Time to Rest –
Signals in Shoot Apex Developmental Transitions
Underlying Dormancy

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Academic dissertation

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This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.


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# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
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<tr>
<td>AP</td>
<td>APETALA</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
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<tr>
<td>CENL</td>
<td>CENTRORADIALIS-LIKE</td>
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<td>CO</td>
<td>CONSTANS</td>
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<tr>
<td>CRY</td>
<td>CRYPTOCHROME</td>
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<tr>
<td>CTR</td>
<td>CONSTITUTIVE TRIPLE RESPONSE</td>
</tr>
<tr>
<td>CZ</td>
<td>central zone</td>
</tr>
<tr>
<td>DSC</td>
<td>dormancy sphincter complex</td>
</tr>
<tr>
<td>EIN</td>
<td>ETHYLENE INSENSITIVE</td>
</tr>
<tr>
<td>ETR</td>
<td>ETHYLENE RECEPTOR/ETHYLENE RESISTANT</td>
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<tr>
<td>FR</td>
<td>far-red light</td>
</tr>
<tr>
<td>FT</td>
<td>FLOWERING LOCUS T</td>
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<tr>
<td>IM</td>
<td>inflorescence meristem</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
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<tr>
<td>LD</td>
<td>long day length</td>
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<tr>
<td>LFY</td>
<td>LEAFY</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>PD</td>
<td>plasmodesmata</td>
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<tr>
<td>PHY</td>
<td>PHYTOCHROME</td>
</tr>
<tr>
<td>PZ</td>
<td>peripheral zone</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative real-time reverse-transcription polymerase chain reaction</td>
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<tr>
<td>R</td>
<td>red light</td>
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<tr>
<td>RM</td>
<td>rib meristem</td>
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<td>RZ</td>
<td>rib zone</td>
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<tr>
<td>SAM</td>
<td>shoot apical meristem</td>
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<tr>
<td>SD</td>
<td>short day length</td>
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<tr>
<td>SEL</td>
<td>size exclusion limit</td>
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<tr>
<td>TFL</td>
<td>TERMINAL FLOWER</td>
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SUMMARY

The shoot apical meristem (SAM) displays a high degree of autonomy and robustness, while simultaneously showing sensitivity to signals arriving from the shoot. In particular, photoperiod-induced graft-transmissible signals evoke developmental transitions at the SAM of both annuals and perennials. In trees, the seasonal habit to cycle between growth and rest is a key feature synchronized by photoperiod, which is detected by phytochrome pigments residing in the leaves. The perception of a short day length (SD) at the end of the season triggers considerable physiological and developmental adjustments which are necessary for over-wintering.

We investigated the SD-induced transition to dormancy in hybrid poplar (\textit{Populus tremula} L. x \textit{P. tremuloides} Michx.) and the absence of such transition in transgenic hybrid poplar (\textit{P35S:AsPHYA}), which over-expresses a heterologous \textit{PHYTOCHROME A} (\textit{PHYA}) gene. To dissect if leaf-derived signals dictate the apical responses to SD, graft systems were produced. In SD-exposed heterografts, both \textit{P35S:AsPHYA} and the wild-type scions arrested growth and set buds, but only wild-type assumed dormancy while the \textit{P35S:AsPHYA} scions flushed repeatedly. This indicates that the shoot apex plays an active role in the responses to SD. Physiological and molecular analyses showed that in the wild type, \textit{CENTRORADIALIS-LIKE1} (\textit{CENL1}), a poplar ortholog of \textit{Arabidopsis thaliana} (L.) Heynh. \textit{TERMINAL FLOWER1} (\textit{TFL1}), was markedly down-regulated coincident with the SD-induced growth cessation and the transition to dormancy. By contrast, \textit{P35S:AsPHYA}, which accumulated \textit{PHYA} transcripts in the rib meristem (RM) and subjacent tissues but not in the SAM, up-regulated \textit{CENL1} in the RM area coincident with an acceleration of stem elongation under SD. Collectively, the results suggest that the RM and SAM influence the transition to dormancy in poplar. Accordingly, up-regulation of \textit{CENL1/TFL1} might promote stem elongation in poplar as well as in \textit{Arabidopsis} during bolting, indicating that the RM may be sensitive to photoperiodic signals.

Photoperiod-induced modifications in growth and development may involve plant hormonal signaling. To investigate the involvement of ethylene signaling in trees, birch (\textit{Betula pendula} Roth.) was rendered ethylene insensitive by expressing the \textit{Arabidopsis} ethylene receptor gene \textit{ETR1} carrying a dominant mutation \textit{etr1-1}. Wild-type and transgenic, ethylene-insensitive birch (\textit{BPetr1-1}) were used to study the role of ethylene in SD-induced responses in the SAM. Under SD, \textit{BPetr1-1} ceased growth, but the transition to dormancy was delayed as compared to the wild type. By contrast, the formation of terminal buds was
abolished in *BPetr1-1*. This indicates that ethylene is required for bud set and that it facilitates growth arrest under SD. Furthermore, *BPetr1-1* did not accumulate abscisic acid (ABA) in the apices under SD, suggesting that ethylene promotes ABA accumulation early during the development of dormancy. In addition, the up-regulation of a *BETA-XYLOSIDASE* in the shoot apices, which was typical for the SD-exposed wild type, was abolished in *BPetr1-1*, indicating that ethylene may be involved in the modification of cell walls during growth cessation. Alterations in SAM behavior were further evident from a reduced apical dominance and early flowering in *BPetr1-1*. Taken together, ethylene is involved in bud set and the timely suppression of SAM activity in birch. In summary, we show that SD-induced bud set and dormancy are distinct developmental phenomena which may involve processing of leaf-generated signals in the morphogenetic areas, RM and SAM, of the shoot apex.
1 INTRODUCTION

Plants fine-tune their growth and development in a fluctuating and changing environment. At the end of the growing season, the perception of the ambient cues is translated into genetically based developmental and acclimation responses. The complexity of the underlying sequence of events is high, and appropriate responses require a sensitive perception of ambient cues as well as an adequate relaying of endogenously generated signals through various pathways. As some of the final responses may take place in tissues remote from the site of perception, long-distance transport of signals is a crucial requirement for the adjustment of growth and development, and the coincident acclimation processes.

This thesis concentrates on the involvement of photoperiod- and ethylene-dependent signaling pathways in the regulation of the perennial growth habit in tree ramets. As signaling takes place in the context of a functionally differentiated and continuously expanding plant body, the general pattern of primary growth is first described. Subsequently, the signaling processes that underlie seasonal, photoperiod-induced developmental phenomena are depicted.

1.1 Vegetative growth and development of plants

1.1.1 The shoot apex

1.1.1.1 The shoot apical meristem, the headquarter

Primary growth and development are orchestrated by the shoot apex. The shoot apex contains the shoot apical meristem (SAM) which produces the meristematic tissues that give rise to all the aerial organs, including the elongating stem. The basic cellular organization of the SAM is similar in all angiosperms (Steeves and Sussex 1989). The SAM characteristically contains undifferentiated cells that in some important aspects resemble animal stem cells (Laux 2003; Sablowski 2004). Molecularly, these cells are first apparent at the globular stage of the embryo (Long et al. 1996; Long and Barton 1998), and they play a vital role in maintaining the proliferative potential of the SAM (Fletcher et al. 1999; Lenhard
et al. 2002; Sablowski 2007). As a whole, the SAM has remarkable properties, as an isolated SAM might have the capacity to continue shoot formation in vitro (Smith and Murashige 1970; Ball 1980; Steeves and Sussex 1989). In addition, surgically split SAMs can regenerate complete SAMs when left on the stem (Steeves and Sussex 1989). This indicates that the SAM has an autonomous organization, although it is clearly responsive to signals arriving from other parts of the plant (Rinne and van der Schoot 1998).

The cell divisions of the densely cytoplasmic, meristematic cells occur in well-organized patterns. Commonly, the cells of the superficial cell layers divide in an anticlinal direction, thereby形成ing a sheet of cells termed tunica. In contrast, the underlying corpus cells divide in all directions, thereby producing a 3-dimensional corpus (Steeves and Sussex 1989). Collectively, tunica and corpus constitute the SAM (Figure 1A). In cytohistological terms, the SAM is subdivided into radial zones (Figure 1B), classically visualized by differential staining of the center and the periphery of the meristem. Within the central zone (CZ), the rate of cell division is relatively slow compared to that in the peripheral zone (PZ), while the cellular organelle distribution might also differ between the zones (Steeves and Sussex 1989). Although all cells in the SAM are interconnected by plasmodesmal channels, they are not all in the same state. Rather, the pattern of symplasmic continuity reflects the subdivision of the SAM into cytohistological zones (Rinne and van der Schoot 1998). The symplasmic separation of the CZ from the primordia producing PZ has been proposed to safeguard the undifferentiated state of the centrally located cells (Rinne and van der Schoot 1998; van der Schoot and Rinne 1999), which might be identical to the stem cells. This could play a role in the phenomenon that under permissive conditions, growth and development at the apex are indeterminate and repetitive in nature.

**Figure 1.** Organization of the meristematic regions in the shoot apex.

Tunica-corpus (A) and the cytohistological zonation (B) model of the shoot apical meristem (SAM). t, tunica; c, corpus; CZ, central zone; PZ, peripheral zone; RM, rib meristem. Adapted from Rinne and van der Schoot (2003).
1.1.1.2 The rib meristem, to reach up to the sky

Underlying the SAM (i.e., the apical dome) resides a small group of meristematic cells, that are often considered to be a part of the SAM. In the classic literature, this group of meristematic cells, denoted subapical meristem or rib meristem (RM) (Figure 1), was regarded as a region with a distinct function (Esau 1977). The RM produces the cell files or ribs that give rise to the pith of the stem. It therefore has an important role in permitting and sustaining stem elongation. The terms RM and subapical meristem have been used in an inconsistent way, not always denoting precisely the same areas. Here, the concept that the RM is the restricted meristematic area that produces longitudinal files of elongating and differentiating cells is followed.

The RM is composed of a few layers of small, densely cytoplasmic cells that divide in periclinal direction, i.e., parallel to the longitudinal axis of the plant body (Esau 1977). The RM corresponds to a meristematic layer in gymnosperms, that is situated below the central mother cells, and which may resemble a cambium by appearance. The RM releases cells into a rib zone (RZ) where the cells become more vacuolated and stretched. In the RZ, the cells also divide but less frequently. In this zone, anticlinal divisions regularly increase the number of ribs to accommodate the increase in width of the growing stem. As the RM and the RZ are destined to solely produce the inner parts of the stem, whereas the SAM produces the aerial lateral organs while maintaining the undifferentiated core, the RM/RZ and the SAM are clearly distinct morphogenetic areas.

1.1.1.3 Leaf development, scouts come and go

Lateral organs arise from primordia at the periphery of the apical dome. The primordia emerge from founder cells at a certain distance and angle from each other resulting in characteristic phyllotactic patterns. The phyllotactic pattern may vary between species or even within the same species according to the developmental stage of the plant (Steeves and Sussex 1989; Sachs 1991; Jean 1994). Procambial strands develop shortly after the emergence of the leaf primordia to connect the growing primordia to the vasculature of the stem. During the outgrowth of the leaf primordia, a dorsiventral blade, which is a strong sink of photosynthates produced in the source leaves, is formed. In the enlarging leaf, photosynthesis and the capacity for photosynthesize production proceeds in a basipetal
direction (i.e., from tip to base) while the import capacity gradually ceases. During this transitional phase between sink and source, the leaf exports and imports simultaneously while continuous RM/RZ activity displaces the apical region upward, away from the developing leaf.

As the leaf becomes more expanded, up to about 50% of the final size, it stops phloem import and becomes a true source leaf that provides photosynthates for the growing areas of the plant (Turgeon 1989). The younger source leaves export photosynthates and other signals predominantly towards the shoot apex. As the shoot system expands, newly created source leaves gradually take over the supply function for the apical parts while the lower leaves increasingly supply the lower stem and roots (Turgeon 1989; Oparka et al. 1999).

1.1.2 Communication in the plant body and SAM

1.1.2.1 Phloem and plasmodesmata, highway to the headquarter

Despite its robust organization, the SAM is responsive to signals arriving from the leaves. The majority of the signals arrive via the vasculature in the translocation streams of the phloem which contains water, nutrients, proteins, phytohormones and nucleic acids (Citovsky and Zambryski 2000; Lough and Lucas 2006; Atkins and Smith 2007). The sieve elements of the phloem are interconnected by sieve-plate pores, which are modified plasmodesmata (PD), forming an extensive continuum throughout the plant. The phloem terminates in the vicinity of the shoot apex, and signals destined for the SAM need to exit the phloem ends and traffic via plasmodesmal connections to the SAM (Rinne and van der Schoot 2003).

The symplasm is a continuous system in which two ontogenically different PD take part: primary PD that originate in conjunction with cell division at the cell plate, and secondary PD that are made through existing cell walls (Lucas et al. 1993). The secondary PD are essential to unite the cells of different cell lineages into a single symplasmic network (Rinne and van der Schoot 1998). Both type of PD possess a plasmodesmal channel that contains a strand of Appressed Endoplasmic Reticulum (AER), also called desmotubule, through which the endomembrane system is continuous from cell to cell (Figure 2). Proteinaceous molecules that link the AER to the PD membrane tube subdivide the PD channel into subchannels of an estimated 2-3 nm (Lucas et al. 1993). Small molecules
(below 1 kDa), such as inorganic ions and metabolites, may diffuse through PD. However, PD are not passive holes in the cell walls but they function dynamically, and modification of the plasmodesmal pore may allow passage of macromolecules.

In the phloem, the specialized PD of the pore complexes connect the companion cells with the adjoining sieve elements. The companion cells motorize the loading of the phloem and produce the proteins necessary for maintenance of sieve tube functions. They also produce the signaling molecules that can be relayed to the apex. The PD between the companion cells and sieve elements, and those in the entire path between source and sink, perhaps, might be in a continuous dilated state as they allow the passive diffusional passage of the green fluorescent protein (27 kDa) produced in the companion cells (Oparka et al. 1999; Imlau et al. 1999).

**Figure 2.** Ultra-structure of a plasmodesm.
A sketch of a plasmodesm in longitudinal (A) and transverse (B) sections. 1, middle lamella; 2, cell wall; 3, plasma membrane; 4, proteinaceous particles embedded in the plasma membrane; 5, spoke-like structures which link the Appressed Endoplasmic Reticulum (ER appressed in the pore) to the plasmamembrane cylinder; 6, putative macromolecular transport complex; 7, ER lumen. Adapted from van der Schoot and Rinne (1999).

### 1.2 Changing gears during end-of-season development

#### 1.2.1 Flowering and dormancy: annuals and perennials

During vegetative growth, developmental patterns are robust and the SAM produces internode-leaf–segments continuously. However, at the end of the season this might change, and development takes a new course. Also the youngest emerging leaves may assume a developmental task that is different from photosynthesis. Although this reprogramming of
the SAM has a genetic and endogenous basis, exogenous signals are crucial in triggering and modulating these events. The SAM not only responds to signals supplied from other parts of the plant, but it might also take autonomous decisions affecting its own fate, as e.g. temperature may be detected in the meristem as in the case of chilling-promoted flowering (vernalization) (Bernier 1988). It is also possible that the SAM, despite being hidden beneath developing leaves, can directly detect light (Wareing 1956; Gressel et al. 1980; Knapp et al. 1986).

Perception of environmental cues, such as light and temperature, may result in divergent responses reflecting the different life strategies of plants. Whereas an annual plant ends its life in flowering and seed production, a juvenile perennial plant enters a resting period at a predicted point of the season. In both cases correct timing is crucial. In annuals, flowering must be synchronized with optimal pollination-conditions in such a way that there remains time to set seed. In perennials growing in temperate areas, dormancy development must be initiated in a timely manner to allow buds to develop dormancy and a basic level of hardiness, as only then survival through winter is feasible. The fact that harsh conditions are perceived and required for restoring SAM proliferation in the spring illustrates how well perennials are adapted to the sequential changes of the seasons (Rinne et al. 1997; Rinne et al. 2001; Horvath et al. 2003; Welling and Palva 2006; Rohde and Bhalerao 2007).

Apical transitions are developmental shifts that require changes in gene expression as well as in cellular collaboration. The latter requires signal exchange via a variety of routes that carry signals across cell membranes or through PD. During the transition to dormancy in birch (Betula pubescens Ehrh.), PD connections within the SAM gradually become dysfunctional and eventually blocked (Rinne and van der Schoot 1998; Rinne et al. 2001). This arrests symplasmic signaling and breaks down metabolic coupling within the SAM, resulting in a dissociation of the overall signaling network of the SAM. A similar physical blockade gradually impairs phloem function during dormancy, as the sieve tubes may become blocked by callose (Esau 1977; Aloni et al. 1991). During entirely different apical transitions, cellular coupling within the SAM, and between SAM and stem, may also be modified. For example, during photoperiod-induced flowering of the annual mustard (Sinapis alba L.), the capacity to transmit signals and sugars to the SAM may be dramatically enhanced as the number of PD in the inflorescence meristem (IM) triples (Ormenese et al. 2000). The identity of the signals, however, and the precise mechanisms by which they are relayed toward the apex remain largely unresolved.
1.2.2 What is out there to watch: seasonal light

Over the season, the Earth rotates systematically, making the duration of the daily light and dark period (photoperiod) the most reliable indicator of the time of year. The daily light/dark cycles are also important for entraining the endogenous time-keeping mechanism, the biological clocks (Pittendrigh and Minis 1964; Millar 2004). The significance of photoperiod to developmental transitions was described by Garner and Allard (1920) who found that day length controls the initiation of flowering in several annual species. On the other hand, in trees, the perception of a critical day length in the autumn triggers physiological and developmental adjustments, such as growth cessation, terminal bud formation, development of dormancy and freezing tolerance (Wareing 1956; Howe et al. 1995; Rinne et al. 1998; Horvath et al. 2003; Rohde and Bhale[er]ao 2007). Both in annuals and perennials, the mature leaves relay signals to the apex upon perception of the photoperiod (Thomas and Vince-Prue 1997). Grafting experiments have indicated that the signals are transmitted via the phloem.

1.2.3 Photoperiod-responsive pathways

1.2.3.1 Phytochromes keep track of day and night

Seasonal changes are accompanied by changes in light regime which are monitored by means of light-perceiving pigments. Among the best characterized of the photoreceptors are phytochromes, which predominantly perceive red (R) and far-red (FR) light, and cryptochromes which most effectively absorb blue light (Smith 2000; Jiao et al. 2007). The principle of phytochrome action was described in the 1950’s when germination of lettuce (Lactuca sativa L.) was shown to be promoted and inhibited by R and FR, respectively (Borthwick et al. 1952). Successive light pulses could alter the response, suggesting that the pigment could be reversibly activated and inactivated depending on the light quality. Later, the pigment was isolated and named phytochrome (Butler et al. 1959; Borthwick and Hendricks 1960), and the complex was shown to function as a dimer with a light-absorbing tetrapyrrolle chromophore attached to each apoprotein (reviewed by Rockwell et al. 2006).

In the natural light environment, the R:FR ratio of daylight is typically 1.15, and at twilight the ratio decreases (Smith 1982). When monitoring the photoperiod, especially the length of the diurnal darkness is of importance. Interruption of a long night by a pulse of R abolishes the normal growth response which, however, can be rescued by complementing the
nigh break with a pulse of FR (Howe et al. 1996; Thomas and Vince-Prue 1997). Nonetheless, light quality may also affect the growth responses, as for example a short exposure to FR at the end of the day can accelerate elongation growth (Downs et al. 1957; Olsen et al. 1997). Thus, phytochromes may function in the photoperiodic control of growth by detecting the quality of the light and the length of the night.

The phytochrome genes encode a small family of photoreceptors. In Arabidopsis thaliana (L.) Heynh., which is the most intensively studied annual model plant, five PHYTOCHROME genes (PHYA-E) encoding the apoproteins have been identified (Clack et al. 1994; Mathews and Sharrock 1997). All phytochromes are synthesized in their inactive, R absorbing form. PHYA represents a photolabile type I phytochrome, which accumulates in darkness, whereas PHYB-E fall in the type II phytochromes which are more stable upon illumination (Sharrock and Clack 2002). Phytochromes have differential, albeit partially overlapping, functions in light-regulated events throughout the plant’s life cycle. For example, both PHYA and PHYB are involved in seedling deetiolation, but, while PHYB is activated by continuous R, PHYA is monitoring continuous FR (Quail et al. 1995; Smith 2000). Appropriate growth responses require perception of the light signal and a subsequent signal transduction network, which leads to changes in gene expression (Figure 3). This may involve nuclear import of the phytochrome in a light quality-dependent manner (Sakamoto and Nagatani 1996; Kircher et al. 1999; Kevei et al. 2007).

Figure 3. A simplified scheme of components involved in PHYA-mediated photomorphogenesis.

PHYApfr and PHYApf represent the photointerconvertible R and FR absorbing forms of PHYTOCHROME A, respectively. The light labile, biologically active form PHYApf may be translocated in the nucleus. In the nucleus, PHYApf may interact with transcription factors, such as PIFs, which can bind to different LREs to induce transcription of target genes. Additional signaling intermediates may also effect the signal transduction in the nucleus. PIF, phytochrome interacting factor; LRE, cis-acting light regulatory element; R, red light; FR, far-red light.

Adapted from Nagy et al. (2001).
1.2.3.2 Flowering-associated genes downstream of PHYA

Flowering of annuals and dormancy-cycling of trees may seem disparate processes. However, both involve graft-transmissible leaf-to-apex signaling, alterations in cell-to-cell-communication via PD, and meristem transitioning (e.g. Thomas and Vince-Prue 1997; Rinne and van der Schoot 1998; Ormenese et al. 2000). Moreover, they are regulated by a photoperiodic pathway involving phytochromes and may share some aspects of molecular regulation (recently reviewed by Kobayashi and Weigel 2007; Rohde and Bhalerao 2007).

In the photoperiod-induced flowering of Arabidopsis, PHYA and CRYPTOCHROME2 (CRY2) mediated perception of long day length (LD) accelerates flowering via functioning of the clock-regulated CONSTANS (CO) gene (Puttill et al. 1995; Samach et al. 2000; Suárez-López et al. 2001; Yanovsky and Kay 2002; Takada and Goto 2003; Ayre and Turgeon 2004; An et al. 2004). CO mRNA levels are high at the end of the light period under LD and during the night under short day length (SD) -conditions (Suárez-López et al. 2001; Yanovsky and Kay 2002). As CO protein is labile in darkness, it accumulates under LD only (Valverde et al. 2004). Under LD, CO induces the transcription of the FLOWERING LOCUS T (FT) gene in the companion cells of source leaves (Kardailsky et al. 1999; Kobayashi et al. 1999; Suárez-López et al. 2001; Yanovsky and Kay 2002; Takada and Goto 2003; An et al. 2004). Recently, it was shown that the FT protein can move from source leaves to the apex and induce flowering (Corbesier et al. 2007; Tamaki et al. 2007), suggesting that FT may act as a phloem mobile “florigen”. As FT is a small, globular protein of approximately 20 kDa (Kardailsky et al. 1999; Kobayashi et al. 1999; Ahn et al. 2006), it may freely diffuse through PD connecting companion cells to sieve elements where the size exclusion limit (SEL) can be considerably higher than at most cell-cell interfaces (Imlau et al. 1999; Lough and Lucas 2006).

In addition to FT, TERMINAL FLOWER1 (TFL1) is up-regulated during LD-induced flowering in Arabidopsis (Bradley et al. 1997; Kardailsky et al. 1999). FT and TFL1 are close homologs, and structurally similar to mammalian phosphatidyl-ethanolamine binding-proteins (PEBPs) or Raf-kinase-inhibitor proteins (RKIPs) (Yeung et al. 1999; Hanzawa et al. 2005; Ahn et al. 2006). FT/TFL may be involved in ligand binding and signaling (Yeung et al. 1999; Banfield and Brady 2000; Ahn et al. 2006) and interact with bZIP transcription factors (Pnueli et al. 2001; Wigge et al. 2005; Abe et al. 2005). Despite the structural similarities, their effect on flowering time is opposite: FT promotes and TFL1 delays the
initiation of flowering in *Arabidopsis* (Shannon and Meeks-Wagner 1991; Koornneef et al. 1991; Alvarez et al. 1992; Bradley et al. 1997). Moreover, the spatial gene expression patterns are different: *FT* is primarily expressed in the companion cells, and *TFL1* expression localizes in a patch of cells subjacent to the SAM (Bradley et al. 1997), which corresponds closely to the RM (Esau, 1977). *TFL1* is generally linked to SAM/IM identity and thought to protect the indeterminate growth pattern of the IM (Shannon and Meeks-Wagner 1991; Alvarez et al. 1992; Bradley et al. 1997) by restricting the expression of the determinate floral meristem identity genes *LEAFY (LFY)* and *APETALA1 (AP1)* to the peripheral zone of the shoot meristem (Mandel et al. 1992; Weigel et al. 1992; Bowman et al. 1993; Gustafson-Brown et al. 1994; Ratcliffe et al. 1998; Ratcliffe et al. 1999; Liljegren et al. 1999).

Also in woody plants *FT* may induce flowering, as over-expression of genes homologous to *Arabidopsis FT* remarkably accelerate flowering time in trifoliate orange (*Poncirus trifoliata* L. Raf.) (Endo et al. 2005) and in poplar (*Populus* species) (Böhlenius et al. 2006; Hsu et al. 2006). Interestingly, *FT* appears to play a role also in the seasonal growth habit of trees, as the transcript levels of poplar *FT1FT2* are reduced at the photoperiodic induction of growth cessation (Böhlenius et al. 2006; Hsu et al. 2006). Collectively, this indicates that *FT* is involved in the timing of photoperiodic responses in annuals and perennials.

### 1.3 Plant hormones have a role in growth and development

Although environment affects SAM behavior, it is mediated by hormonal signals arriving from the shoot system. The responsiveness of SAMs to hormone supply is convincingly demonstrated in vitro, where proliferation of isolated SAMs is facilitated by application of auxin and/or cytokinin (Shabde and Murashige 1977; Steeves and Sussex 1989). This suggests that these hormones may not be synthesized within the meristem at sufficient levels, and that import from young leaves may be necessary for proper functioning (Ljung et al. 2001; Reinhardt et al. 2003). Moreover, alterations of hormone concentrations may accompany developmental meristem transitions. For example, in rosette plants, such as *Arabidopsis*, flowering may be promoted by gibberellins (Wilson et al. 1992; Blázquez et al. 1998).

Little is known of hormonal regulation associated with bud dormancy in perennials. Abscisic acid (ABA) was first thought to play a regulatory role in dormancy of woody plants
(Eagles and Wareing 1963; Eagles and Wareing 1964) as it can inhibit growth when applied exogenously. However, the mechanism of ABA action in bud dormancy has remained obscure, and ABA may function in cold acclimation (Welling et al. 1997; Rinne et al. 1998). Nonetheless, studies in other dormant systems, such as seeds of annuals and buds of potato (Solanum tuberosum L.) tubers, are supportive of a role for ABA and ethylene in seeds and buds that develop dormancy. There is also evidence that these hormones may interact in some processes. For example, in seeds of Arabidopsis, high levels of ABA may contribute to deep dormancy whereas ethylene may promote germination by decreasing the sensitivity to ABA (Beaudoin et al. 2000; Ghassemian et al. 2000; Chiwocha et al. 2005). In potato microtubers, which are modified underground stems, application of ethylene and ABA may induce dormancy and ABA may also participate in the maintenance of dormancy (Suttle and Hultstrand 1994; Suttle 1998). However, although ABA clearly retards growth, it remains unclear if and how it is involved in dormancy in woody plants.

1.3.1 Ethylene receptors and signaling

Hormone perception and subsequent transcriptional regulation is necessary for coordinated growth and development, and meristem transitioning also involves hormone perception. This is demonstrated, for example, by the fact that ethylene-insensitive Arabidopsis mutants are late-flowering, suggesting that in wild type ethylene may facilitate the transition to flowering (Bleecker et al. 1988; Guzmán and Ecker 1990).

In case of ethylene, mechanisms involved in perceiving and reacting to this gaseous plant hormone are relatively well characterized by molecular genetic studies. In Arabidopsis, ethylene receptors comprise a small gene family of five members, ETHYLENE RECEPTOR/ETHYLENE RESISTANT (ETR)1-2, ETHYLENE RESPONSE SENSOR (ERS)1-2, and ETHYLENE INSENSITIVE (EIN)4 (Bleecker et al. 1988; Hua et al. 1995; Sakai et al. 1998; Hua et al. 1998). They may share redundant functions in ethylene signaling (Hua and Meyerowitz 1998). Of the ethylene receptors, ETR1 was the first that was characterized in detail (Bleecker et al. 1988; Chang et al. 1993; Schaller and Bleecker 1995). ETR1 exists as a membrane-associated dimer, and functioning of the receptor involves attachment of a copper ion as a cofactor and conformational changes of the protein (Schaller et al. 1995; Rodriguez et al. 1999; Hirayama et al. 1999). Ethylene is believed to bind in the amino-terminal domain of the receptor which has transmembrane structures (Schaller and Bleecker
ETR1 predominantly localizes to the endoplasmic reticulum (ER), suggesting that the ER is the site where ethylene is perceived (Chen et al. 2002).

Several components of the ethylene signaling pathway have been identified and their sequence of action has been determined. In general, ETR1, and other ethylene receptors, function as negative regulators (Hua and Meyerowitz 1998). This implies that in the absence of ethylene, ETR1 is normally in an active state and represses ethylene responses. CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), which is a direct target of ETR1, also acts as a negative regulator of ethylene responses (Kieber et al. 1993). In contrast, in the presence of ethylene, ETR1 and CTR1 are inactivated and the ethylene signal is mediated to the nucleus via positive regulators such as EIN2 (Alonso et al. 1999) and EIN3 (Chao et al. 1997) (Figure 4). In the nucleus, EIN3 induces ethylene-responsive element binding factors (ERFs) (Ohme-Tagaki and Shinshi 1995) which, in turn, regulate the ethylene responsive genes. Analyses of genomic sequences in Arabidopsis and poplar (Populus trichocarpa Torr. & Gray) show that ERFs constitute a large family of transcription factors with approximately 125 and 175 members, respectively (Riechmann et al. 2000; Tuskan et al. 2006), indicating a redundant role for ethylene in modulating gene expression. The particularly large number of ERFs found in poplar may suggest the involvement of ethylene in processes specific to trees (Tuskan et al. 2006).

Figure 4. A simplified scheme of the ethylene signaling pathway in the regulation of gene expression.

In the presence of ethylene, the negative regulators ETR and CTR1 are inactivated. This releases EIN2 and the transcription factors EIN3 and EILs, which accumulate in the nucleus. The induction of the secondary transcription factors, ERFs, allows normal ethylene responses. A MAPK cascade can be involved in the signal transduction between CTR1 and EIN2, and between CTR1 and EIN3. Moreover, the levels of EIN3 are regulated by F-box proteins EBF1 and 2 whose transcription is ethylene-inducible. ETR, ETHYLENE RECEPTOR; CTR1, CONSTITUTIVE TRIPLE RESPONSE1; MAPK, mitogen-activated protein kinase; EIN, ETHYLENE INSENSITIVE; EIL, EIN3-LIKE; EBF, EIN3-BINDING FACTOR; ERF, ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR.

Adapted from Guo and Ecker (2004) and Yoo et al. (2008).
1.3.2 Mutants and transgenics as tools: revealing cross-connections

Valuable information of plant responses and protein functions can be obtained by using mutants and transgenic plants. Ideally, a single factor is changed in comparison to the wild type, allowing the potential identification of the gene function in planta. Such studies have been important when identifying several components of the ethylene signaling pathway. Furthermore, they have indicated potential functional similarities between light and ethylene signaling pathways. The mutant approach has taken advantage of the ethylene-mediated inhibition of hypocotyl elongation in dark-grown seedlings, i.e., the so-called triple response which was first described by Neljubow in 1901 (see Knight and Crocker 1913). Normally, when seedlings are exposed to ethylene in the darkness, they display exaggerated curvature of the apical hook, inhibition of hypocotyl and root elongation and radial expansion of the hypocotyl. In contrast, the Arabidopsis etr1 mutant lacks the normal response to ethylene, whereas ctr1 displays this phenotype even in the absence of ethylene (Bleecker et al. 1988; Kieber et al. 1993). This suggests that ethylene and light affect the mechanism of hypocotyl elongation in Arabidopsis.

Light and ethylene signaling may affect some functions also in the post-embryogenetic regulation of stem elongation. For example, in tobacco (Nicotiana tabacum L.), functional ethylene signaling is required to time shade avoidance phenomenon in which FR emitted from the neighboring plants need to be detected in order to accelerate stem elongation (Pierik et al. 2003). This was concluded from the responses of transgenic tobacco plants rendered ethylene-insensitive by heterologous expression of an Arabidopsis ethylene receptor gene carrying a dominant mutant allele etr1-1. The etr1-1 mutation, which represents the mutation of a cysteine residue in the second transmembrane domain of ETR1, prevents the binding of ethylene to the receptor (Bleecker et al. 1988; Chang et al. 1993) and has been shown to confer ethylene-insensitivity when heterologously expressed in several species (Wilkinson et al. 1997; Knoester et al. 1998). Thus, the transgenic lines can be viewed as “mutants” that are deficient in ethylene sensing and, consequently, attenuated or blocked in normal responses to ethylene. Here, a similar approach was used to study light-regulated growth responses specific to woody plants. Transgenic trees were employed as a tool to investigate photoperiod and ethylene-dependent signaling associated with the perennial growth habit.
2 AIMS OF THE STUDY

Seasonal responses are synchronized by photoperiod. In trees, perception of a critical day length in the autumn triggers substantial physiological and developmental adjustments, such as growth cessation, bud set and dormancy. These adjustments must be initiated in a timely manner to complete the development of a dormant and freezing tolerant state, as only then the plants can survive through winter. However, the signals underlying the SD-induced cessation of elongation growth, bud set and the transition to dormancy are poorly known.

Earlier, it has been shown that under SD, the action of the light receptor PHYA in the leaves affects the transition to dormancy in hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) (Olsen et al. 1997). However, the role of the shoot apex and its various morphogenetic regions in these processes has been little addressed (Rinne and van der Schoot 1998; Rinne et al. 2001). Furthermore, there were no data available on whether or how ethylene signaling might affect the light-mediated seasonal growth and development in perennials.

The aim of this study was to investigate if and how the shoot apex processes the leaf-derived signals involved in the photoperiod-induced adjustments of growth and development in trees. Specifically, the functioning of the meristematic areas of the shoot apex in the photoperiodic responses was addressed. Furthermore, the coupling of the SD-induced events, such as bud set and transition to dormancy, was evaluated. Particular attention was also given to the role of ethylene as a signaling molecule in developmental transitions.
3 MATERIALS AND METHODS

Details of the materials and methods used to obtain the data presented in this thesis are given in each publication (I-III). Here, a brief description of the plant materials as well as a list of methods employed in the publications (Table 1) is provided.

To study signals involved in SD-induced cessation of elongation growth, bud set and the transition to dormancy, PHYA over-expressing hybrid aspen (Populus tremula L. x P. tremuloides Michx.) (transgenic line 22 called as P35S:AsPHYA) (Olsen et al. 1997) and ethylene-insensitive birch (Betula pendula Roth.) (transgenic lines called as BPetr1-1) (II) along with the reference wild-type ramets were utilized. Wild type indicates the nontransgenic clone that was transformed to create transgenic lines. In hybrid aspen (also indicated as hybrid poplar), clone T89 was the wild type (Olsen et al. 1997), while in birch, clones originating from central and southern Finland (see Pääkkönen et al. 1997) were used for the transformations (II). Furthermore, to facilitate the dissection of events intrinsic to leaf and apex, graft systems of P35S:AsPHYA and the corresponding wild type were produced (I).

Table 1. The methods used in this study. The parentheses indicate that the method was conducted only by the co-authors of the publication.

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<tr>
<td>DNA extraction</td>
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<tr>
<td>Primer design and quantitative real-time RT-PCR (qPCR)</td>
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4 RESULTS AND DISCUSSION

Environmental signals are crucial in shaping the growth and development of plants. To time developmental transitions, such as flowering in annuals and dormancy in perennials, day length is the most reliable cue. In the annual model species *Arabidopsis*, the photoperiodic pathways regulating flowering are relatively well defined. However, in trees it has remained unclear how perception of a critical day length at the end of the season results in developmental transition that brings about a dormant state. This problem is addressed in the present study.

4.1 Fast responses to SD in trees

4.1.1 PHYA in the leaves triggers developmental adjustments

Leaves comprise a large area of aerial tissues in deciduous trees. Depending on their developmental stage, leaves may act as suppliers of sucrose or other growth-modulating molecules, but simultaneously also as effective sensors of the environment. Thus, it is conceivable that seasonal processes, such as dormancy development, may be triggered in the leaves from where signals can be transmitted to inform the other parts of the plant about the environmental changes (Wareing 1956; Thomas and Vince-Prue 1997).

In hybrid poplar (*Populus tremula x P. tremuloides*), PHYA plays a prominent role in the photoperiodic regulation of growth cessation. Over-expression of oat (*Avena sativa* L.) PHYA under the control of the Cauliflower Mosaic Virus 35S promoter disturbs day length detection and prevents dormancy establishment in the strongly transgenic line 22 (denoted here as *P35S:AsPHYA*) (Olsen et al. 1997). *P35S:AsPHYA* is dwarfed (Olsen et al. 1997; Welling et al. 2002; I), but the plastochron, i.e., the time between the formation of successive leaves, is comparable to the wild type (I). The SAM of *P35S:AsPHYA* proliferates and leaf primordia emerge in phyllotactic patterns characteristic of the wild type. This might be contributed by the wild-type -like expression pattern of poplar´s endogenous PHYA and the absence of *AsPHYA* transcripts in the SAM and youngest primordia, as shown by in situ hybridization (I). However, later in leaf development, *AsPHYA* transcripts are present in high amounts (Olsen et al. 1997; I). On the other hand, the 35S-driven high accumulation of *AsPHYA* mRNA in the area of the RM/RZ correlates with dwarfism (I).
As described, in *P35S:AsPHYA*, growth arrest under inductive photoperiod is abolished. Similarly, in potato, PHYA delays tuberization (Yanovsky et al. 2000) which occurs in the young stem when the underground shoot apex ceases elongation. In *Arabidopsis*, in turn, over-expression of PHYA results in early flowering (Bagnall et al. 1995) whereas *phyA* mutants flower late (Johnson et al. 1994; Reed et al. 1994). Collectively, this indicates that PHYA is an important trigger of photoperiod-induced events in the apex both in perennials and annuals.

4.1.2 FT expression levels in the leaves cease rapidly

When poplar is exposed to SD, various cellular and molecular responses are apparent. Among the fastest ones is the decrease in expression levels of *FT1/FT2* (Böhlenius et al. 2006; Hsu et al. 2006; I) and *CO2* (Böhlenius et al. 2006; I) in the leaves. Down-regulation of *FT1/FT2* may occur within a few days after the beginning of the SD exposure, and before any alterations in stem elongation or the rate of leaf formation takes place. qPCR analysis showed that also in *P35S:AsPHYA* the *FT2* transcript levels are initially down-regulated. This indicates that *P35S:AsPHYA* shows some degree of response to SD, although subsequently the expression level of *FT2* partly recovers (I).

*FT* is involved in photoperiod-mediated responses also in annuals. During the photoperiodic induction of flowering in *Arabidopsis*, *FT* and *CO* are activated in response to the functioning of the photoreceptors PHYA and CRY2 (Yanovsky and Kay 2002). Flowering of the rosette plant *Arabidopsis* involves emergence of the inflorescence stem (increased RM activity) and transitioning of the SAM into an IM. Thus, both in *Arabidopsis* and in juvenile poplar, *FT* expression in the source leaves is associated with changes in the RM activity. In *Arabidopsis*, the up-regulation of *FT* coincides with bolting. By contrast, in poplar, *FT1/FT2* might primarily assist the timing of growth cessation as the expression levels decrease much ahead of any physiological changes that can be measured (Böhlenius et al. 2006; Hsu et al. 2006; I).

4.1.3 Symplasmic connections in the shoot apex become restricted

PD are channels that provide cytoplasmic continuity to adjoining cells, thereby creating a symplasmic space (Lucas et al. 1993). In a growing shoot apex, several symplasmic fields
can be distinguished by following the spreading of a small-molecular-weight fluorescent dye (Lucifer Yellow CH, LYCH) via PD from a microinjected cell. In hybrid poplar, the central part of tunica and corpus form distinct symplasmic compartments in the SAM, and the PD between the RM and the adjacent corpus are narrowed, not allowing the passage of LYCH (I). Furthermore, the tunica layer is divided into two distinct fields: a central field in the CZ and a complete or partial ring-like field in the PZ, as demonstrated in birch (*Betula pubescens*) (Rinne and van der Schoot 1998). Taken together, the RM is symplasmically distinct, and within the SAM there are several morphogenetic units which may collaboratively coordinate shoot development (Rinne and van der Schoot 1998; van der Schoot and Rinne 1999).

When the day length shortens below a critical value, cellular collaboration in the shoot apex undergoes alterations. Under SD, the SEL of PD rapidly decreases, as revealed by restricted diffusion of LYCH in the SAM (Rinne and van der Schoot 1998; Rinne et al. 2001; I). In hybrid poplar, the diminished symplasmic connectivity of SAM cells could be observed just prior to the first visual signs of cessation of stem elongation, that is between 2-3 weeks of SD under the experimental conditions used (I). In the slowly dividing CZ, the communication could diminish a few days earlier than in the PZ (I), suggesting that after the CZ has diminished its symplasmic connectivity, the PZ may continue its morphogenetic activity of leaf primordia initiation. Similar SD-induced cell isolation may occur also in the RM. Eventually, after 7-8 weeks of exposure to SD, the uncoupling of all cells became fixed by dormancy sphincter complexes (DSCs) in wild-type hybrid poplar (I). DSCs, visualized by using tannic acid staining and Transmission Electron Microscopy, firmly close PD and characterize the (endo-)dormant state which can be reversed only by a dormancy-removing treatment such as chilling (Rinne and van der Schoot 1998; Rinne et al. 2001; I). By contrast, in *P35S:AsPHYA*, the symplasmic connectivity was not compromised and DSCs were not formed under SD (I), correlating with the absence of dormancy.

In summary, under SD, the *FT1/FT2* transcript levels decrease very fast in the leaves of poplar. This might result in a dramatic reduction of leaf-derived FT1/FT2 protein in the apex, as the FT protein has been shown to traffic long distances to apex in several flowering-induced herbaceous species (Corbesier et al. 2007; Tamaki et al. 2007; Lin et al. 2007; Jaeger and Wigge 2007; Mathieu et al. 2007). Moreover, under SD, the symplasmic connectivity rapidly decreases in the SAM, reducing cellular interactions and morphogenetic signaling, and anticipating further adjustments in growth and development.
4.2 **SAM and RM in the photoperiodic control of growth and development**

4.2.1 **Grafts indicate a role for the shoot apex**

Although SD can trigger signaling processes in the leaves, considerable molecular, hormonal and morphogenetic changes take place in the shoot apex. Visually, elongation growth and leaf production cease, and a terminal bud forms. The morphological changes, as well as the transition to dormancy, proceed gradually and may overlap in time, making it difficult to distinguish whether they are independent or not. To assess if the leaf-derived signals solely determine the responses of the apex, or whether the apex itself plays a role in the morphological modifications and the development of dormancy, various types of graft systems were exposed to SD conditions.

Grafting experiments with wild type and *P35S:AsPHYA* clearly showed that the apex plays an important role in shaping the plants’ responses to SD (I). When the scion (apical part) of the heterograft (wild-type scion on *P35S:AsPHYA* stock, or vice versa) was defoliated, thus enforcing the movement of stock-leaf derived signals into the apex, both types of heterografts eventually ceased growth and formed a terminal bud under SD. This shows that *PHYA* over-expression may delay but not prevent photoperiod-induced cessation of elongation growth and bud set once appropriately stimulated. Interestingly, however, only the defoliated wild-type scion entered dormancy while the *P35S:AsPHYA* scion displayed recurrent flushing accompanied by stem elongation under SD. In *P35S:AsPHYA* scions, the over-expression of *PHYA* particularly in the RM area might have contributed to the repetitive flushing and stem elongation. In some cases, also the defoliated wild-type scion could flush, but the flushing did not involve stem elongation. This indicates that the SAM and RM activities might function semi-independently, as in the wild-type scion the RM proliferation was permanently blocked although the SAM could still respond to growth-promoting signals proceeding from the *P35S:AsPHYA* source leaves.

4.2.2 **TFL1/CENL1 may modulate RM and SAM behavior**

The *Arabidopsis TFL1* gene has been associated with plant architecture and timing of floral transition, as the *tfl1* mutant is early flowering and characterized by a short inflorescence stalk bearing determinate floral meristems (Shannon and Meeks-Wagner 1991; Alvarez et al. 1992; Bradley et al. 1997). The functioning of the gene was further verified by over-
expression of \textit{TFL1}, which produced enlarged shoot and inflorescence systems in the transgenic \textit{Arabidopsis} plants (Ratcliffe et al. 1998). This could be re-produced by heterologous over-expression of \textit{TFL1} relatives (Jensen et al. 2001; Nakagawa et al. 2002; Pillitteri et al. 2004; Kotoda and Wada 2005; Böhlenius et al. 2006), indicating that these genes are functionally conserved in a range of species. However, although it was early on described that upon floral induction the up-regulation of \textit{TFL1} concentrates in a patch of cells just below the apical dome (Bradley et al. 1997) which corresponds to the RM area (Esau 1977; I), the putative dual role of \textit{TFL1} in regulating both RM activity and SAM determinacy was not recognized.

We found that the expression levels of \textit{CENTRORADIALIS-LIKE1} (\textit{CENL1}), a poplar ortholog of \textit{Arabidopsis TFL1} (Böhlenius et al. 2006; I), correlated with RM activities of wild type and \textit{P35S:AsPHYA} under LD and SD (I). According to the qPCR analyses, in wild-type apices the transcript abundance of \textit{CENL1} remained at LD levels or somewhat elevated during the first three weeks of SD exposure when growth still continued, but then rapidly declined coinciding with growth cessation, bud set and dormancy establishment. As compared to the wild type, in \textit{P35S:AsPHYA} the \textit{CENL1} expression was lower under LD but continued to increase towards the end of the SD exposure (I). In \textit{P35S:AsPHYA}, the relatively low expression level of \textit{CENL1} under LD (I) correlates with the fact that short internodes have reduced cell numbers (Olsen et al. 1997) whereas under SD the gradual increase in expression coincides with the increase in internode length (I). In wild type and \textit{P35S:AsPHYA}, the strongest expression of \textit{CENL1} was found in the RM/RZ area (I), as consistently shown also in other species (Bradley et al. 1997; Ratcliffe et al. 1998; Kardailsky et al. 1999; Ratcliffe et al. 1999; Zhang et al. 2005; Conti and Bradley 2007). Collectively, this suggests that RM activity might be modulated locally at a molecular level and that SAM and RM might co-operate at developmental transitions both in annuals and in perennials.

In \textit{Arabidopsis}, \textit{TFL1} appears non-compatible with the floral identity genes \textit{LFY} and \textit{AP1} (Ratcliffe et al. 1999; Liljegren et al. 1999). Moreover, it was recently shown that the TFL1 protein moves from the RM to the IM in flowering \textit{Arabidopsis}, and that it may have a role in endomembrane trafficking (Conti and Bradley 2007; Sohn et al. 2007). Similar mechanisms might function also in woody plants. In apple (\textit{Malus x domestica} Borkh.) and navel orange (\textit{Citrus sinensis} L.), the expression patterns of genes homologous to \textit{LFY} and \textit{AP1} may contrast that of \textit{TFL1} after floral induction (Pillitteri et al. 2004; Kotoda and Wada
Furthermore, in poplar, *CENL1* expression levels are decreased (Ruttink et al. 2007; I) while *AP1* and *LFY* are up-regulated at bud maturation (Ruttink et al. 2007). If CENL1 protein had a non-cell-autonomous role, the elevated expression of *CENL1* in wild-type hybrid poplar after 2-3 weeks under SD might indicate increased traffic of the protein into the SAM. At this point diffusional movement of morphogens is already restricted as the PD are narrowed. Nonetheless, the PD can still be gated, i.e., the SEL can increase to permit passage of molecules. In the SAM, CENL1 might protect the indeterminate state, at least until DSCs arrest non-cell-autonomous function. However, to test this hypothesis, localization studies of the CENL1 protein would be required.

### 4.3 Events coinciding with growth cessation in the shoot apex

The SD-induced physiological and morphological changes in the shoot apex of trees involve cessation of stem elongation and formation of a terminal bud. These changes tend to occur gradually in a programmed manner. For example in birch (*Betula pendula* Roth.), once the cessation process was initiated by a short-term SD treatment, it progressed for some time under subsequent LD before growth accelerated again (III). The time lag before growth resumption might represent a time required to either re-activate the growth promoting machinery in the leaves, such as FT2 production, or reverse of the early processes initiated in the apex. Given that leaf formation could resume sooner than stem elongation (III), SAM properties might have been less affected by the transient SD exposure as compared to RM activity.

Under the controlled SD conditions, the strongest decrease in the rate of stem elongation, as well as the first visual signs of bud formation, took place at around 12 days in birch (III) and 3 weeks in hybrid poplar (I). Several other phenomena occurred at this time point. *CENL1* expression in the apex of hybrid poplar increased, possibly to safeguard the indeterminate state of the SAM during the development of dormancy (I). At the same time the plant prepared itself to arrest growth. This included, as analyzed by qPCR, the up-regulation of a gene encoding for a *BETA-XYLOSIDASE* in the apex of birch (III) and hybrid poplar. Beta-xylosidases are enzymes involved in the hydrolysis of xylan, which is a hemicellulosic component in the primary and secondary cell walls (Goujon et al. 2003; Somerville et al. 2004; Collins et al. 2005). This indicates that the properties of the cell walls may alter in the early phases of growth cessation. The bud burst capacity was decreased in
broadleaf birch (III) and hybrid poplar (I) at 2 and 3 weeks of SD, respectively, although it took about four additional weeks for dormancy to become established in both species. Thus, the initiation of growth cessation may represent a critical time-point for the establishment of crucial changes at the apex. Some of these changes may be mediated by the plant stress hormones ethylene and ABA. The roles of these hormones, particularly that of ethylene, in the regulation of meristem transitions and other developmental processes will be discussed in the following sections.

4.4 Ethylene in the photoperiodic control of growth and development

4.4.1 Generation of ethylene-insensitive birch

Plant hormones are widely associated with seasonal growth and development. In herbaceous plants, evidence has accumulated that gibberellins, ABA and ethylene play a role in light-regulated seasonal events, such as tuberization in potato (Suttle and Hultstrand 1994; Suttle 1998; Horvath et al. 2003) and flowering in Arabidopsis (Mouradov et al. 2002; Razem et al. 2006; Achard et al. 2007). However, less is known about the role of hormones in dormancy development of forest trees. To explore the involvement of ethylene signaling in growth and development of trees, we generated ethylene-insensitive birch (Betula pendula) lines (BPetr1-1) (II) by Agrobacterium-mediated transformation (Lemmetyinen et al. 1998) with the Arabidopsis dominant mutant allele etr1-1 under its own promoter (Chang et al. 1993). Ethylene-insensitivity was tested by exposing actively growing wild type and transgenic lines to high doses of ethylene gas (II). In wild type, all leaves abscised within the three days of ethylene exposure (II) and subsequently the apex turned necrotic and died. On the contrary, in BPetr1-1, hardly any leaves abscised (II) and there was no visible damage in the apex after the ethylene exposure. Later, a similar role for endogenous ethylene in natural leaf shedding was demonstrated, as leaf abscission was impaired in BPetr1-1 in autumn (III).

Southern and northern analyses showed that the transgenic lines with diminished or absent responses to ethylene exposure carried at least one active copy of the Arabidopsis etr1-1 mutant allele (II, III). Furthermore, northern analysis showed that the induction of some ethylene-responsive genes, such as a MITOCHONDRIAL PHOSPHATE TRANSLOCATOR1 (Kiiskinen et al. 1997), was attenuated in the leaves of BPetr1-1 (II). Together, this indicates that the expression of Arabidopsis etr1-1 confers ethylene-
insensitivity in birch. Furthermore, the role of ethylene in end-of-season leaf abscission (Jackson and Osborne 1970) was experimentally confirmed in a deciduous tree species.

4.4.2 Ethylene as a primer and timer of dormancy development

In order to study the role of ethylene signaling in photoperiodic responses of trees, wild type and BPetr1-1 were exposed to SD for variable periods. All lines responded to transient SD exposure by cessation of stem elongation, although in BPetr1-1 the rate of cessation was slightly slower and the resumption of growth faster when compared to the wild type (III). The development of dormancy was assessed by testing the bud burst capacity of single node-cuttings that were grown in water culture under growth-promoting conditions. In wild type, bud burst capacity gradually decreased with increasing duration of SD exposure and was completely abolished at 6 weeks of SD (III). By contrast, in the strongly transgenic line BPetr1-1-35, the bud burst capacity was not compromised during the first 5 weeks of exposure; however, eventually also this line entered dormancy (III). This indicates that impaired ethylene signaling did not prevent the perception of SD, but it did shorten the phase of reduced meristem activity and delayed the entry into dormancy. In conclusion, the results indicate that ethylene has an important role in the correct timing of growth arrest and meristem transitioning under SD. This is supported by recently published gene expression analyses showing that several genes related to ethylene biosynthesis and signaling are up-regulated in poplar (Populus tremula L. x P. alba L.) apices before growth ceases under SD (Rohde et al. 2007; Ruttink et al. 2007). Timing of growth cessation might be conducted partly via ethylene-dependent modulation of cell walls, as the up-regulation of the BETA-XYLOSIDASE typical of wild type was absent in BPetr1-1 (III). Collectively, this indicates that ethylene may act as an important signal in the early phases of growth cessation and dormancy development.

4.4.3 Might ethylene traffic along with FT2/CENL1 to regulate growth?

Signals that have the ability to move long distances and act non-cell-autonomously might have a positive (FT2, CENL1) or negative (ethylene) effect on stem elongation in trees, with a specific temporal and spatial range of action. These effects on stem elongation could also be reflected in SAM behavior. CENL1 expression was down-regulated in the apex of hybrid
poplar coincident with growth arrest (I). On the other hand, FT1/FT2 (Böhlenius et al. 2006; Hsu et al. 2006; I) and ethylene (III; Rohde et al. 2007; Ruttink et al. 2007) may rather act as early signaling molecules to time photoperiodic growth responses. While FT is produced in the leaves and subsequently transported to the shoot apex (see recent reviews by Kobayashi and Weigel 2007; Turck et al. 2008), it is less clear where the ethylene is produced that affects apex functioning. Ethylene pathways might be activated locally in the apex or more widely in the leaves from where ethylene or its precursor has to be transported to the apex.

All plant tissues may have the capacity to synthesize ethylene and most likely low basal levels are always present. Usually, elevated ethylene levels are found only in response to certain environmental stimuli or during specific natural processes such as the ripening of climacteric fruits (Bleecker and Kende 2000). However, the responses to ethylene may not be directly dependent on the absolute levels but also on the plant’s ability to perceive the signal and on the subsequent transduction events through the signaling pathways. Ethylene is released from plants as a volatile, and a targeted mechanism for movement within the plant has not been described. Yet, as ethylene is readily soluble in aqueous and lipid environments, it might diffuse over distances within the plant. This suggests that the crucial regulatory role in ethylene responses would rather be the perception of ethylene, which can vary locally between tissues and cells. Nevertheless, as precursors in ethylene biosynthesis, such as 1-aminocyclopropane-1-carboxylic acid, can be transported in the phloem or xylem, this could be an additional means to regulate ethylene responses via ethylene concentrations (Yang and Hoffman 1984; Walz et al. 2004).

4.4.4 Could ABA play a role in dormancy establishment together with ethylene?

In addition to potential crosstalk between light and ethylene signal transduction, SD-induced growth cessation may also involve interactions among different hormones within the apex. A transient increase in ABA content of apices is typical for birch (Welling et al. 1997; III) and poplar (Rohde et al. 2002) coinciding with growth cessation under SD. BPetr1-1, however, did not accumulate ABA as the wild type, and the development of dormancy was delayed under SD (III). This suggests that ABA, which acts downstream of ethylene, might be dependent on ethylene directly or indirectly.

The involvement of ABA in bud dormancy development has remained unclear (Welling et al. 1997; Rinne et al. 1998), but it seems possible that ethylene and ABA interact
in the bud to facilitate growth arrest in a timely manner. Like ethylene, ABA might affect meristem transitions through sensitivity changes. For example, the *Arabidopsis* mutant *fca*, which is late-flowering under LD conditions (Koornneef et al. 1991), carries a mutation in a gene which has been shown to encode an ABA receptor involved in flowering (Macknight et al. 1997; Razem et al. 2006). However, *fca* responds to vernalization (low temperature) in a normal manner (Koornneef et al. 1991). In poplar, the expression of a gene homologous to the *Arabidopsis* FCA increases rapidly under SD while other genes related to ABA signaling and biosynthesis are up-regulated later during growth cessation, concomitant with the increase in ABA (Rohde et al. 2002; Ruttink et al. 2007). This indicates that ABA perception increases already at an early phase before any change takes place in ABA biosynthesis. As suggested, the later increase in ABA levels could facilitate the acclimation to low temperatures rather than modulate dormancy development at the SAM (Welling et al. 1997). Thus, it can be concluded that increased ABA sensitivity might act together with ethylene sensitivity to time growth cessation and dormancy development and, secondly, participate in late events such as bud maturation and cold acclimation via concentration changes. In any case, ethylene-dependent alterations in ABA content or sensitivity might not be crucial for over-wintering of birch, as the *BPetr1-1* buds could survive conditions mimicking early winter. However, it has not been tested if their actual freezing tolerance is diminished. Furthermore, *BPetr1-1* needed fewer chilling units than the wild type to remove dormancy, suggesting that ethylene is involved also in sustaining the dormant state in birch.

### 4.4.5 Bud set is ethylene-dependent and distinct from dormancy

While in case of growth cessation and dormancy development ethylene seems to primarily affect the timing, it has a dramatic effect on bud set. Terminal buds were not formed in *BPetr1-1* under SD (III). This phenotype is somewhat similar to that described in poplar (*Populus tremula* x *P. alba*) over-expressing *ABSCISIC ACID-INSENSITIVE3 (ABI3-OE)* which results in arrested apices with large embryonic leaves and small bud scales (Rohde et al. 2002). Thus, both ethylene and ABA signaling may affect the SD-induced formation of terminal buds in trees. Moreover, as both *BPetr1-1* (III) and *ABI3-OE* (Ruttink et al. 2007) can enter dormancy, photoperiod-induced bud set is clearly distinct from dormancy. This is further confirmed by the fact that the defoliated *P35S:AsPHYA* scions grafted on the wild-
type stocks could temporarily arrest growth and even form a terminal bud without entering dormancy under SD (I).

Leaf development associated with SD-induced bud set includes several events. Normally, leaf primordia develop into embryonic leaves and stipules into bud scales while a small leaf just below the bud turns yellow and abscises (Goffinet and Larson 1981; Rohde et al. 2002; III). At the same time, the young sink leaves continue to expand below the prematurely abscised leaf. In BPetr1-1, all these events were abnormal: (1) the sink leaves did not expand to the same degree while the embryonic leaves expanded more than the comparable wild-type leaves, (2) the local leaf abortion was absent, (3) and the bud scales did not expand to cover the bud (III). The premature senescence and development of an abscission zone in a single petiole suggests that the cause is a local accumulation of ethylene or an increased sensitivity to ethylene.

In general, ethylene seems to regulate the onset of leaf senescence in a variety of species (Aharoni and Lieberman 1979; John et al. 1995; Grbic and Bleecker 1995). In BPetr1-1, the fully expanded leaves showed delayed but not prevented senescence based on both visual observations and measurements of total chlorophyll content (III). A similar situation has been reported for ethylene-insensitive Arabidopsis mutants (Bleecker et al. 1988; Grbic and Bleecker 1995). This suggests that ethylene signaling is not necessary for the execution of leaf senescence but rather that it is involved in the timing of the senescence process. The central role of ethylene in the timing of autumn leaf senescence is further supported by the rapid increase in the transcript levels of genes associated with ethylene biosynthesis and perception in birch and poplar (Valjakka et al. 1999; Andersson et al. 2004; Sillanpää et al. 2005). However, ethylene appears to be required for the programmed death of a sink leaf typical for bud set (III) which takes place much ahead the yellowing and shedding of mature leaves.

4.5 Ethylene modifies the architecture and flowering time in birch

Characterization of ethylene-insensitive birch revealed that ethylene affects bud development in many ways. This was also strongly reflected in the shoot architecture. Characteristically, BPetr1-1 had a bushy appearance, as due to vigorous outgrowth of axillary buds the number of branches was significantly increased as compared to wild type (III). Moreover, while the apices of branches eventually died in the wild type, those of
BPetr1-1 survived throughout the season (III). This indicates that ethylene signaling is
decisive not only for bud set but also for the fate of branch apices under controlled SD
conditions. Thus, ethylene may contribute to the sympodial growth pattern, which is typical
of birch (Maillette 1982), by facilitating the abscission of shoot apices.

An additional trait characteristic of BPetr1-1 was the formation of inflorescences
several years earlier as compared to the wild type (III). All these features - reduced apical
dominance, survival of apices without bud set and early flowering - were unpredicted.
Possibly they relate to the perennial growth habit of birch and for this reason may contradict
with the previously reported phenotypes of ethylene-insensitive annuals. For example, etr1-1
Arabidopsis is late-flowering (Bleecker et al. 1988). Moreover, the degree of apical
dominance in ethylene-insensitive tobacco or Arabidopsis is comparable to the wild type
(Cline 1991; Knoester et al. 1998). Commonly, basipetal auxin transport is associated with
apical dominance (Thimann and Skoog 1933; Gälweiler et al. 1998; McSteen and Leyser
2005). However, as auxin does not enter the bud (Hall and Hillman 1975; Morris 1977),
secondary messengers are required to control the outgrowth of axillary buds. While in
Arabidopsis the auxin effect does not seem to involve ethylene when tested using excised
node assays (Chatfield et al. 2000), it remains to be established how in birch ethylene and
auxin might co-operate to control the activity of axillary meristems. Collectively,
considering also the involvement of ethylene in growth cessation and bud dormancy
development under SD (III), woody perennials are promising models for assessing the
various roles of ethylene.

In terms of life strategies, annual weeds and long-living trees are very different. This is
reflected even in their organogenetic properties. For example, in Arabidopsis, bud primordia
do not appear at the axils of young rosette leaves. However, once flowering is initiated,
vigorous cell division starts in the RM/RZ giving rise to an inflorescence stem, and cauline
leaves as well as the youngest rosette leaves are accompanied with axillary meristems. At
this IM stage, the axillary meristems grow out to form inflorescence branches, and additional
meristems, termed accessory meristems, may later develop between the branch and its
subtending leaf (McSteen and Leyser 2005). By contrast, trees consistently produce a bud in
the axil of each leaf. In addition, in trees the active and inactive phases of the RM can cycle
during their juvenility for multiple years. In view of this, it is interesting that the function
of the proposed “florigen”, FT, seems similar in Arabidopsis and in trees (Kardailsky et al.
1999; Kobayashi et al. 1999; Endo et al. 2005; Böhlenius et al. 2006; Hsu et al. 2006). In FT
over-producing poplar (Böhlenius et al. 2006; Hsu et al. 2006), or in the most pronounced cases of ethylene-insensitive birch (III), inflorescences are formed already during the first season as in Arabidopsis, i.e., without a dormant period before floral initiation. However, in case of BPetr1-1, the considerable shortening of the juvenile phase might be an indirect effect of ethylene in the normal growth form of birch. In BPetr1-1, inflorescences often emerged from branches that in the wild type of corresponding age had not grown out from axillary buds, or from apical buds of branches that aborted in wild type. Nevertheless, in BPetr1-1 sometimes even the main axis terminated in an inflorescence, while in the wild type a terminal bud would form. It is not known precisely how flowering in deciduous trees is regulated, but it has been postulated that the FT levels increase as the tree ages (Böhlenius et al. 2006; Hsu et al. 2006). Thus, the role of FT in flowering might be more direct, accelerating flowering in a dose-dependent manner. By contrast, the role of ethylene in delaying flowering of birch might result from its involvement in axillary bud outgrowth and shoot architecture.

4.6 Dormancy might adopt defense mechanisms

In this study, growth arrest and bud set were described as events typically associated with the SD-induced transition to dormancy in trees. Nevertheless, these events may occur as a response to adverse conditions independent of day length. For example, nutrient deficiency may provoke growth cessation and bud formation in wild-type birch. Moreover, exposure to low temperature arrests stem elongation in wild-type and P35S:AsPHYA hybrid poplar (Welling et al. 2002). However, in these cases growth resumed as soon as permissive conditions were brought back. A chemical inhibition of gibberellin biosynthesis together with chilling prevented any elongation growth irrespective of photoperiod in hybrid poplar (Mølmann et al. 2005). Under these treatments, P35S:AsPHYA showed a bud-like structure under SD conditions (Mølmann et al. 2005). Thus, growth arrest and marked changes in the morphology of the shoot apex can be induced also as a response to harsh treatments.

Common strategies between dormancy and defense can be noted at a supracellular level. In trees, bud dormancy is characterized by DSCs, and callose may accumulate also in response to several stresses (Rinne and van der Schoot 2003 and references therein). Moreover, ethylene, a plant stress hormone, is here suggested to function as an early signal in bud dormancy development (III), and a similar role as a signaling molecule has been
proposed in oxidative stress responses, as propagation of an ozone-induced lesion may require ethylene (Overmyer et al. 2000; Ahlfors et al. 2004; II; Vahala 2003). Collectively, this indicates that similar mechanisms may be recruited in photoperiod-dependent regulation of growth and development as well as various stress responses, and that ethylene might have an important role for plant survival in natural conditions.

4.7 Concluding remarks

Flowering in Arabidopsis and seasonal growth cessation in perennials are such vital processes that they must be regulated through several overlapping and/or interacting pathways to secure a successful outcome. Here, it was suggested that flowering and dormancy may be governed by overlapping molecular mechanisms, including those involved in leaf-to-apex signaling (Figure 5). It was found that homologs of several genes commonly associated with PHYA-mediated flowering of Arabidopsis may also be regulated during SD-induced development of bud dormancy in trees (I). Moreover, the results suggest a novel role for ethylene in the priming and timing of bud dormancy, and reveal that ethylene is required for terminal bud formation (III) (Figure 5). In these events, ethylene may act upstream of, or together, with ABA. In addition, it was described that ethylene regulates meristem functioning, bud fate and abscission in birch (II, III). Whether the photoperiodic and ethylene pathways are independent or interacting in the seasonal regulation of growth in trees is not clear. It might be interesting to examine if, for example, the temporal expression patterns of genes homologous to CO2/FT2 and CENLI deviate in the ethylene-insensitive birch as compared to the wild type.

Furthermore, it was shown that for dormancy to develop, the photoperiod-generated leaf-to-apex signaling may be processed in the distinct meristematic regions of the shoot apex. Specifically, it was suggested that a major target of the signal cascade is the RM (I), which is a meristematic area subjacent to the SAM. The involvement of the two distinct morphogenetic areas, RM and SAM, reflects and supports the notion that cessation of stem elongation and bud formation on one hand, and dormancy development on the other, are biologically distinct processes (I, III).
Figure 5. Putative signaling (A) and the time-frame of events (B) involved in the photoperiod-induced transition to dormancy, as indicated in the present study.

(A) Light is detected by photoreceptors, such as PHYA, in the source leaves which signal to the shoot apex via the phloem. Collectively, sieve-plate pores and PD extend the symplasmic continuum from the leaves to the SAM, thereby providing a route for symplasmic signaling. Under LD, FT2 protein may travel from the leaves to the shoot apex to promote growth, while under SD, the supply of FT2 protein ceases. The expression domain of CENL1 concentrates in the RM area, but the CENL1 protein might move into the SAM to protect indeterminacy. In the apex, ethylene regulates the expression of a BETA-XYLOSIDASE gene and may promote ABA accumulation under SD. In addition to the shoot apex, the ethylene effect might localize in the leaves.

(B) Dormancy development is divided in three phases according to visual recording of the meristem activities (1, active proliferation; 2, decreased activity; 3, arrested activity). Events in the second phase are partially overlapping.

SAM, shoot apical meristem; RM, rib meristem; PHYA, PHYTOCHROME A; PD, plasmodesmata; FT, FLOWERING LOCUS T; CO, CONSTANS; CENL1, CENTRORADIALIS-LIKE1; ABA, abscisic acid; DSC, dormancy sphincter complex; LD, long day length; SD, short day length.
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REFERENCES


