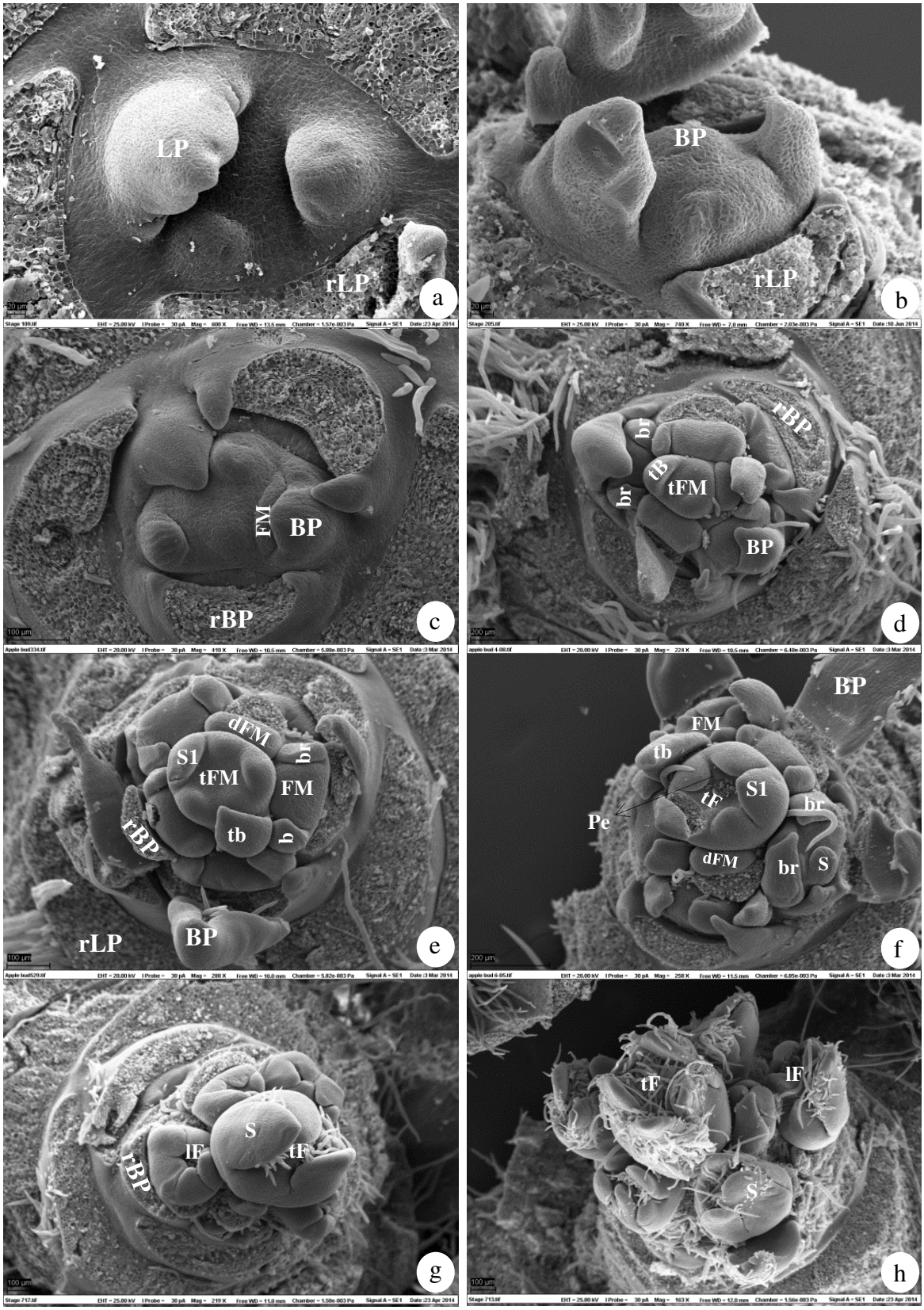

Figure 12. *Developmental stages of the SAM during floral morphogenesis in spurs of 'Summerred' collected in 2013. a) Stage 1, narrow and flat apex; b) Stage 2, domed apex with initiation of the first bracts; c) Stage 3, inflorescence meristem and floral meristems with bracts; d) Stage 4- terminal and lateral floral meristems has developed bractlets; e) Stage 5- terminal and proximal floral meristems has initiated sepals; f) Stage 6, terminal floral meristem has initiated petals and lateral floral meristems have initiated sepals; g) and h) Stage 7, all floral meristems have developed sepals. LP- leaf primordium; rLP- removed LP; BP- bract primordium; rBP- removed BP; tB- terminal bract; FM- floral meristem; tFM- terminal FM; dFM- distal FM; br- bractlet; S- sepal; tF- terminal (king) flower; Pe- petal.*

3.2.3. FBF in spurs and extension shoots

FBF was followed in spurs of 14 cultivars and in extension shoots of six cultivars by dissecting buds throughout the 2013 growing season. Fig. 13a,b show average percentage of generative meristems (stage ≥ 2) in spurs and extension shoots, respectively. The percentage of generative meristems was higher in spurs and increased with time. Generative meristems were first observed in spurs of 'Discovery' and 'Summerred', followed by 'Gravenstein' 'Aroma', 'Franskar' and 'Elstar', and the percentage increased rapidly in successive dates. By late August, almost all buds collected had generative meristems. 'Franskar' was an exception, and from mid- August until the last sampling date just 20% of the buds collected had generative meristems. In extension shoots, the percentage of generative meristems was variable between cultivars, position within the shoot and sampling date (Fig. 13b). The highest percentages were found in 'Discovery', 'Elstar' and 'Summerred' (> 30% on average) on the middle and upper parts of the shoots, and the lowest percentages were found in 'Gravenstein', 'Aroma' and 'Franskar' (< 20%).

When comparing all cultivars and correlating the percentage of generative meristems on extension shoots with the length of the shoots, a moderately negative correlation between the two variables was found (r = -0.5, meaning that shorter shoots had slightly higher percentage of generative buds). However, individual tests showed a weak negative correlation for 'Discovery' and 'Summerred', weak positive correlation for 'Franskar' and 'Gravenstein' and no correlation for 'Aroma' and 'Elstar'.

The average progress of the floral morphogenesis in spurs and extension shoots is illustrated by the development curves in Fig. 14a. As shown, there was a linear increment in the stage of development of the buds from the first sampling date until the maximum stage was reached. On average, the maximum stage reached in spurs was 7 (all flower primordia had developed sepals) and 5 in extension shoots (both terminal and proximal flower meristems had developed sepals). The average date for the first visible sign of FBF (domed meristems, stage 2) in spurs was approximately July 25, 2013, equivalent to 61.8 DAFB, and August 17, in extension shoots (Fig. 14a), equivalent to 80.4 DAFB (Table 4). When the development of the flower buds once had started, it continued rather fast (approximately 30 days from stage 2 to stage 7 for both types of buds). By late summer (approximately 90.4 DAFB), the first stage 7 was observed in spurs, and by early autumn (approximately by the last sampling date, 110.4 DAFB) the first stage 7 was observed in extension shoots.

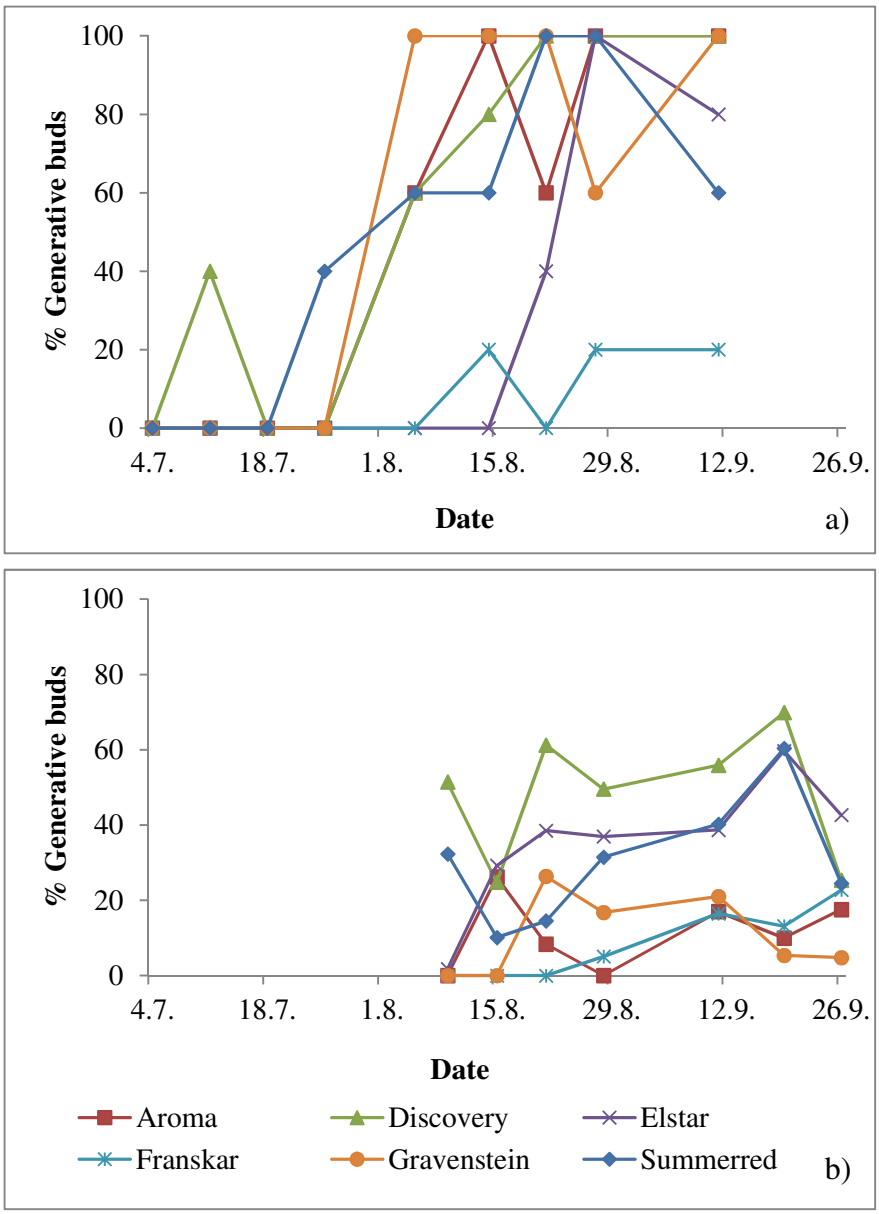

Figure 13. *Average percentage of generative meristems (≥ stage 2) on: a) spurs and b) extension shoots in 2013. Dataset was corrected and vegetative buds (stage 1) were excluded from the average values. n= 5 for spurs and n= 3 for extension shoots.*

Individual development curves for spurs are shown in Fig. 14b. In general, the pattern of development was similar in all cultivars. Development started slowly from stage 1 (vegetative meristem) to stage 2, and once stage 2 was reached the subsequent development was relatively rapid. There were differences in the amount of days between the first sampling date and the first observed stage 2 (Table 4). For instance, 'Sävstaholm', 'Quinte', 'Julyred', 'Lobo', 'Summerred' and 'Franskar' reached stage 2 first, between 51-59 DAFB. 'Prins', 'Gravenstein', 'Aroma' and 'Vista Bella' reached all stage 2 between 61-68 DAFB and 'Mutsu' and 'Elstar' reached stage 2 last, between 72-83 DAFB. Similarly, the amount of days from stage 2 to stage 7 varied between cultivars. For example, 'Summerred',

‘Gravenstein’ and ‘Sävstaholm’ had the shortest period of development between stages 2 and 7 (11-18 days). For ‘Vista Bella’, ‘Elstar’, ‘Lobo’ and ‘Prins’ the period was 22-28 days, for ‘Julyred’, ‘Mutsu’ and ‘Aroma’ was 31-37 days and ‘Franskar’ and ‘Quinte’ had the longest development period (48-52 days). No correlation was found between the earliness of reaching stage 2 and the amount of days between stages 2 and 7 (data not shown).

The first visible stage 2 was observed in ‘Discovery’, approximately 42 DAFB, and the development period between stages 2-7 was 47 days. ‘Åkerø’ had the first visible stage 2, 72 DAFB and the period between stages 2 and 7 was rather short in comparison to the rest of the cultivars (17 days). It is important to point out that, as for the vegetative measurements, these two cultivars were excluded from the average results in Table 4.

The progress of floral morphogenesis followed a similar pattern in extension shoots (Fig. 14c), where in general, the stage of development increased with time. On the first sampling date, the majority of the cultivars had buds in stages 1-2, and hereafter development continued at a rather fast rate (denoted by the slope of the curve) in ‘Summerred’, ‘Discovery’ and ‘Gravenstein’, and at a slower rate in ‘Elstar’, ‘Aroma’ and ‘Franskar’. It can be noted that just a fraction of the cultivars had reached stage 7 by the last sampling date, for instance, ‘Elstar’ and ‘Franskar’ reached stages 4 and 2, respectively. Regarding the amount of days between the first sampling date and the first observed stage 2, there were cultivar differences (Table 4). For example, extension shoots of ‘Summerred’, ‘Aroma’ and ‘Elstar’ reached stage 2 first (between 69-77 DAFB), while extension shoots of ‘Gravenstein’ and ‘Franskar’ reached stage 2 last (between 86-93 DAFB). Similarly, the amount of days from stage 2 to stage 7 varied between cultivars. For example, ‘Summerred’, ‘Elstar’, ‘Franskar’ and ‘Gravenstein’ had a period of development between stages 2-7 of 23-29 days, and ‘Aroma’ had the longest development period (42 days). ‘Discovery’ had the first visible stage 2 at 71 DAFB, and the development period between stages 2 and 7 was 33 days.

When data from spurs and extension shoots are compared, it can be noticed that the former type of buds started differentiation of flower buds before the latter (20 days earlier on average), and the period of development between stages 2 and 7 was similar between them (30 days on average). In general, by the time when the first stage 2 was observed in extension shoots, spurs had already reached stage 4 (Fig. 14a). ‘Summerred’ reached stage 2 on both spurs and extension shoots 58 and 69 DAFB, respectively (a difference of approximately 10 days between bud types). ‘Aroma’ had a similar period between stage 2 in spurs and

extension shoots (64 DAFB and 77 DAFB, respectively). For ‘Discovery’ and ‘Franskar’ the difference was quite large, approximately 30 days. ‘Elstar’ had a rather small difference (approximately 4 days) in the occurrence of stage 2 between bud types; for this cv. stage 2 was first observed in extension shoots and therefore the difference is negative. Regarding the period of development between stages 2 and 7, in general, spurs developed faster into stage 7 than extension shoots. ‘Franskar’ and ‘Discovery’ were exceptions in which the development period was longer in spurs.

Similarly to the dataset of vegetative measurements, a cluster analysis (by observations) was performed using generative data, in order to group cultivars with similar characteristics. The analysis included cultivars in which both spur and extension shoots data were available, e.g. ‘Aroma’, ‘Elstar’, ‘Gravenstein’ and ‘Summerred’. Since considerable differences were found between shoot types, individual analyses were performed for spur and extension shoot data, independently. The parameters used were the period between full bloom and stage 2 (‘Stage 2’ columns in Table 4) and the period between stages 2 and 7 (‘Diff. 2-7’ columns in Table 4). The former parameter represents the earliness of the differentiation of flower buds and the latter represents the rapidness of the development from stage 2 to 7. The cluster analyses were performed using a hierarchical agglomerative clustering procedure.

For spurs (Fig. 15a), the dendrogram shows two main branches separated at a similarity level of 0.0, meaning highly dissimilar groups. The first branch (from right to left) contains one sub-cluster with ‘Summerred’ and ‘Gravenstein’ (with similarity level of 72.5, based upon the fact that stages 2 and 7 were reached in the shortest period of time). The second branch is further divided into two sub-clusters at a similarity level of 12.5. The first sub-cluster on that branch (from right to left) contains only ‘Elstar’. The remaining cluster includes ‘Franskar’ and ‘Aroma’ at a similarity level of 65.6 (based upon the fact that these cultivars reached stage 7 in the longest period of time). For extension shoots (Fig. 15b), two main branches are separated at a similarity level of 0.0. The first branch (from right to left) contains one sub-cluster with ‘Summerred’ and ‘Elstar’ (with similarity level of 61.2, based upon the fact that stages 2 and 7 were reached in the shortest period of time). The second branch is further divided into two sub-clusters at a similarity level of 30.9. The first sub-cluster on that branch (from right to left) contains ‘Gravenstein’ and ‘Franskar’. The remaining cluster includes only ‘Aroma’ at a similarity level of 30.9 (based upon the fact that this cultivar reached stage 7 in the longest period compared to the rest of the cultivars in the analysis).

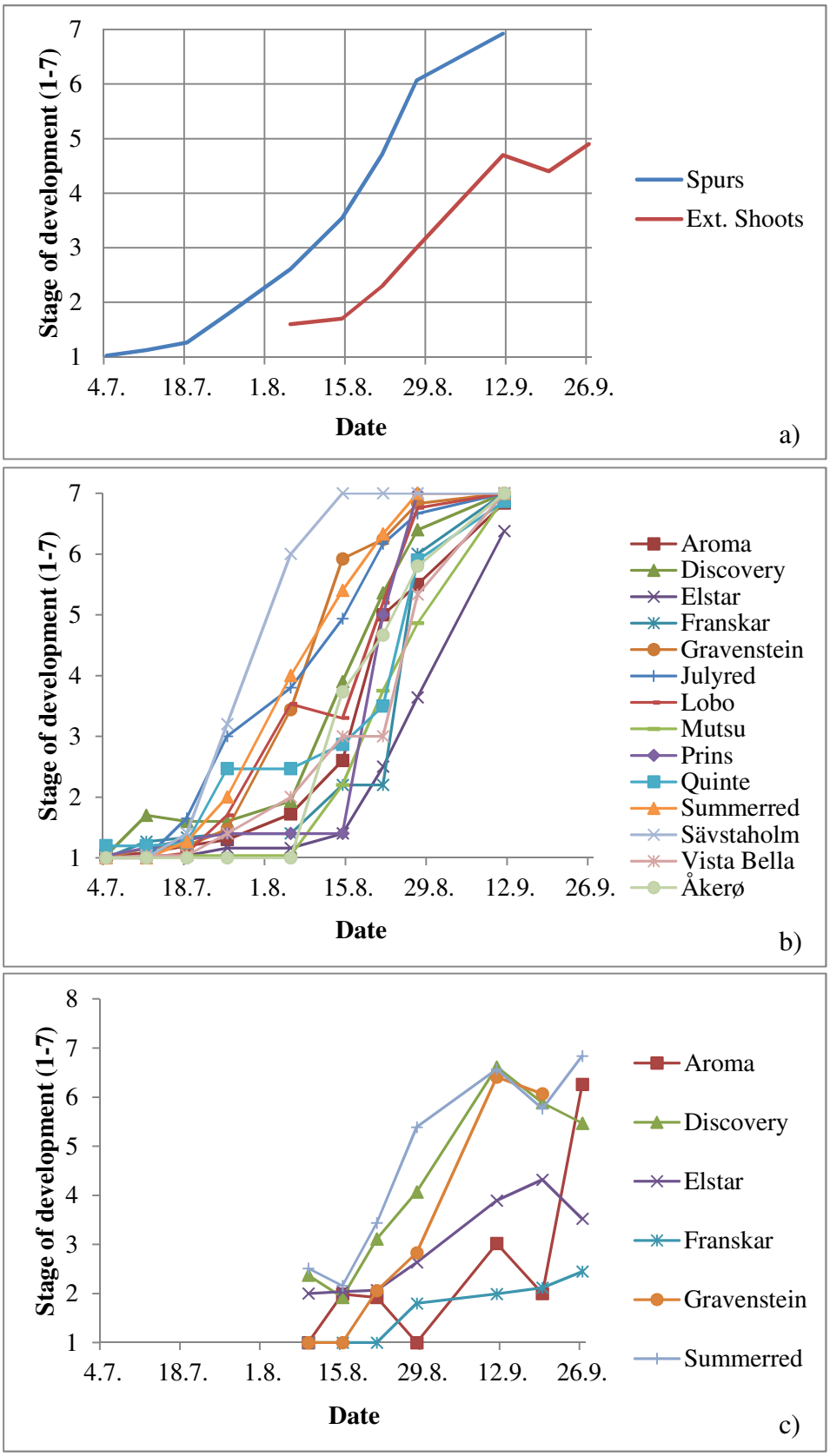

Figure 14. *Progress of floral morphogenesis in apple cultivars in Åsbakken during the growing season in 2013. a) Total average for spurs and extension shoots; b) individual curves for spurs; c) individual curves for extension shoots. Dataset was corrected and vegetative buds (stage 1) were excluded from the average values.*

Table 4. Periods of generative development in spurs and extension shoots. Data is expressed as the amount of days between full bloom and the actual reached stage (DAFB).

Cultivar	Spurs			Extension shoots		
	Days to stage 2	Days to stage 7	Diff. 2-7	Days to stage 2	Days to stage 7	Diff. 2-7
'Aroma'*	67	104	37	77	119	42
'Discovery'*	42	89	47	71	104	33
'Elstar'*	81	104	23	77	104	27
'Franskar'*	59	107	48	93	122	29
'Gravenstein'*	64	79	15	86	115	29
'Julyred'	52	83	31	-	-	-
'Lobo'	56	83	27	-	-	-
'Mutsu'	72	104	32	-	-	-
'Prins'	61	89	28	-	-	-
'Quinte'	52	104	52	-	-	-
'Summerred'*	58	69	11	69	92	23
'Sävstaholm'	51	69	18	-	-	-
'Vista Bella'	68	90	22	-	-	-
'Åkerø'	73	90	17	-	-	-
Selected average*	65.8	92.6	26.8	80.4	110.4	30.0
Total average**	61.8	90.4	28.7	80.4	110.4	30.0

Diff. 2-7 represents the amount of days between the first observed stage 2 and the first observed stage 7. Selected average includes cultivars for which both spurs and extension shoots were collected. Total average** excludes 'Discovery' and 'Åkerø'.*

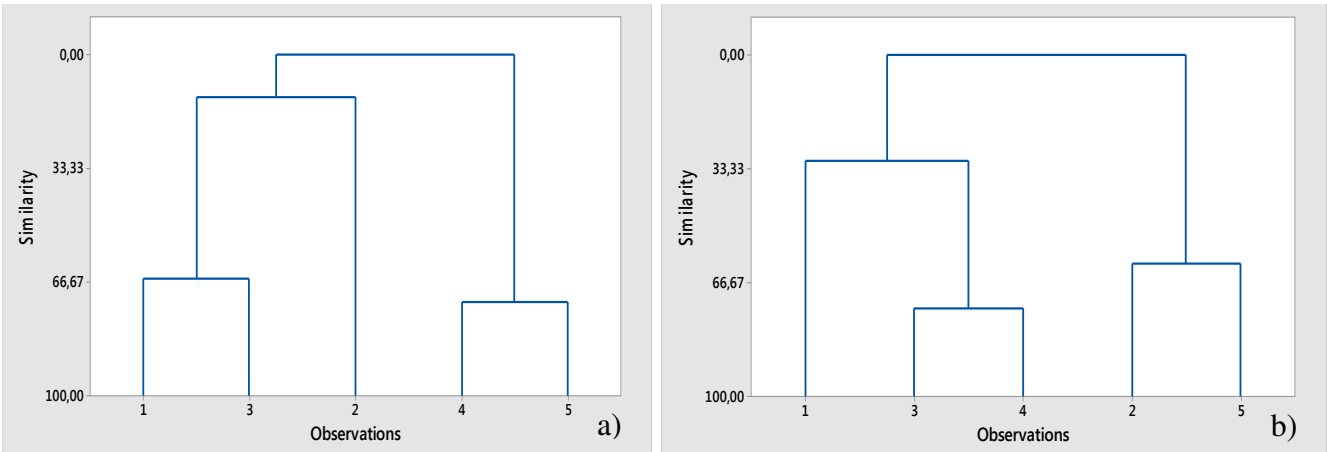

***Figure 15**. Diagrams of relationships (dendrogram) between six apple cultivars in Åsbakken based on periods of development of flower buds on a) spurs and b) extension shoots. Observations on the X-axis correspond to cultivar code numbers: 1= 'Aroma', 2= 'Elstar', 3= 'Franskar', 4= 'Gravenstein', 5= 'Summerred'. Distance on the Y-axis shows how close joined clusters are by a similarity level (0 = dissimilar, 100 = identical).*

3.2.4. Anthesis in 2013- 2014

Anthesis in Åsbakken was visually assessed in 2013-2014 by recording the dates on which 80% of opening buds were in the phenological stages 'green tip' (GT), 'pink' (P) and 'full bloom' (FB). The total period of flowering for each year was defined as the time between the first GT and the last observed FB for each cultivar. Fig. 16 summarizes average flowering periods (by calendar date) for each stage, cultivar and year. The figure shows that flowering started earlier and was extended for the longest period (weeks) in 2014 compared to 2013. Moreover, the stages GT and P were separated by almost 4 weeks on average (more than doubled compared to 2013). Subsequently, the stages P and FB were separated by similar time intervals in both years, and their occurrence was earliest in 2014. In general, the earliest cultivars to break bud and reach full bloom were 'Franskar', 'Gravenstein', 'Prins', 'Summerred' and 'Sävstaholm' and the latest cultivars were 'Aroma', 'Julyred', 'Lobo' and 'Mutsu'. Flowering on the remaining cultivars occurred on intermediate dates.

Fig. 17a shows mean air temperature (T) curves during flowering periods in 2013 and 2014. Mean air T for the stages GT, P and FB were higher in 2013 (10.2, 16.6 and 16.5°C, respectively) compared to 2014 (3.4, 13.8 and 15.9°C, respectively). It is apparent that there was more variability in both temperature and date on which the earliest stage of bud-break was reached.

In order to compare flowering between years in terms of accumulation of heat during the season, growing degree days (GDD) were calculated. The best results were obtained with a base temperature of 4°C (Table 5 and Fig. 17b). The earliest stage of bud-break (green tip) occurred at 39 and 27.6 GDD in 2013 and 2014, respectively, and subsequent stages occurred at considerably higher values of accumulated GDD. The average difference in GDD (Diff. 2013-2014 in Table 5) was positive and ranged from 11.4 and 28.9 GDD for GT and FB, respectively. In addition, a positive correlation between accumulated GDD and the occurrence of the different stages (R^2= 0.91 and R^2= 0.90 in 2013 and 2014, respectively) was found.

Figure 16. Average flowering periods for cultivars in Åsbakken in 2013-2014 (by calendar date). For each cultivar, the first row (in orange) corresponds to 2013 and the second row (in green) corresponds to 2014. GT= 'green tip', P= 'pink' and FB= 'full bloom'.

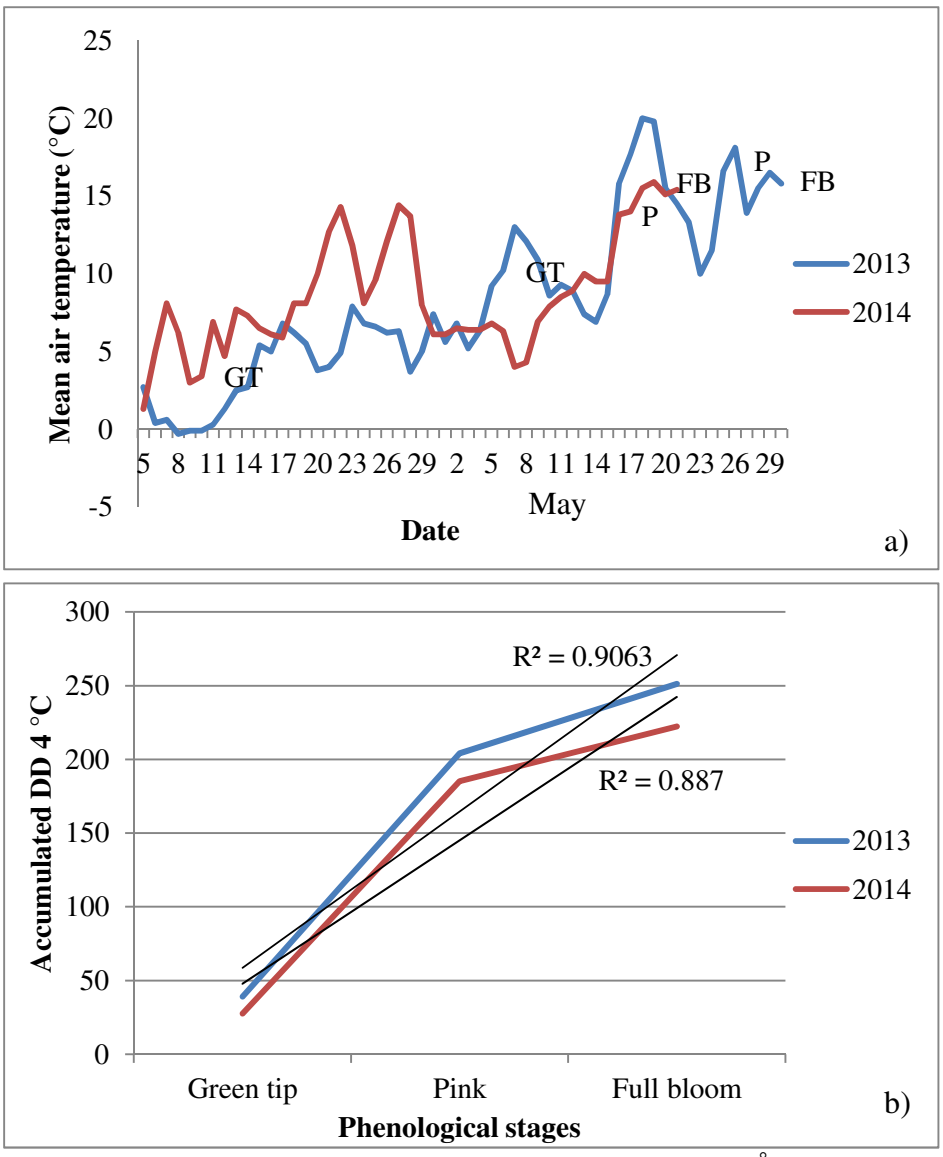

Figure 17*. a) Daily mean T during flowering in 2013 and 2014 in Åsbakken. Average dates for the occurrence of the phenological stages green tip, pink and full bloom are indicated on each curve; b) Progress of the stages of bud-break in 2013 and 2014 related to* $GDD_{T = 4°C}$ *(growing degree days). Coefficient of determination (R²) for the linear regression performed is shown for each curve.*

Individual curves for the progress of the stages of bud-break for each cultivar in 2013 and 2014, are shown in Fig. 18. As shown, there were variations between cultivars and such variations were consistent within the same year. For instance, in 2014 'Franskar', 'Gravenstein', 'Summerred' and 'Sävstaholm' reached all phenological stages of bud-break at the lowest amount of GDD, and 'Elstar', 'Lobo', 'Mutsu' and 'Prins' at the highest amount of GDD. In 2013, the pattern was similar, however more variability was observed in the order in which cultivars reached the different stages. In general, 'Franskar', 'Granvenstein' and 'Savstaholm' reached all stages at the lowest amount of GDD and 'Elstar' and 'Prins' at the highest amount of GDD.

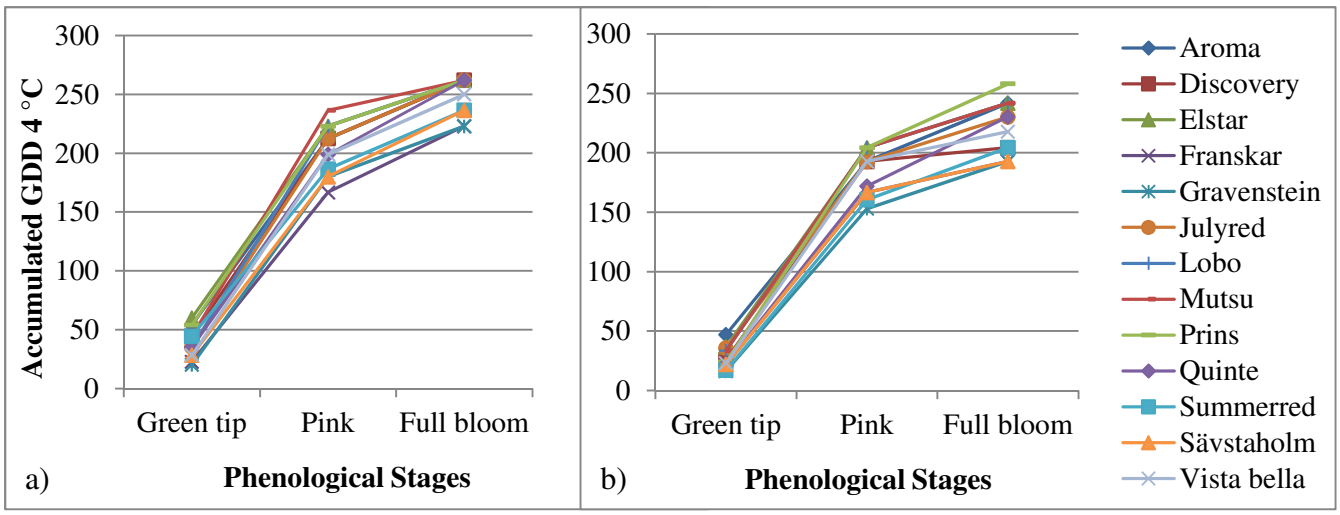

Figure 18. *Progress of the stages of bud-break related to* $GDD_{T = 4°C}$ *(growing degree days) in a) 2013 and b) 2014.*

Table 5. Accumulated $GDD_{T = 4°C}$ (growing degree days) at which the phenological stages green tip (GT), pink (P) and full bloom (FB) occurred in 2013 and 2014 in Åsbakken.

Cultivar	2013 ($GDD_{T = 4°C}$)			2014 ($GDD_{T = 4°C}$)			Diff. 2013-2014		
	GT	P	FB	GT	P	FB	GT	P	FB
'Aroma'	54.1	212.5	262.0	47.1	192.7	241.8	7.0	19.8	20.3
'Discovery'	44.6	212.5	262.0	25.8	192.7	204.4	18.8	19.8	57.7
'Elstar'	60.0	223.1	262.0	36.9	204.4	241.8	23.1	18.7	20.3
'Franskar'	22.7	166.6	223.1	21.9	166.7	192.7	0.8	-0.1	30.4
'Gravenstein'	20.2	180.1	223.1	16.4	153.0	192.7	3.9	27.1	30.4
'Julyred'	35.2	212.5	262.0	35.7	192.7	230.1	-0.5	19.8	31.9
'Lobo'	35.2	223.1	262.0	33.6	204.4	241.8	1.6	18.7	20.3
'Mutsu'	44.6	236.4	262.0	33.6	204.4	241.8	11.0	32.1	20.3
'Prins'	54.1	223.1	262.0	24.0	204.4	258.1	30.1	18.7	3.9
'Quinte'	35.2	199.0	262.0	22.3	171.8	230.1	12.9	27.1	31.9
'Summerred'	44.6	186.6	236.4	17.0	160.6	204.4	27.6	26.0	32.1
'Sävstaholm'	28.4	180.1	236.4	21.9	166.7	192.7	6.5	13.4	43.7
'Vista Bella'	28.4	199.0	250.1	23.1	192.7	217.8	5.3	6.3	32.4
Average	39.0	204.2	251.2	27.6	185.1	222.3	11.4	19.1	28.9

Combining accumulated GDD data (Fig. 18) and calendar dates data (Fig. 16), the 13 cultivars studied can be grouped into 1) early flowering: 'Gravenstein', 'Franskar', 'Summerred' and 'Sävstaholm'; 2) mid-season flowering: 'Discovery', 'Julyred', 'Quinte' and 'Vista Bella' and 3) late flowering: 'Aroma', 'Elstar', 'Lobo', 'Mutsu' and 'Prins'.

In addition to the general flowering data presented above, the development of overwintered extension shoots (from 2013) was registered in selected cultivars the following spring. Diagrams of each of the 10 shoots were made indicating the total amount of generative and vegetative buds that burst, and their position on the shoot (data not shown). Fig. 19 shows combined data for extension shoots on the percentage of flower buds observed in 2013 and the percentage of open flowers registered in 2014. The percentage of generative buds varied between cultivars without a clear pattern. The highest percentage of generative buds was observed in 'Elstar', 'Discovery' and 'Summerred' both before (2013) and after winter dormancy (spring 2014). The lowest percentage of generative buds was obtained in 'Aroma' and 'Franskar' in 2014 and 'Gravenstein' in 2013 and 2014. Moreover, it was qualitatively observed on those cultivars that developed flower buds in extension shoots, that the buds were positioned in the middle to top region of the shoot. As for the results in 2013, the percentage of flowers in 2014 was not correlated to the length of the shoot (data not shown).

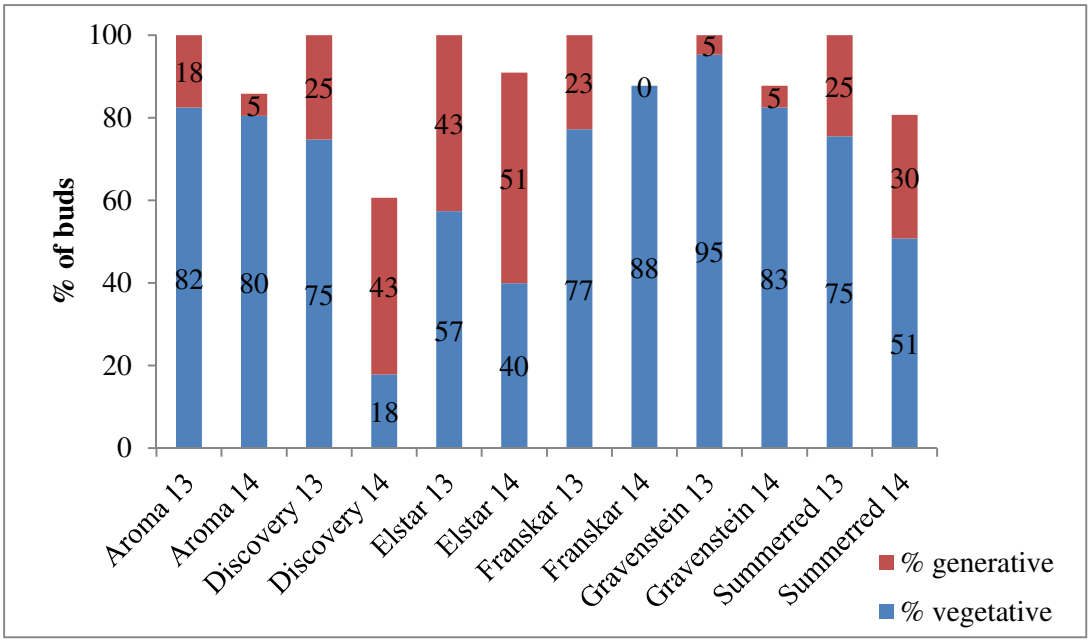

Figure 19. *Percentage of generative and vegetative buds on extension shoots in 2013 and 2014. Data from 2013 correspond to dissections of three extension shoots on the last sampling date of the season (September 26, 2013). Data from 2014 correspond to visual assessment of flowering on 10 overwintering extension shoots from 2013.*

3.2.5. Leaf abscission

The progress of leaf abscission was followed from October 1 until November 25, 2013, and as shown in Fig. 20a, cultivar differences were observed. The onset of leaf abscission was earliest in 'Julyred', 'Prins', 'Quinte' and 'Vista Bella'. By October 1, 2013, these cultivars had visible signs of leaf senescence (leaf yellowing) and about 20% of the leaves had already been abscised. The onset of leaf abscission in the rest of the cultivars occurred later. For instance, visible signs of leaf senescence and abscission in 'Discovery', 'Lobo', 'Mutsu', 'Sävstaholm' and 'Åkerø' were first observed by October 10, and in 'Franskar', 'Gravenstein' and 'Summerred' by October 24. For 'Aroma' and 'Elstar' the onset of leaf abscission was rather late, about 30 calendar days after the first registration date. By November 25, total leaf abscission was observed in almost all cultivars, except for 'Aroma' and 'Elstar' which abscised just 40% of their leaves by this date. In addition, there was a moderately negative correlation between weekly mean T and weekly percentage of leaf abscission (r = -0.67).

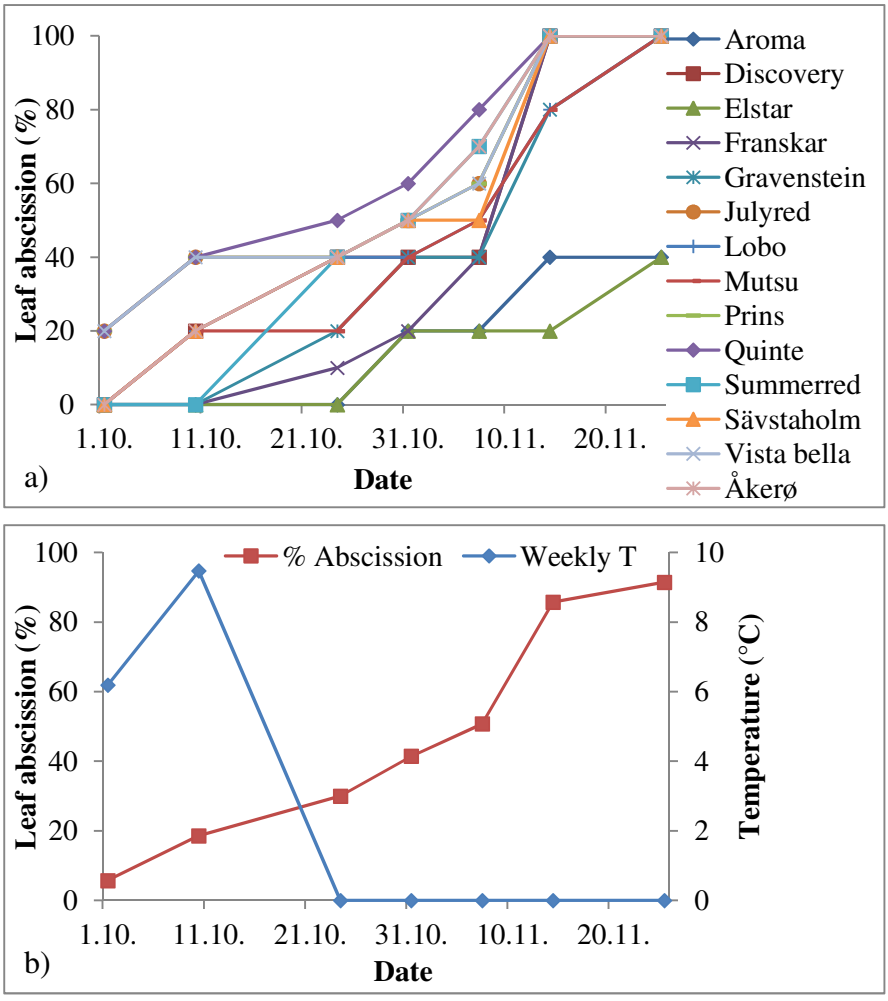

***Figure 20**. Leaf abscission by the end of 2013. a) weekly percentage of leaf abscission; b) total average leaf abscission and temperature.*

3.2.6. FBF in 2013 and anthesis in 2014

FBF was assessed by dissecting all extension shoots of one tree per cultivar per location at the end of the growing season in 2013. Table 6 and Fig. 21 show average growth data including the total number of buds per tree, shoot length, percentage of vegetative and generative buds, and mean generative stage. In general, shoot length and percentage of vegetative buds were highest in 'Gravenstein'. The total amount of buds, percentage of generative buds and mean generative stage were highest in 'Aroma' trees.

An ANOVA was performed to the growth data collected, and the grouping information for this analysis is shown in Table 6. Significant differences were found in the percentage of vegetative and generative buds between cultivars, and locations within the same cultivar. The percentage of vegetative buds was highest in 'Aroma' from Stjørdal and Landvik, and lowest in trees from Kapp. For 'Gravenstein', the highest percentage of vegetative buds was found in trees from Ullensvang and Stjørdal, and the lowest in trees from Kapp. For both cultivars, most of the locations overlap, indicating that significant differences were only found between locations with the lowest and highest percentages of vegetative buds. The highest percentage of generative buds was found in 'Aroma' from Kapp and Ås, and this was significantly different from Stjørdal. 'Aroma' from Ullensvang and Landvik had middle percentages of generative buds, and these were significantly different from Stjørdal and Kapp. Similarly, for 'Gravenstein', the highest percentage of generative buds was observed at Kapp and Ås, and the lowest was found at Stjørdal and Ullensvang. Trees from Landvik had a middle percentage of generative buds, but this was not significantly different from Ås, Ullensvang or Stjørdal.

There was also a trend of increasing mean generative stage from the northernmost locations to the southernmost locations. For instance, the mean generative stage of buds of 'Aroma' and 'Gravenstein' from Landvik was 6.1 and 5, respectively, compared to 3.3 and 3.9 in trees from Stjørdal (Table 6). However, these differences between cultivars and locations were not statistically significant.

Table 6. Growth data for 'Aroma' and 'Gravenstein' as results of dissections at the end of the growing season in 2013.

Cultivar	Location	Tot. buds/tree	Shoot length (cm)	% veg. buds	% gen. Buds	Mean gen. stage
'Aroma'	Landvik	57.0	22.6	79.6_{ab}	20.4_{b}	6.1
	Ås	184.0	50.1	51.8_{bc}	48.2_{ab}	4.5
	Ullensvang	102.0	24.8	64.5_{b}	35.5_{b}	4.2
	Kapp	196.0	52.5	26.3_{c}	73.7_{a}	4.9
	Stjørdal	124.0	42.6	98.1_{a}	1.9_{c}	3.3
	Mean	*132.6*	*38.5*	*64.1*	*35.9*	*4.6*
'Gravenstein'	Landvik	122.0	41.4	77.8_{ab}	22.2_{bc}	5.0
	Ås	171.0	54.5	61.8_{bc}	38.2_{ab}	5.2
	Ullensvang	133.0	55.0	100.0_{a}	0.0_{c}	0.0
	Kapp	122.0	54.9	50.5_{c}	49.5_{a}	5.3
	Stjørdal	100.0	54.8	96.5_{a}	3.5_{c}	3.9
	Mean	*129.6*	*52.1*	*77.3*	*22.7*	*4.9*
P-value (ANOVA)	Cultivar	ns	ns	0.035	0.035	ns
	Location	ns	ns	ns	Ns	ns

The data are means of all shoots of one tree per cultivar per location. Mean values within each column for each cultivar followed by a different lower-case letter are significantly different (P ≤0.05). ns (not significant).

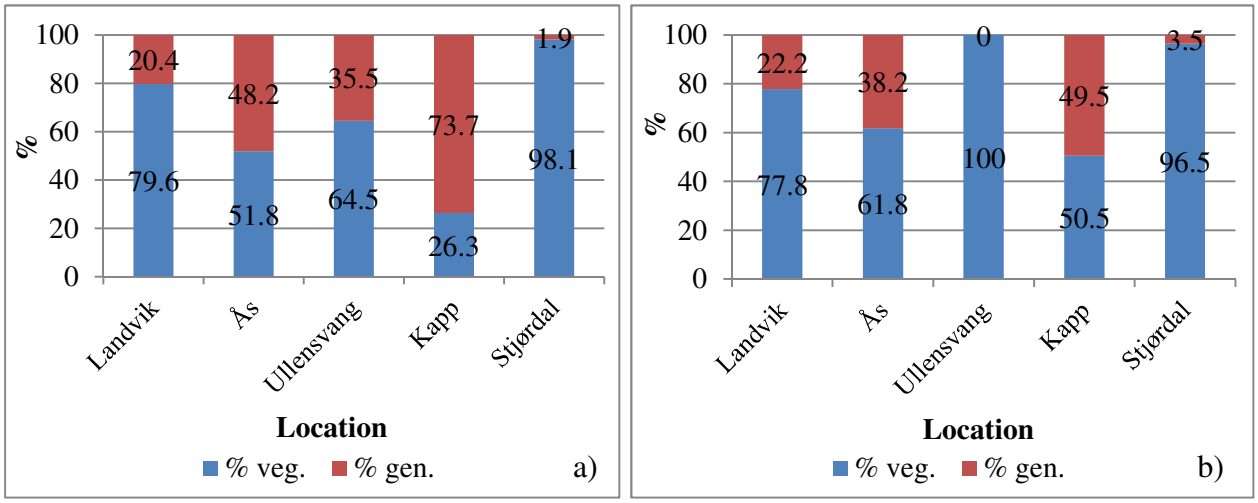

***Figure 21**. Percentage of vegetative and generative buds on extension shoots of a) 'Aroma' and b) 'Gravenstein' grown at different locations in 2013 and registered at the end of the season in the same year.*

Trees that were not dissected in autumn 2013 (n= 5 per cultivar per location) overwintered under controlled conditions (0-1°C, 90% RH) and flowering was assessed after forcing under outdoor conditions in spring 2014. Average growth data for these trees is shown in Table 7 and Fig. 22, and includes total number of buds per tree, shoot length, percentage of vegetative, nonbreaking and generative buds, and total number of flowers. Similarly to the results from dissections in 2013, the mean percentage of vegetative buds and shoot length were higher in 'Gravenstein', and the mean percentage of generative and nonbreaking buds, and the total number of flowers was higher in 'Aroma' trees.

An ANOVA was performed, and considerable differences for the parameters percentage of vegetative, nonbreaking and generative buds, and total number of flowers between cultivars and locations were obtained (Table 7). The percentage of vegetative buds was significantly higher in trees of both cultivars from Stjørdal. Trees of both cultivars from Ullensvang had the lowest percentage of vegetative buds. The percentage of nonbreaking buds for 'Gravenstein' was significantly higher in trees from Ullensvang. For 'Aroma', the highest percentage was found at Ullensvang and the lowest at Kapp, although the differences were not significant between the remaining locations. Regarding generative buds, the highest percentage was found at Ås and Kapp, and the lowest at Stjørdal. Statistically significant differences for this parameter were only found between Kapp and Stjørdal for 'Aroma', and between Ås, Kapp and the rest of the locations for 'Gravenstein'. Similarly, the total number of flowers was significantly lower at Stjørdal and higher at Ås and Kapp.

A cluster analysis was performed in order to divide the multivariate dataset into groups of locations with similar characteristics (Fig. 23). Both cultivars had a similar partition of locations with two main branches separated at a similarity level of 0.0, meaning highly dissimilar groups. The first branch (from right to left) contains the locations Kapp and Ås. Trees from these locations had the largest total number of flowers and percentage of generative buds and the lowest percentage of nonbreaking buds (Table 7, Fig. 22). The second branch is further divided into two sub-clusters at similarity levels of 48.3 and 23.2 for 'Aroma' and 'Gravenstein', respectively. For 'Aroma', the first sub-cluster (from right to left) only contains Stjørdal. This location had the highest percentage of vegetative buds and the lowest of generative buds and total number of flowers. For 'Gravenstein', the first sub-cluster contains Stjørdal and Ullensvang, locations that had the lowest percentage of generative buds and total number of flowers and the highest percentage of vegetative buds. The remaining cluster includes Landvik and Ullensvang for 'Aroma' and only Landvik for 'Gravenstein'.

Trees from Ullensvang had the lowest percentage of vegetative buds and the highest percentage of nonbreaking buds, while trees from Landvik had middle values for the same parameters in this study.

Table 7. Growth data for 'Aroma' and 'Gravenstein' recorded in spring 2014.

Cultivar	Location	Tot. buds/tree	Shoot length (cm)	% veg. buds	%nonbreaking buds	% gen. buds	*Tot. no.* of flowers
'Aroma'	Landvik	128.4	43.6	51.3_{ab}	27.1_{ab}	21.6_{ab}	98.4_{abc}
	Ås	190.0	44.6	52.4_{ab}	29.0_{ab}	18.6_{ab}	116.0_{ab}
	Ullensvang	150.8	46.0	42.7_{b}	44.0_{b}	13.3_{ab}	57.8_{bc}
	Kapp	154.0	53.0	52.8_{ab}	20.2_{ab}	27.0_{a}	120.0_{a}
	Stjørdal	126.2	43.3	66.5_{a}	22.5_{ab}	11.0_{b}	38.2_{c}
	Mean	*149.9*	*46.1*	*53.1*	*28.6*	*18.3*	*86.1*
'Gravenstein'	Landvik	108.4	44.3	72.4_{a}	20.7_{b}	6.9_{b}	38.0_{bc}
	Ås	176.4	46.9	63.6_{ab}	13.3_{b}	23.1_{a}	143.0_{a}
	Ullensvang	136.8	54.5	54.9_{b}	35.1_{a}	10.0_{b}	39.4_{bc}
	Kapp	124.8	43.5	56.2_{b}	20.9_{b}	22.9_{a}	86.6_{b}
	Stjørdal	111.4	45.7	77.9_{a}	19.1_{b}	3.0_{b}	9.4_{c}
	Mean	*131.6*	*47.0*	*65.0*	*21.8*	*13.2*	*63.3*
P-value (ANOVA)	Cultivar	ns	ns	0.00	0.00	0.017	0.018
	Location	ns	ns	0.00	0.00	0.00	0.00

The data are means of all shoots of five trees per cultivar per location. Mean values within each column for each followed by a different lower-case letter are significantly different (P ≤0.05). ns (not significant).

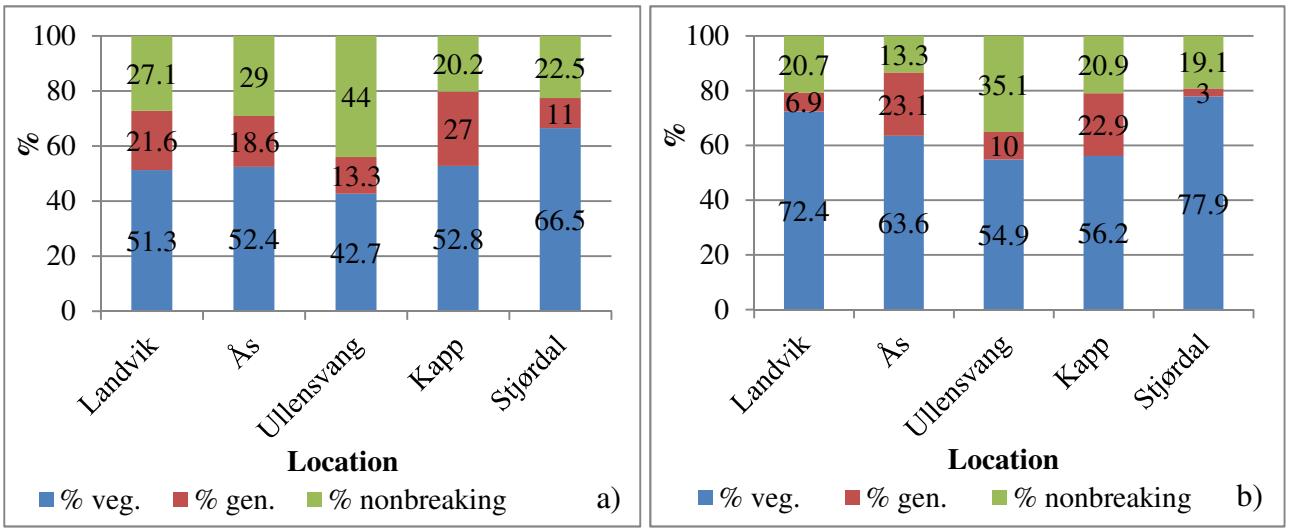

***Figure 22**. Percentage of vegetative, generative and nonbreaking buds in trees of a) 'Aroma' and b) 'Gravenstein' grown at different locations in 2013 and recorded in spring 2014.*

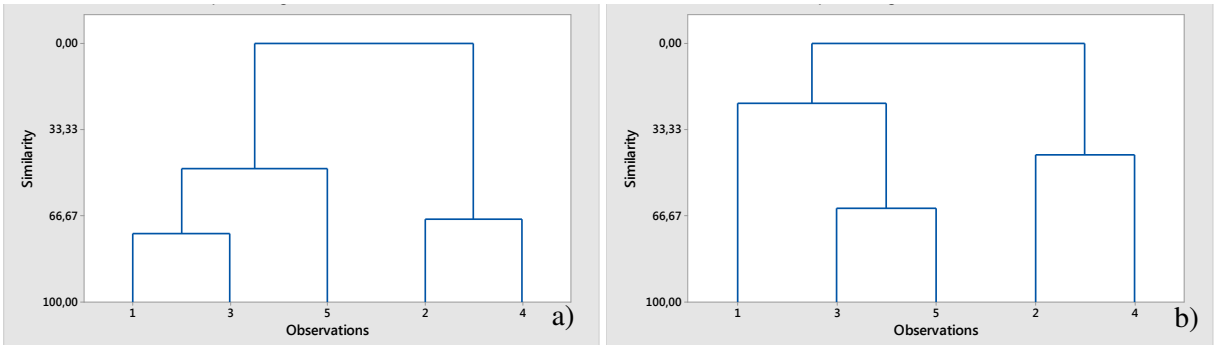

***Figure 23**. Diagrams of relationships between five locations based on transformed growth data for a) 'Aroma' and b) 'Gravenstein'. Observations on the X- axis correspond to location code numbers: 1= Landvik, 2= Ås, 3= Ullensvang, 4= Kapp, 5= Stjørdal. Distance on the Y-axis shows how close clusters are by a similarity level (0 = dissimilar, 100 = identical).*

Based on the growth data collected and analyzed in 2013 and 2014, trees from Stjørdal formed the lowest amount of flower buds, both as percentage of generative buds before and after winter rest, and as total number of flowers opened in the spring, and consequently had the highest amount of vegetative buds. Trees from Kapp and Ås formed the highest amount of flower buds, both before and after winter rest, and had the highest amount of opened flowers in the following spring.

It is important to point out that qualitatively, anthesis in overwintered trees was irregular and the period of flower registration was extended for over 3 weeks. In 'Gravenstein' trees from the southernmost locations (Landvik and Ås), bud-break started first and in trees from Stjørdal occurred much later. Abortion of flower and vegetative buds before and after anthesis was observed and the number of opened flowers per bud was highly variable, ranging from 1-7 flowers per cluster (data not shown).

3.2.7. Effect of temperature and precipitation on FBF and anthesis

Temperature and precipitation data for the five locations is presented in Table 8 and Fig. 24. In general, average monthly temperatures from June to August were higher at Landvik (14.3 – 17.7°C) and lower at Stjørdal (13.1 – 14.1°C). Average mean daily precipitation varied between locations and months. Landvik, Ås and Kapp had similar amount of precipitation in the period from June 1 to August, 1, 2013. From June to July, there was a decrease in precipitation, followed by a slightly increment from July to August. From August onwards, precipitation was stable, ranging from 1 to 4 mm at Ås, Kapp and Stjørdal, and considerably higher at Landvik and Ullensvang (ranging from 2-16 mm).

In order to see the relative effect of these external factors on the flowering outcome, a simple linear regression analysis was performed between average monthly temperatures and precipitation (Table 8 and Fig. 24), and FBF data (Fig. 21 and 22 and Table 7 and 8). The analysis was performed with average temperatures and precipitation during the following periods: June, July, August, September, June-July, July-August, August-September, June-August and July-September.

Coefficients of determination (R^2) for this analysis were lower than 0.5 and therefore, it can be concluded that average monthly temperatures explained very little of the variability obtained in the FBF and flowering data. Considering these results, growing degree days (GDD) with base temperatures from 15 to 20°C were calculated, in order to see if accumulated heat during the summer could explain the variability observed (Table 9). The temperature range was chosen according to the optimal reported in the literature for the FBF process in apple buds (see section 2; literature review). The highest correlation values were found with a base temperature of 15°C. Table 10 shows R^2 values for the linear regression between FBF, flowering data and $GDD_{T=15°C}$ for the different periods during summer 2013. In general, the variation in accumulated GDD during July explained a high percentage of the variability in the amount of flower buds formed. For instance, 73 and 77% of the variability in percentage of generative buds in 2014 for 'Aroma' and 'Gravenstein', respectively, was explained by the variation in accumulated GDD in July 2013. Less variability in total number of flowers was explained by accumulated GDD (67 and 39% in 'Aroma' and 'Gravenstein', respectively). 86 and 84% of the variability in percentage of generative buds observed in 2013 in 'Aroma' and 'Gravenstein' was explained by the variability in accumulated GDD during July 2013.

Variations in daily mean precipitation during July 2013 accounted for 84 and 75% of the variability in percentage of generative of buds for 'Aroma' and 'Gravenstein', respectively. Moreover, variations in precipitation in June-July explained 95% of the variability in total flower number and 86% of the variability of generative buds (dissections before winter dormancy) in 'Aroma'. Less significant relationships were found between precipitation and percentage of generative buds and total number of flowers in 'Gravenstein' trees.

Table 8. Monthly mean temperatures (T in °C) and daily precipitation (Prec. in mm) from June to November at five locations in Norway.

	June		July		August		September		October		November	
Location	T (°C)	Prec. (mm)	T (°C)	Prec. (mm)	T (°C)	Prec. (mm)	T (°C)	Prec. (mm)	T (°C)	Prec. (mm)	T (°C)	Prec. (mm)
Landvik	14.3	5.3	17.7	0.4	16.0	1.8	12.8	7.0	8.9	5.6	4.3	4.4
Ås	14.0	4.1	17.1	0.6	15.2	2.3	10.8	2.1	6.8	3.3	3.6	0.0
Ullensvang	14.1	2.4	16.8	2.8	15.1	5.0	11.8	3.4	8.4	7.7	4.9	12.7
Kapp	13.4	4.1	17.2	0.2	14.9	2.8	10.7	0.8	5.8	1.5	2.3	2.3
Stjørdal	13.1	4.0	14.4	3.5	14.1	2.5	11.3	1.4	6.3	2.6	2.7	0.8

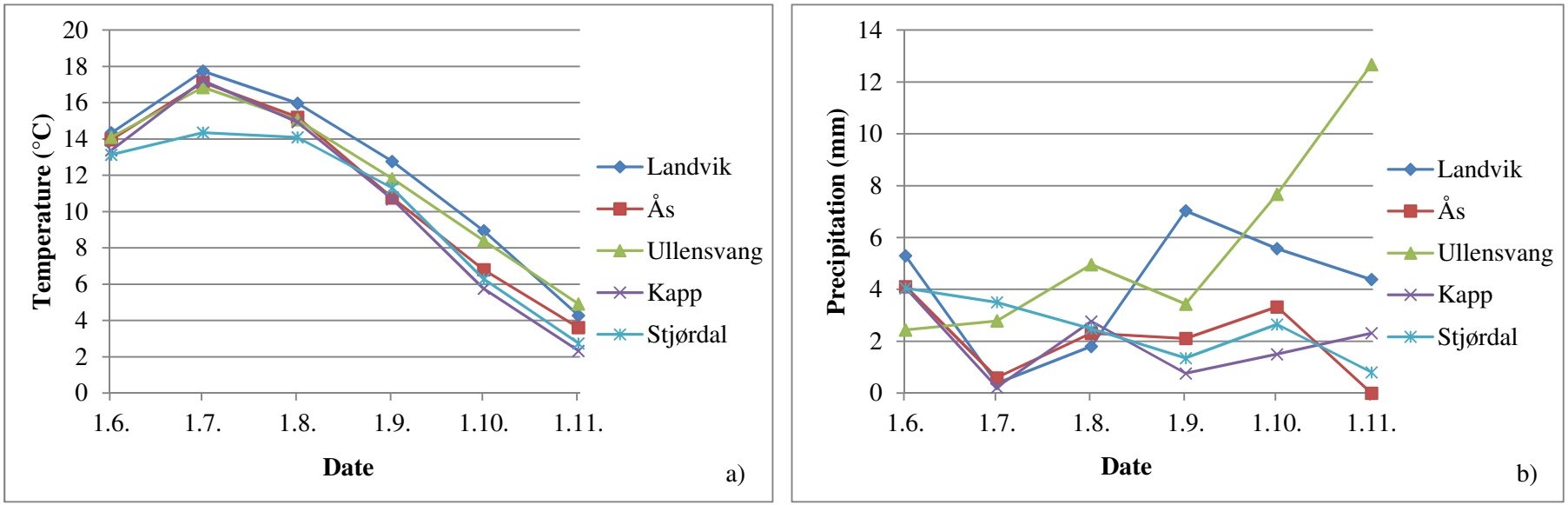

***Figure 24**. a) Monthly mean T and b) daily mean prec. at five locations in Norway during the period June 1 –November 1, 2013.*

Table 9. Growing degree days with base temperature of 15°C ($GDD_{T=15°C}$) during the summer months in 2013 at five locations in Norway.

Month	Landvik	Ås	Ullensvang	Kapp	Stjørdal
June	9.3	12.8	10.5	8.1	12.0
July	52.8	65.9	47.8	94.3	41.3
August	13.7	36.2	26.0	37.8	36.4
September	12.6	13.6	3.1	12.8	20.4

Table 10. Coefficients of determination (R^2) for the simple linear regression analysis between percentage of generative buds in 2013 (data from dissections) and 2014 (assessment of flowering), total number of flowers and $GDD_{T=15°C}$ (growing degree days) during summer 2013. 0 = no relationship between the variables, 1= 100% of the variability of percentage generative buds is explained by variability in accumulated GDD.

	'Aroma'			**'Gravenstein'**		
Period	%gen. buds 2013	%gen. buds 2014	Tot. no. flowers	%gen. buds 2013	%gen. buds 2014	Tot. no. flowers
June	0.12	0.50	0.13	0.12	0.01	0.03
July	0.86	0.77	0.67	0.84	0.73	0.39
August	0.13	0.02	0.01	0.10	0.24	0.15
September	0.13	0.03	0.06	0.03	0.02	0.07
June-July	0.86	0.73	0.68	0.86	0.78	0.46
July-August	0.75	0.45	0.43	0.70	0.73	0.41
August-Sept.	0.01	0.33	0.00	0.09	0.08	0.06
June-August	0.11	0.10	0.12	0.12	0.43	0.66
July-Sept.	0.55	0.39	0.37	0.69	0.60	0.35

In general, taking into consideration all the results presented above, accumulated temperatures of 15°C in July accounted for a large part of the variability found in the amount of flower buds formed. Moreover, precipitation in July, which for most locations was the lowest during the summer in 2013, showed a strong relationship with the amount of flower buds formed in 2013.

4. Discussion

The results presented in this thesis partially illustrate a year in the growth cycle of apple trees, from bud-break in early spring, shoot extension and formation of flower buds throughout the summer, to the onset of growth cessation and dormancy in the autumn, and bud-break the following spring. Special attention was paid to the process of flower bud formation (FBF), and its relationship to vegetative growth, anthesis and climate, i.e. temperature and precipitation, under outdoor conditions.

Growth and development of apple cultivars in the experimental orchard at Ås

4.1. Shoot growth

The average progress of shoot growth followed a normal sigmoid growth pattern (Fig. 8a), as also reported by Abbott (1984) for 'Cox orange Pippin' in field works in the southwest of England. Shoot growth started right after full bloom and extended on average for 66.8 days after full bloom (DAFB), at a rate of 5.8 cm/week (Table 3). Since shoot growth was first recorded after full bloom, Fig. 8a shows only the linear part of the growth curve, the active growth period, and a plateau that indicates the date of growth cessation.

Considerable differences were observed between cultivars in the duration, rate and extent of shoot growth, and based on these differences the cultivars were grouped into fast, middle and slow growing (Table 3 and Fig. 11). Lauri et al. (1995) also reported differences in vegetative growth patterns between 10 apple cultivars in France, and partially ascribed such differences to the ploidy level of the scions. Lespinasse and Noiton (1986) reported that shoot growth, in terms of internode length, leaf area and stomatal density was enhanced in clones of 'Red Delicious' with high ploidy levels. Taking this into consideration, larger growth rates and total shoot length would be expected in triploid cultivars compared to diploid ones. In the present study, triploid cultivars (i.e. 'Gravenstein' and 'Mutsu') were classified as middle growing based on growth rate, total shoot length and duration of the growth period. Conversely, some of the fastest growing cultivars were diploid (e.g. 'Sävstaholm', 'Aroma' and 'Lobo').

Webster (2005b) pointed out that, in addition to the ploidy level of the cultivar, other internal factors such as the nature of the rootstock may regulate the response of the whole tree to environmental conditions, and thus, affect shoot growth. For instance, dwarfing rootstocks (e.g. M9 and B9) induce earlier termination of shoot growth and promote compact and slow

growth (Webster 2005b; Jackson 2003). In the current study, the trees of 'Discovery' and 'Åkerø' selected for measurements, had the shortest growth period, the lowest growth rate and the shortest shoot length of all cultivars (Table 3 and Fig. 8b,c). Compared to other trees of the same cultivars, these selected trees had considerably weaker growth. The 'Discovery' trees recorded were grafted on the dwarfing rootstock M9, and this may have caused the weak growth observed. Since 'Discovery' is a slow growing cultivar, grafting on dwarfing rootstocks has been reported to be detrimental for the vegetative vigor of the tree. For 'Åkerø', the rootstock information was unavailable, thus it is difficult to determine the cause of the weak growth observed. However, trees of the same cultivar grafted on different rootstocks had considerably higher number of leaves and longer shoots than the recorded trees (Fig. 25, parallel experiment not part of this study). For instance, average shoot length and number of leaves in these trees was 52 cm and 27 leaves, respectively. These values are considerably higher than for the 'Åkerø' trees recorded (Table 3). It can also be speculated that the health of the recorded trees was compromised and therefore, its overall vegetative performance was weak.

In addition to internal factors, shoot growth is affected by external factors such as temperature, soil conditions and tree management (Webster 2005b). Temperature has a large influence on shoot growth, however, its effect is complex since it also affects other processes in the tree (e.g. photosynthesis, transpiration, source/sink relations, dormancy, flower bud initiation and fruit development), and the effect may vary in different stages of the growing cycle of the tree (Calderón et al. 2002; Jackson 2003; Webster 2005b). Observations by Abbott (1984), Barlow (1975) and Johnson & Lakso (1985) in apple orchards, suggested that shoot growth is a function of temperature. This is supported by Abbott (1984), Calderón et al. (2002), Tromp (1976), Tromp and Boertjes (1996) and Verheij (1996). These authors reported that under controlled conditions, temperatures in the range of 10-26°C were positively correlated with shoot growth. Furthermore, Johnson & Lakso (1985) reported that under optimal temperatures, shoot growth is linearly related to accumulated GDD. In the present study, similar results were found, and accumulated GDD over 10°C were strongly and positively correlated with extension shoot growth (Fig. 9b). The physiological basis of this response is that cell division and growth are temperature dependent processes (Hänninen & Tanino 2011).

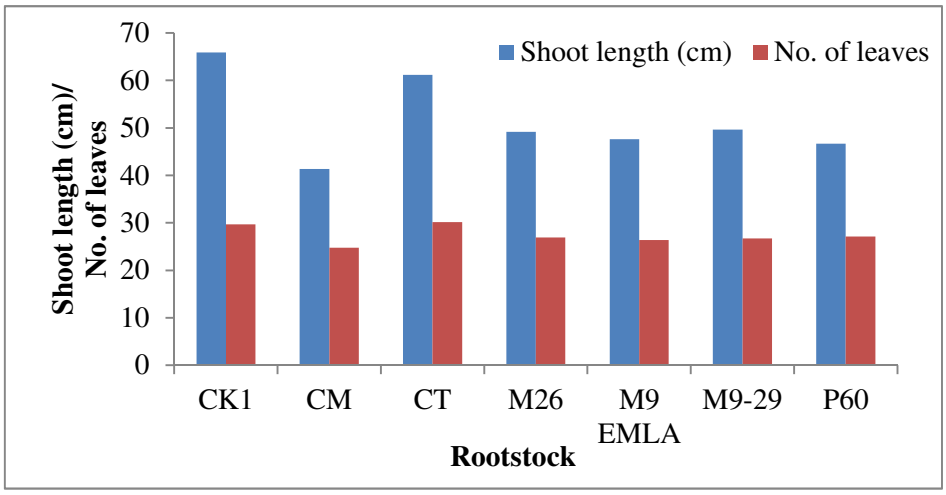

Figure 25. *Growth in 'Åkerø' trees grafted on seven different rootstocks in Åsbakken. Data was recorded in 2013.*

4.2. Stages of development of the shoot apical meristem (SAM)

Floral morphogenesis in apple trees (i.e. FBF) is a continuous process that takes place over an extended period of time during the growing season. In the present study, the progress of FBF was followed by identifying morphological changes in the SAM using the scale proposed by Foster et al. (2003). In this scale, the authors considered the broadening of the SAM as the first sign of floral development. Dadpour et al. (2011) disagreed with the use of the broadening of the apex as the only sign of floral development. Instead, these authors proposed the broadening of the SAM and the formation of a furrow as the first signs of floral development. Buban and Faust (1982), Fulford (1965a), Hanke et al. (2007), Hoover et al. (2004), Huang et al. (1986), Jackson & Sweet (1972), Luckwill (1974) and Westwood (1988) considered all, the doming of the SAM and the formation of bract primordia (stage 2) as the first signs of floral development. In the present study, the first sign of floral development was considered to be stage 2 and not the broadening of the SAM. This due to the fact that stage 2 is easily recognized by removing bud scales, leaf and bract primordia under the stereomicroscope, in contrast to meristem diameter, in which laborious measurements must be done in order to determine the meristem identity.

Seven of the eight morphological stages proposed by Foster et al. (2003) were identified in the current study (Table 2, Fig. 12). Stage 2 was identified based on the rounded, protuberant appearance of the SAM, and subsequent stages of development (3-7) were identified based on the order of appearance of floral meristems (Fig. 12c-e). The last identified stage of development was the differentiation of sepals on all lateral flowers (stage 7, Fig. 12g,h).

4.3. The onset of FBF and growth cessation

The onset of FBF, indicated by the date of the first observed stage 2, has been reported to vary between shoot type (i.e. spurs and extension shoots) and cultivars (Forshey & Elfving 1989; Hirst & Ferree 1995; Hoover et al. 2004; Jackson 2003; McArtney et al. 2001; Tromp 2005b). In the current study, FBF was first observed in spurs (by mid-July, equivalent to 61.8 DAFB), and about 20 days later in extension shoots (by mid-August, equivalent to 80 DAFB) (Fig. 14a). This delay in the onset of FBF in extension shoots has been reported to be a consequence of the apical dominance exerted by actively growing shoot apices, which prevents the outgrowth of lateral buds (Tromp 2005b). The basis of this dominance are hormonal, especially the direct inhibitory effect of auxins and modification by gibberellins from growing shoot tips and seeds on FBF (Bangerth 2005a).

The onset of FBF in apple trees may take place before growth cessation, but also after (Hanke et al. 2007). Furthermore, it has been reported to vary depending on shoot type (e.g. spurs and extension shoots), bud position and age of the tree (Tromp 2005a; Hirst & Ferree 1995; Jackson 2003; Verheij 1996; Zhu et al. 1997). Zhu et al. (1997) found that the onset of FBF in extension shoots took place six weeks before growth cessation, in lateral buds of young trees under controlled conditions. Hirst and Ferree (1995) found that the onset of FBF occurred after growth cessation in spurs of trees that were at least 10 years old. Verheij (1996) showed that, under controlled conditions, there was a good correlation between growth cessation and FBF in spurs of young trees. From these results, it is clear that growth cessation may not be an absolute prerequisite for FBF, at least in spurs. Nevertheless, the fact that growing shoot tips are active sites of GA production, and that these hormones inhibit FBF, reinforces the idea of a positive effect of growth cessation on FBF in extension shoots (Faust 1989; Hanke et al. 2007; Tromp 2005b). In the current study, the first signs of FBF were observed before or after growth cessation in spurs and only after growth cessation in extension shoots (Table 3 and 4). This is in accordance with the idea that the relationship between growth cessation and FBF is indefinite. Instead, it may be circumstantial depending on the shoot type and vigor of the tree (Verheij 1996). For instance, in slow growing cultivars, FBF started very close to or after growth cessation in both spurs and extension shoots, while in vigorous cultivars, FBF occurred invariably before growth cessation in spurs and after growth cessation in extension shoots. It seems that the onset of FBF in spurs may be under internal control, different than the apical dominance of extension shoots.

In the present study, growth cessation in spurs was not evaluated and therefore, it is difficult to determine with certainty whether this was related to FBF. Nevertheless, Fullford (1966a; 1966b) and Abbott (1977) confirmed that growth cessation is related to FBF in spurs. Fullford (1966a; 1966b) showed that in spurs, the first sign of growth cessation is the formation of bud scales, which takes place before the onset of floral development. Abbott (1977) also reported that FBF in spurs took place after growth had stopped and bud scales had been formed in these shoots.

Regarding the average period for the onset of FBF, the results in Table 4 are consistent with results reported by Buban & Faust (1982), Hoover et al. (2004), Koutinas et al. (2010) and Tromp (1984, 2005) for spurs, and Buban and Faust (1982), Faust (1989), Pogorelov (1970) and Tromp (2005b) for extension shoots. Considerably longer periods have been reported by Abbott (1977) (doming at approximately 84 DAFB in South England), Hirst & Ferree (1995) (85-109 DAFB in Ohio, USA), McArtney et al. (2001) (70-100 DAFB in New Zealand) and Dadpour et al. (2011) (90-120 DAFB in Tabriz, Iran). Shorter periods have been reported by Foster et al. (2003) (broadening at 39-53 DAFB in New Zealand). The variations between different studies may be a consequence of differences in the date of full bloom and the stage of development chosen as the transition to floral development. First, the date of full bloom, used as the point of reference from which days were counted, has been reported to be cultivar and climate dependent (Grauslund 1996; Jackson 2003; Wertheim & Schmidt 2005). Therefore, different results are expected when a large range of cultivars are studied at geographical locations that differ greatly in climate. Second, the stage of development chosen as the transition to floral development varies between studies, hence the estimation of the onset of FBF is expected to vary. For instance, Foster et al. (2003) found a period of 39-53 DAFB for the onset of FBF when considering the broadening of the apex as the first sign of FBF. Since the broadening of the apex takes places before its doming (Dadpour et al. 2011), the onset of FBF is shorter than when considering doming of the apex as the first sign of FBF.

Hanke (1981) showed no correlation between the stages of differentiation of the shoot apex and the date of full bloom, and therefore, disagreed on the use of DAFB as the unit to describe the onset of FBF. This author reported that the onset of FBF in Germany took place in mid-July (similar dates has also been reported by Hirst & Ferree (1995) in USA and in the current study), and stated that the floral differentiation of the shoot apex seems to be more stable according to calendar date.

4.4. Duration and progress of floral differentiation

In general, the duration of the FBF process (i.e. period between first observed stages 2-7) was approximately 30 days for both shoot types (Table 4). Similar results have been reported by Buban & Faust (1982) (at least 30 days in Hungary and Chile) and McArtney et al. (2001) (21-50 days in New Zealand). Hirst & Ferree (1995) and Luckwill & Silva (1979) reported periods of 20 days in USA and 7 days in England.

The progress of FBF, in terms of the stage of development and the proportion of generative meristems (stage ≥ 2), increased with time in spurs (Fig. 14b and 13a), and either increased or decreased in extension shoots without a clear pattern (Fig. 13b and 14c). Once stage 2 was reached, the development of flower buds into subsequent stages was rapid and by the last sampling date, a large proportion of buds were in stage 7. There were significant differences in the stages of development reached in spurs and extension shoots on the last sampling date. On this date, spurs were either in stage 1 or 7, indicating that stages 2-6 may be temporary and that all domed meristems reached stage 7 by the end of the growing season. Similar results has been reported by Foster et al. (2003), Hoover et al. (2004) and Faust (1989). In contrast, a variety of stages were found on the last sampling date in extension shoots, possibly indicating that not all buds that started floral differentiation reached stage 7 by the end of the growing season (Fig. 14b). These results are in accordance with findings by Costes (2003), Faust (1989), Jackson (2003), Tromp (2005) and Zeller (1960), and may indicate that differentiation of buds from stages 2-7 in extension shoots takes longer time, and may extend throughout the autumn, winter and the following spring, as long as temperatures are high enough to allow organogenic activity in the meristem. In the present study, temperatures after mid-October were below 0°C, therefore, it may be speculated that the differentiation of flower buds in extension shoots resumed the following spring, when T exceeded 0°C.

Floral differentiation in extension shoots started in buds from the middle of the shoot and progressed to peripheral buds towards both ends, i.e. a centrifugal gradient of floral differentiation. A similar gradient has been reported by Buban and Faust (1982), Costes (2003), Hanke et al. (2007) and Tromp (2005). Furthermore, Costes (2003) reported that terminal buds were in a more advanced stage of development than lateral buds. Conversely, in the current study, buds from the middle of the shoots were more advanced stages of development than terminal buds (data not shown).

4.5. Proportion of generative buds and cultivar differences

Considerable differences were found in the proportion of flower buds between shoot types (Table 4 and Fig. 13a,b and 14b,c). For both spurs and extension shoots, the proportion of generative buds varied between sampling dates and cultivars, and in order to perform statistical analyzes, the data had to be corrected by taking into account the generative buds (stages ≥ 2) only. Even after correction of the dataset, the proportion of generative buds and the stages of development were not completely stable. For instance, in extension shoots (Fig. 13b and 14c), sudden decreases in the percentage of floral buds and the stage of development were observed on successive dates without a clear pattern. Such results reflect the lack of synchrony in the FBF process, which results in a heterogeneous population of buds in different stages of development (Buban & Faust 1982; Jackson 2003; McArtney et al. 2001).

The heterogeneity within a tree is brought about by the fact that whether or not a bud becomes floral, depends on a series of factors. The most important factors are the age of the bud (younger spurs and extension shoots tend to differentiate flower buds earlier than older ones), its maturation (since a critical number of appendages must be formed before FBF occurs), the position within the tree (shaded sites of the tree are usually poor in terms of FBF, basal buds tend to stay vegetative) and the presence of fruit on adjacent branches (whose seeds inhibit FBF) (Jackson 2003; Webster 2005b). In the current study, buds were collected randomly, and caution was taken to ensure that the sampling site was on well exposed, south facing branches of the tree. This may have helped minimize the effect of shading. However, since no other considerations such as shape and diameter of the buds (which may have given a hint on the identity of the bud), and the presence of fruits on adjacent branches were taken, it can be speculated that these factors could explain some of the variation observed.

Part of the variation may also be a consequence of the sampling method used. In the present study, the sampling method was destructive, meaning that the fate of individual buds was impossible to follow throughout the growing season. On every sampling date, new buds in potentially different stages of development were collected, hence contributing to the overall variation.

Large cultivar differences were also found, and based on the cluster analysis performed the cultivars were categorized into three groups (Fig. 15). The groups obtained were different depending on the shoot type, and this makes the classification of cultivars by means of generative data from different shoot types difficult. Nevertheless, the general

differences obtained may be explained by intrinsic properties of the trees, such as the nature of the rootstock, the vigor of the scion and the biennial bearing tendency of each cultivar.

Shoot growth, especially growth vigor, is widely described in the literature and by fruit growers as antagonistic to FBF (Faust 1989; Jackson 2003; Tromp 2005b). Growth vigor of the scion is usually controlled by the rootstock, for instance, scions grafted on weak rootstocks have shorter shoots, tend to stop growth earlier, and for certain rootstock-scion combinations an increase in the proportion of floral buds has been reported (Luckwill 1970; Swarbrick 1929 cited by Hirst & Ferree 1995; Webster 2005b). Most of the cultivars in the current study were grafted on M9 rootstock (see section 3.1. Plant material), and since the performance of the same cultivars on different rootstocks was not compared, it is difficult to conclude whether the observed differences can be ascribed to rootstock effect only. Nevertheless, for 'Discovery', this effect may have been more pronounced since sampled trees were grafted on M9 rootstock. As pointed out previously, 'Discovery' is a cultivar with weak vigor and therefore, grafting on dwarfing rootstocks, such as M9, should be avoided. Weaker vigor was observed in these trees, in terms of considerably earlier cessation of growth and shorter extension shoots (length $\leq$ 16 cm), compared to other trees of the same cultivar in the orchard (Fig. 25). This may have been the cause of the early onset of FBF and the higher proportion of flower buds on extension shoots for this cultivar.

The nature of the scion has also been reported to account for differences in the proportion of flower buds formed. This is related to the tendency of some cultivars to differentiate more floral buds in either spurs or extension shoots (Buban & Faust 1982; Tromp 2005b; Webster 2005b), and the tendency to biennial bearing (Jackson 2003). In the present study, a higher proportion of floral buds were found in 'Summerred' and 'Elstar' (Fig. 13b). For 'Summerred', Zhu et al. (1997) reported a higher proportion of floral buds on extension shoots under controlled conditions. Also, qualitative observations by fruit growers in Norway confirmed that one-year-old 'Summerred' trees grafted on M9 rootstock exhibit enhanced flowering capacity compared to older trees grafted on different rootstocks (Myren, G., pers. comm.). These observations may be a result of the effect of the rootstock-scion combination together with the age of the tree. For 'Elstar', Jackson (2003) and Tromp (2005) reported enhanced FBF in extension shoots as an intrinsic feature of the cultivar.

Regarding the tendency to biennial bearing, Jackson (2003) pointed out that the amount of flower buds formed also depends on the tendency of certain cultivars to produce a

large crop one year, and a light or absent crop the following year (due to the inhibitory effect of hormones, namely GAs from the seeds of the large cropping year). In the present study, FBF data was collected for only one year, and the cropping and management history of the cultivars was neither recorded nor controlled. Therefore, it is difficult to determine whether the differences in the proportion of floral buds between cultivars were caused by their biennial bearing tendency.

4.6. FBF and climate

The proportion of floral buds formed in different cultivars has also been ascribed to differences in the local climate, especially temperature and precipitation (Buban & Faust 1982; Jackson & Sweet 1972; Tromp 2005b). In the current study, the variation observed in the proportion of buds formed was not directly correlated to either variations in temperature or to precipitation during the growing season (data not shown).

Decreasing temperatures from mid-August coincided with the termination of shoot growth (Fig. 8a and 10) and the commencement of FBF in extension shoots, probably due to the release of the apical dominance. Low temperatures have been reported to control growth cessation in apple trees (Heide & Presterud 2005), and since growth cessation was positively correlated with FBF in extension shoots, it is possible that the onset of FBF was affected by decreasing temperatures during mid-August. The average daily temperatures at which growth cessation and differentiation of floral buds started in spurs and extension shoots was in the range of 15-20°C (Fig. 10 and 14a). This range is in agreement with the optimal temperatures of 13-20°C reported by Tromp (1984), Verheij (1996) and Zhu et al. (1997) for stimulation of FBF under controlled conditions.

Possibly, there may be two different effects of temperature on FBF in extension shoots; one indirect and one direct effect. Firstly, high temperatures may have an indirect effect on FBF through stimulation of shoot growth, and secondly, after having reached a state of maturity, low temperatures may promote FBF through induction of growth cessation (Heide, O.M., pers. comm.).

4.7. Anthesis

Based on the flowering data collected (Fig. 16), the apple cultivars studied were classified into early flowering: 'Gravenstein', 'Franskar', 'Summerred' and 'Sävstaholm'; mid-season flowering: 'Discovery', 'Julyred', 'Quinte' and 'Vista Bella' and late flowering: 'Aroma', 'Elstar', 'Lobo', 'Mutsu' and 'Prins'. These results are in agreement with reports by Stedje and Skard (1939) and Bø et al. (1998) for 'Discovery', 'Franskar', 'Gravenstein', 'Julyred', 'Lobo', 'Prins', 'Summerred', 'Sävstaholm' and 'Vista Bella' in Norway. Grauslund (1996) proposed a similar classification for 'Summerred', 'Gravenstein', 'Discovery', 'Aroma', 'Mutsu' and 'Elstar' based on flowering dates over a 10 years period in Denmark. A similar classification has also been proposed by Borrie and Chaussee (2015) for most of the cultivars studied, based on information from fruit growers around the world.

In general, there was a similar pattern of bud-break between both years in terms of order of flowering (i.e. early cultivars were early in both years and vice versa). This seasonal pattern in apple and other temperate fruit species is considered to be hereditable and related to climate (Jackson 2003). Two aspects of climate are reported in the literature as determinant for the seasonal pattern of bud-break; heat accumulation during spring and chilling temperatures during the preceding autumn-winter period (Jackson 2003; Tromp 2005a).

Accumulated heat above 4°C, as growing degree days (GDD, Fig. 17b), was positively correlated with the occurrence of the different phenological stages of bud-break. As discussed previously, this correlation is in accordance with the fact that the processes involved in bud development, i.e. cell division and growth, are temperature dependent (Hänninen & Tanino 2011). For instance, the first stage of bud-break (green tip, GT) occurred earlier and was extended for a longer period in 2014 compared to 2013 (Fig. 16). Such differences may be explained by differences in mean air temperature between years. The fact that temperatures in 2014 were considerably higher during April (Fig. 17a), resulted in higher accumulated GDD that led to an earlier occurrence of GT. From GT onwards, temperatures were variable and considerably lower in 2014 resulting in lower GDD (17a,b), and possibly accounting for the extended period between GT and subsequent stages (Fig. 16). As pointed out by Jackson (2003) and Landsberg (1979), the rate of growth after bud-break is determined by prevailing temperatures, so that lower temperatures slow down the rate of development, while higher temperatures lead to a more rapid development.

Regarding chilling temperatures, a rough calculation of chilling days from September 1 to December 1 (data not shown), showed that there was more accumulated chilling in 2013. Moreover, the amount of GDD at which the different stages of bud-break were reached was on average higher in 2013 compared to 2014. From these results it is evident that chilling and accumulated GDD were negatively correlated. Cannell (1989) also reported a negative correlation between the number of GDD needed to reach full bloom and days with temperatures below 3°C from October 1-December 1 in south England. Such negative correlation may be ascribed to the effect of chilling temperatures in releasing buds from dormancy, by increasing their potential of growth development after a certain threshold (characteristic of each cultivar) has been reached (Jackson 2003; Landsberg 1979; Tromp 2005a).

The occurrence of the different stages of bud-break also differed between cultivars. Such differences may be ascribed to variations in the particular chilling and heat requirements of each cultivar (Hänninen & Tanino 2011; Jackson 2003), and possibly to temperatures during the period of FBF (Chuine & Cour 1999). Particular chilling and heat requirements were not evaluated in the current study, and flowering data was collected in two years only. Nevertheless, the results obtained may suggest that early and late flowering cultivars have low and high chilling requirements, respectively. This suggestion is in agreement with the model proposed by Powell (1985), in which the date of bloom of a cultivar is determined by its chilling and subsequent heat requirements. For instance, cultivars with high chilling requirements bloom later than cultivars with lower chilling requirements, because more time is needed to meet the high chilling requirements even under cold climates (i.e. temperatures under freezing point contribute very little to physiological chilling (Powell 1985; Tromp 2005a)).

Regarding the effect of temperature during FBF on anthesis the following year, the supporting literature is limited and inconclusive (Chuine & Cour 1999; Tromp 2005b). Hanke (1981) and Hoover et al. (2004) found no correlation between the onset of FBF and the date of full bloom. Moreover, Chuine & Cour (1999) reported that 82-100% of the variation observed in flowering dates was explained by heat and chilling requirements and not by temperatures under which floral buds were formed. Tromp (2005b) has also stated that the relationship between the time of flowering and FBF is weak based on qualitative observations. In the present study, the results obtained show that some cultivars started FBF in spurs first and opened flowers first the following spring (e.g. ‘Franskar’ and ‘Sävstaholm’),

while others started FBF in spurs last and opened flowers last (e.g. 'Elstar') (Table 4, Fig. 16). Similar results have been reported by Abbott (1984) in South England, and they may suggest an indirect relationship between temperature under FBF and anthesis, mediated by the cessation of shoot growth, the completion of pre-dormancy development and the subsequent development of endo-dormancy (Landsberg 1979; Tromp 2005a).

When comparing the proportion of flower buds observed in 2013 and 2014, the results were inconclusive (Fig. 19). The slightly higher percentage of generative buds in 2014 for 'Elstar' and 'Summerred' may be related to natural variation, and the enhanced capacity of these cultivars to differentiate flowers in extension shoots. The considerably higher proportion of generative buds obtained in 'Discovery' may be related to the large amount of shorter extension shoots and earlier growth cessation. In addition, it may be speculated that for these three cultivars, FBF may have taken place early in the spring, thus accounting for the higher percentage of generative buds in 2014. Variation in the material due to the relative low number of extension shoots sampled (n = 3 in 2013 and n= 10 in 2014) may also explain the differences between years.

4.8. Effect of geographical location on growth and development of 'Aroma' and 'Gravenstein'

The results indicate that local climate at the five locations studied had a considerable effect on the development of vegetative buds and the proportion of flower buds and flowers formed. More specifically, variations in accumulated temperatures and average mean daily precipitation during July accounted for more than 70% of the variability observed (Tables 9 and 10). In fact, results from Åsbakken showed that the onset of FBF occurred on average at the end of July, beginning of August, so it is not surprising that accumulated temperatures and precipitation during this period had an effect on both generative parameters.

The decreasing trend in the stage of development of flower buds from the southernmost (Landvik) to the northernmost location (Stjørdal) observed, could be explained by means of differences in temperature during the summer months (Table 10 and Fig. 24a). Trees of both cultivars from Stjørdal formed the least amount of flower buds, and such buds were in less developed stages compared to trees from the other locations. These results may indicate that temperature during the period July-October were too low for the initiation and differentiation of more flower buds at this location (Fig. 24a). Moreover, dissections

performed at the end of the growing season in 2013 revealed that flower buds on trees from Stjørdal were less developed compared to the other locations (Table 7), and were the last ones to open in the following spring (qualitative observation). The negative effect of low temperatures on FBF has also been reported by Verheij (1996) for 'Cox's Orange Pippin', and Zhu et al. (1997) for 'Summerred' under controlled conditions. These authors found that temperatures of 13°C had a negative effect on both the proportion of buds formed and the rate of development.

For the remaining locations (Landvik, Ås, Ullensvang and Kapp), considerable differences were found in the proportion of flower buds and flowers formed, despite the very similar average temperatures (Table 8, Fig. 24a). In general, the highest proportion was found in trees of both cultivars from Kapp and Ås, and these locations had the highest amount of accumulated GDD during July (Table 9). Since more than 70% of the variation in the proportion of flower buds and flowers formed could be explained by accumulated temperatures of 15°C during July (Table 10), it can be hypothesized that these locations had optimal conditions for FBF. It has been reported that under controlled conditions the optimal temperature for FBF is around 16°C (Tromp 1976; Tromp 1984; Verheij 1996), and this is consistent with the positive correlation between accumulated $GDD_{T=15°C}$ and FBF found in the current study. However, since average temperatures in the different locations were close to the optimal reported in the literature, part of the variation observed may also be attributed to the combined effect of other external factors, such as precipitation and gardener practices (differential treatment of potted trees regarding irrigation, fertilization and placement).

In the current study, low precipitation during June-July could explain more than 75% of the variation in the proportion of flower buds formed (data not shown). In general, moderate water stress has been associated with early cessation of shoot growth and increased FBF (Salter & Goode 1967; Tromp 1984; Tromp 2005b). As for temperature, experiments dealing with the effect of water stress in FBF are inconclusive and circumstantial (i.e. depending on cultivar, age and nutrient status of the tree, severity of the stress and time of the year) (Jackson 2003). The most common reported effect of water stress on FBF is through altered vegetative growth (Jones 1987; Salter & Goode 1967; Tromp 1984; Verheij 1996), and its physiological basis has been associated to changes in the hormonal balance of the tree (e.g. reduced gibberellin content due to earlier growth cessation (Tromp 2005a) and higher content of drought induced cytokinins (Bangerth 2005a)).

Since the results reported in the literature are inconclusive, and the current study was carried out under outdoor conditions with just one year data, any assumption on the specific effect of both temperature and precipitation on FBF is merely speculative. More research under controlled conditions is needed to give more conclusive results regarding the specific effect of these climatic parameters on FBF and development.

Regarding gardener practices, because apple trees were placed at five different research stations in Norway, and this experiment was in parallel to registrations in Åsbakken, it was not possible to control that the initial conditions were met in all locations. For instance, trees from Ås were placed on concrete floors and it may be speculated that under sunny summer days, temperatures may have been higher on this floor compared to soil. This may have accounted for the enhanced FBF in trees from Ås compared to Landvik, where average temperatures were the highest of all locations (Table 9 and Fig. 24a). Enhanced FBF at increasing temperatures in the range of 13-20°C has been reported previously by Verheij (1996) and Zhu et al. (1997), and the effect was ascribed to direct stimulation of organogenic activity in the meristems.

Regarding anthesis in 2014, trees overwintered at Ås were irregular compared to trees from Ullensvang. The period of forcing was rather long (between three and four weeks), leaf growth was poor and a high proportion of buds aborted, thus making the registration of bud-break impossible. Such symptoms are consistent with delayed released of dormancy by insufficient winter chilling (Heide & Prestrud 2005; Jackson 2003; Powell 1985). Insufficient chilling was possibly caused by differences in the overwintering conditions at Ås and Ullensvang, for instance, instead of cold storage at constant temperature and relative humidity, trees from Ullensvang were overwintered in a barn where the conditions were similar to those outdoors. It can therefore be hypothesized that constant temperatures just above freezing were not satisfactory to meet the chilling requirements, and this resulted in the abnormal growth observed in trees overwintered at Ås. This hypothesis is supported by observations by Erez et al. (1979) under both outdoor and controlled conditions. These authors reported that fluctuating outdoor temperatures were more efficient for the fulfillment of chilling requirements compared to constant temperatures. In order to prove the validity of this hypothesis on the cultivars studied, more experiments under both controlled and outdoor conditions are needed.

4.9. FBF and its relationship to other developmental processes in the tree

FBF was also related to other developmental processes in the tree, such as bud-break, leaf abscission and fruit maturation. An overview of the relationships found is presented in Table 11. In this table, cultivars are classified depending on the earliness of the onset of the different processes evaluated.

Regarding bud-break, the classification includes two stages, i.e. green tip (GT, as the time of bud-break) and full bloom (FB). Relationships between FBF and these stages were observed in some cultivars. For instance, the earliness of 'Franskar' in reaching the stages of bud-break corresponded well to the earliness in differentiating flower buds in spurs. Similarly, 'Vista Bella' was a mid-season bloomer and differentiated flowers in mid-season. 'Elstar' was late for both bud-break and differentiation of flowers in spurs. It has been suggested that these relationships may be indirect and mediated by temperature, which affects the completion of pre-dormancy development, the time of growth cessation, the onset of dormancy and the subsequent emergence from dormancy (related to heat and chilling requirements) (Heide & Prestrud 2005; Landsberg 1979; Tromp 2005a). In the remaining cultivars, no clear relationship was found between the two processes. This lack of correspondence between FBF and anthesis has also been reported by Hanke (1981) and Hoover et al. (2004).

Regarding leaf abscission and its relationship to the onset of FBF, some cultivars were classified similarly (e.g. 'Quinte' and 'Julyred' as early, 'Gravenstein' as middle and 'Elstar' as late). However, this correspondence was not clear and it may be that the processes are not directly correlated. Even if highly hereditable, leaf senescence and abscission are mainly controlled by decreasing temperatures in the autumn (Jonkers 1980). In the present study, by the onset of leaf senescence, initiated floral buds were fully developed (stage 7) in spurs, and in various stages of development in extension shoots.

When comparing the onset of FBF in spurs with fruit harvesting and ripening, some of the cultivars studied were classified similarly (e.g. 'Quinte' and 'Julyred' as early, 'Gravenstein' as middle and 'Elstar' and 'Mutsu' as late). Many factors have been reported to exert combined effects on the physiological maturity (associated with the time of harvesting), the ripening of apple fruits (Tromp 2005c) and the onset of FBF (Jackson 2003; Tromp 2005b). Therefore, the similar classification of cultivars based upon these processes may be artificial and may not represent a causal- relationship between them. Nevertheless, it may be speculated that even if there is not a direct relationship, the rate of the different physiological

processes involved may be similar. This could imply that physiological processes leading to FBF, physiological maturity of the fruits and a certain fruit quality associated with ripening, have similar rates under the local climate.

Leaf abscission correlated well with the period of fruit ripening and this may be related to the effect of leaves on the climacteric response of fruits. It has been proposed that leaves exerts a 'retarding' effect on fruit development compared to when leaves are not present (Tromp 2005c). Therefore, it may be that the time at which the state of physiological maturity and subsequent ripening of the fruit are reached, is associated with the presence of leaves. This assumption may be valid if fruits are retained on the tree.

As concluding remarks, the results in this study give basic knowledge on the phenology and development of apple cultivars adapted to the Norwegian climate, which imposes a rather short growing season compared to other fruit growing regions in Europe. This knowledge may be useful for the timing of horticultural practices towards achieving good, stable crops, i.e. fruit thinning to reduce biennial bearing. Based on these results, it seems that the critical time of fruit thinning would be different for current year's crop, and for FBF, which determines next year's crop. For the former, fruit thinning can have a positive effect even late in the season, whereas the effect on FBF is limited to an early period when seed development and GA production takes place. This knowledge may also be useful for future selection of new cultivars and for further research under orchard and controlled environmental conditions (e.g. to determine whether the onset of FBF is variable between years, and to study the specific effect of temperature on FBF and on the onset and emergence from dormancy in different cultivars).

Table 11. Summary table for all parameters evaluated.

Cultivar	Bud-break		FBF		Vegetative growth			Fruit growth	
	Green tip	Full bloom	Spurs	Ext. shoots	Vigor	Growth cessation	Leaf abscission	Harvesting	Ripening
'Aroma'	Late	Late	Middle	Middle	Vigorous	Late	Late	Middle	Late
'Discovery'	Middle	Middle	-	-	-	-	Middle	Middle	Middle
'Elstar'	Late	Late	Late	Middle	Middle	Middle	Late	Late	Late
'Franskar'	Early	Early	Early	Late	Slow	Early	Middle	Early	Middle
'Gravenstein'	Early	Early	Middle	Late	Middle	Middle	Middle	Middle	Middle
'Julyred'	Middle	Late	Early	Ne	Vigorous	Late	Early	Early	Early
'Lobo'	Middle	Late	Early	Ne	Vigorous	Late	Middle	Late	Middle
'Mutsu'	Middle	Late	Late	Ne	Middle	Middle	Middle	Late	Late
'Prins'	Middle	Late	Middle	Ne	Slow	Early	Early	Mid	Early
'Quinte'	Middle	Late	Early	Ne	Middle	Middle	Early	Early	Early
'Summerred'	Middle	Middle	Early	Early	Slow	Early	Middle	Middle	Middle
'Sävstaholm'	Early	Middle	Early	ne	Vigorous	Late	Middle	Middle	Middle
'Vista Bella'	Early	Middle	Middle	ne	Middle	Middle	Early	Early	Early
'Åkerø'	ne	ne	-	-	-	-	Middle	Late	Late

Flower bud formation (FBF) in spurs. *Early: 50-60 days after full bloom (DAFB); middle: 60-70 DAFB; late: 70-80 DAFB.*
FBF in extension shoots*. Early: >70 DAFB; middle: 70-80 DAFB; late: 80-90 DAFB.*
Vigor. *Based on rate of shoot extension. Slow growing: 3-4 cm/week; middle: 4.5 cm/week; vigorous: > 6.5 cm/week.*
Growth cessation*. Early: >60 DAFB; middle: 60-70 DAFB; late: 70-80 DAFB.*
Leaf abscission*. Based on onset of signs of leaf senescence and abscission in 2013: early: by October 1; middle: October 1 –October 14; late: after October 14, 2013.*
Fruit harvesting*. As the period in which fruits have reached a physiological maturity that will allow them to ripe normally after detachment from the tree. Early: August; middle: September; late: October. Based on Asdal (2014) and Stjede & Skard (1939).*
Fruit ripening. *As the period in which fruits have an acceptable quality after harvest. Early: August-September; middle: October-November; late: November to January. Based on Asdal (2014) and Stjede & Skard (1939).*
ne: not evaluated. Cultivars Åkerø and Discovery were not taken into consideration in the vegetative measurements due to their weak vegetative growth.

5. Conclusions

The results of this study have given an insight into the timing of flower bud formation (FBF), and its relationship to other developmental processes in the annual growth cycle of apple trees. FBF was first observed in spurs of actively growing trees between late June and mid-August, at average air temperatures of 15-17°C. By late summer, all spur buds that initiated flowers had differentiated sepals on all flower primordia. FBF in extension shoots occurred after growth cessation, by late summer, at average air temperatures of 11-15°C, and extended throughout the autumn.

Large variations were found among the proportion of buds that became floral between sampling dates, and this reflects a lack of synchrony in the FBF process. A relationship was observed between the earliness of bud-break, growth vigor and cessation and FBF in spurs and extension shoots. The late flowering cv. Elstar was vigorous and stopped growth and started FBF latest. The early and middle flowering cultivars Franskar and Summerred, were slow growing and stopped growth and started FBF in spurs first. Less clear relationships between the three processes were found in the rest of the cultivars. The period of floral development was on average 90 DAFB, implying that the cultivars studied are adapted to a rather short growing period.

The onset of leaf senescence was correlated to the onset of FBF in some cultivars. However, this relationship was not clear and leaf senescence seemed to be more correlated to decreasing temperatures during autumn. The onset of FBF was somehow related to reported harvesting and ripening periods in some of the cultivars studied.

External factors such as accumulated temperatures of 15°C and low precipitation during July accounted for over 70% of the variation in the proportion of flower buds formed. Between the five locations studied, there was a decreasing trend in the developmental stage of flower buds. Flower buds from the northernmost location (Stjørdal) were in less developed stages compared to buds from the southernmost location (Landvik), and such differences correlated well with temperatures during July. Even if temperature was slightly higher at Landvik, the proportion of flower buds formed was higher at Ås and Kapp, and this may indicate that other factors rather than air temperature were also involved (e.g. gardener practices, placement of trees, etc).

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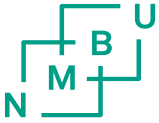

Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no