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The genetics influencing seed cone initiation and development in *Picea abies*

How a SNP changed everything

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Cover: Left: Wild type *P. abies* seed cones after pollination. Right: Homozygous *acrocona* transition shoots after pollination (photos: Nathan Zivi)

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Abstract

Picea abies is an economically important forest tree that commonly dominates Swedish boreal forests. Its tendency towards infrequent seed cone-setting, and therefore low seed production, has been a challenge for research, breeding, and industry. In order to better understand what processes are involved in initiation and development of seed cones, we took a variety of approaches: First we provided evidence that a mutation in the combined *microRNA156/529* binding site of gene encoding a SQUAMOSA BINDING PROTEIN LIKE transcription factor (*PaSPL1*) is the causal factor in the early cone-setting phenotype of the *acrocona* mutant (Paper I). The mutation in the *miR156/529* binding site leads to a misregulation of *PaSPL1* transcripts which promotes female reproductive development and early cone-setting. Second, we performed Spatial Transcriptomics (ST) experiments to create a spatiotemporal atlas of gene expression during early stages of shoot primordia development. In these experiments, we collected vegetative, seed cone, and *acrocona* transition shoot primordia at three time points during development (Paper II). The development of different tissues and gene expression within the different bud types were described and led to the identification of four tissue domains with distinct expression profiles. A ShinyApp was developed to allow easy navigation of gene expression in all bud types and time points. Third, we studied the natural variation in cone-setting ability and effects of Gibberellic Acid (GA) injections in a seed orchard (Paper III). Seed cone production, hormone concentration, and gene expression between treatments and genotypes were analyzed.

Keywords: *Picea abies*, Norway spruce, *acrocona*, PaSPL1, miRNA, Spatial Transcriptomics, seed cone, GA, Gibberellin, seed orchard, development, initiation

The genetics influencing seed cone initiation and development in Picea abies

Abstrakt

I ett svenskt persperktiv är gran (*Picea abies*) ett ekonomiskt viktigt trädslag som dominerar stora delar av den svenska skogen. Trots sin dominans så är produktionen av förädlat växtmaterial inte enkel. Till stor del beror det på att granar genomgår en lång juvenil period innan de börjar sätta kottar, samt att kottsättning därefter sker ojämt mellan olika år. Den låga fröproduktionen är en utmaning både för forskning, förädling och industri. För att öka förståelsen för vilka processer som är involverade i initiering och utveckling av honkottar har vi använt oss av flera olika angreppsätt: I en första studie visade vi att en mutation i den kombinerade inbindningsplatsen för mikroRNA156 (*miRNA156*) och *miRNA529* i genen som kodar för proteinet *Picea abies* SQUAMOSA BINDING PROTEIN LIKE (*PaSPL1*) är kopplad till tidig kottsättning. I dessa försök har vi använt oss av en naturligt förekommande varitet av gran, P. *abies* var. *acrocona*, som är känd för sin tidiga kottsättning. I studien visar vi att mutationen i *miR156/529*-inbindningsstället ökar stabiliteten hos de transkript som produceras från den muterade allelen av PaSPL1; vilket i sin tur ger en tidig och mer frekvent kottsättning. I en andra studie har vi producerat en karta av det samlade genuttrycket under tidiga stadier av vegetativa skott och honkottars utveckling. Metoden för att producera denna karta över genuttrycket kallas Spatial Transcriptomics. I dessa experiment samlade vi tidiga stadier av vegetativa skott, honkottar och så kallade övergångsskott från *acrocona* mutanten. Utvecklingen av olika vävnader och genuttryck inom de olika vävnadstyperna beskrevs och ledde till identifiering av fyra olika domäner med distinkta uttrycksprofiler. Vi utvecklade dessutom ett digitalt användarverktyg för att möjliggöra en enkel visualisering av genuttrycket i olika knopptyper. I den tredje och avslutande studien studerade vi den variation i kottsättningsförmåga som finns i naturliga populationer av P. *abies*. Vi studerade även effekterna av injektioner med växthormonet gibberellinsyra i en fröodling, och analyserade hur hormonbehandlingar påverkade kottsättningen, hormonmetabolismen och genuttryck.

Keywords: *Picea abies*, Norway spruce, *acrocona*, PaSPL1, miRNA, Spatial Transcriptomics, seed cone, GA, Gibberellin, seed orchard, development, initiation

For those who did

For those who will

For Elara and Ezra

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Akhter, S.*, Westrin, K. J.*, **Zivi, N.***, Nordal, V., Kretzschmar, W. W., Delhomme, N., Street, N. R., Nilsson, O., Emanuelsson, O., & Sundström, J. F. (2022). Cone-setting in spruce is regulated by conserved elements of the age-dependent flowering pathway. *New Phytologist*, *236*(5), 1951–1963. https://doi.org/10.1111/nph.18449

II. Saarenpää, S.*, **Zivi, N.***, Masarapu, Y., Herrera-Foessel, S. A., Orozco, A., Englund, M., Sundström, J. F., Giacomello, S. Spatiotemporal developmental gene expression dynamics in Norway spruce shoots (Manuscript)

III. Mishra, L.*, Yadav, S.*, **Zivi, N.**, Westrin, K. J., Almqvist, C., Emanuelsson, O., Sundström, J. F. Natural variation in cone-setting ability in Norway spruce (Manuscript)

The contribution of Nathan Zivi to the papers included in this thesis was as follows:

I. Performed experiments and conducted fieldwork. Contributed to writing the manuscript.

II. Performed the experiments. Analyzed data. Participated in writing the manuscript.

III. Participated in the experimental planning, sample collection, data analysis and writing the manuscript.

List of figures

1. *Picea abies* and Swedish forestry

1.1 Description of the species

Picea abies (L.) H. Karst., commonly known as Norway spruce, is a primarily outcrossing, monoecious coniferous tree with unisexual reproductive cones (Caudullo et al. 2016). Individuals can grow to be over 50 m tall and live hundreds of years (Caudullo et al. 2016). Vegetative buds form on apical meristems on apical branches and any meristem that is not a seed cone or pollen cone bud. Vegetative structures are called needles, and they are green with dotted white lines on the underside. They are generally 2-3 centimeters long and have four sides and a sharp apex (Caudullo et al. 2016). Seed cones are most often formed on apical meristems on second order lateral shoots that are one or two years old. They are erect when emerging, and their color ranges from green to dark purple (Figure 1A). Seed cones are cylindrical and, while receptive to pollen, are 4-8 cm long. After pollination, they become pendant, turn green, and grow in size before eventually becoming brown and wood-like when mature. Pollen cones are often more common than seed cones. They occur on less vigorous branches in the lower ⅔ of a tree and on the hanging, curtain branches (Figure 1B). They can form in a ring at the base of an extending shoot, in the middle of a shoot, or in an apical position. Mature pollen cones before pollen release are generally red to dark red and about 1-2 centimeters long. After releasing their pollen, they die and often fall off the tree. Sexual maturity is often reached in 20-30 years in favorable conditions or up to 40 in unfavorable conditions (Caudullo et al. 2016).

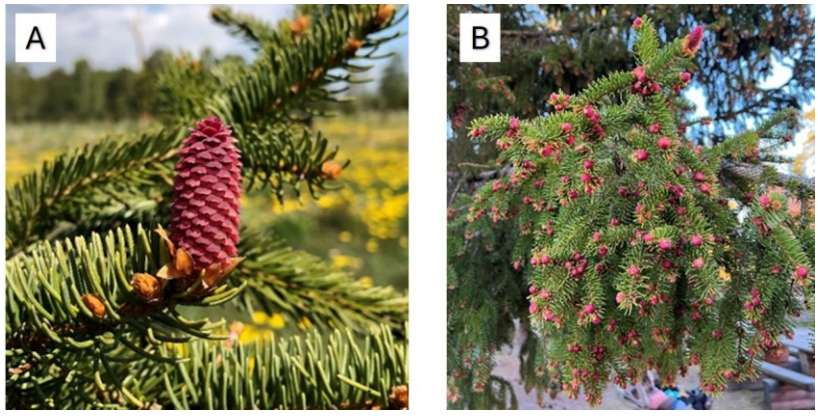

Figure 1. *P. abies* reproductive cones

(A) Receptive seed cone **(B)** Mature pollen cones

The native range of *P. abies* includes mountainous regions of central and southeastern Europe, north through the Baltic countries up to Northern Norway, Sweden, Finland, and Russia, and east across Belarus and Russia to the Ural Mountains (European Environment Agency 2006). In the far east of the *P. abies* range it combines with the range of *P. obovata* and in the Balkans its range overlaps with *P. omorika* (European Environment Agency 2006; Caudullo et al. 2016). *P. abies* is a dominant or commonly found species in boreal, hemiboreal, nemoral coniferous, Alpine coniferous, some types of mountainous beech, and spruce mire forests (European Environment Agency 2006; European Commission: Joint Research Centre 2016). The species is a dominant presence in both natural and planted forests throughout Sweden because it can thrive in the boreal forest climate with short growing seasons in the summers and long, cold winters (European Environment Agency 2006; Caudullo et al. 2016; SLU 2024; Rytter et al. 2016). Other dominant species in the same biotopes are Scots pine, silver birch, and downy birch (Rytter et al. 2016; SLU 2024). Other common species in Sweden are aspen, gray alder, and black alder (Rytter et al. 2016; SLU 2024).

1.2 The importance of forestry in Sweden

Forestry and forest products are a major part of the Swedish economy, making up 9-12% of exports (Forests and Forestry in Sweden 2015). There is 23.473 million ha of productive forest land (58% of all land) in Sweden, and *P. abies* is growing on 26.7% of productive forest land and on 39% of

all land (SLU 2024). Since records began in 1923, *P. abies* has been the most common species over all types of land but was overtaken by *Pinus* sylvestris in the late 2010s (SLU 2024). This is due, at least in part, to high temperatures and drought in 2018 and an increase in damage due to pests and disease forcing forest owners to consider planting sites more carefully than before (Felton et al. 2019; Curt Almqvist, personal communication). Even with potential issues with *P. abies* there is high demand for more seed. New seed orchards are being established to try to alleviate the shortage of improved *P. abies* seeds produced in Sweden, but projections show the planned seed orchards will still not be able to produce enough seed (Almqvist and Wennström 2020). The lack of seed will force forest owners to either source improved seed from outside the country, use wild seed, or plant a different species. Using wild seed makes the regenerated forest less productive and more susceptible to climate and pest problems than it would have been if improved seed was used and having to buy seed from abroad leaves forest owners open to the risk of potentially volatile foreign relations. Despite more than 60 years of breeding, the Swedish *P. abies* breeding program has only recently started its third breeding cycle (Almqvist and Wennström 2020). Even with only two breeding cycles, seed produced by the seed orchards in the breeding program have achieved a genetic gain of 10-25% over unimproved material (Almqvist and Wennström 2020). Having seed specifically bred for the Swedish environment with known parental material is integral to producing the best outcomes for Swedish forestry.

1.3 Threats to Swedish forests

With a changing climate, boreal forests will be majorly affected by rising temperatures (IPCC 2021; ACIA 2005). It is expected that at higher temperatures there will be shorter winters and longer summers which means longer growth periods. Earlier bud burst will happen in response, and trees will be more susceptible to unseasonal frosts (Jönsson and Bärring 2011; Langvall 2011). Increasing amounts of unexpected and severe weather events including droughts, fires, and out of season frosts can cause damage to trees, especially to reproductive structures, which are not prepared or adapted to those events (Kilpeläinen et al. 2010; Jönsson and Bärring 2011; Langvall 2011). Unfortunately, as the climate changes, the ranges of diseases and pests will change too. Warmer temperatures provide the opportunity for

continental pest species to survive further north, and impact trees that have had little to no selective pressure against these threats and potentially have little defense (Jactel et al. 2019; Pureswaran et al. 2018; Sturrock et al. 2011). Further, research already conducted into reproductive phenology may lose a lot of value when the length of growing and dormancy seasons change, making it even harder to predict when seed and pollen cones will form. This makes planning breeding activities more difficult.

1.4 Breeding can be used to deal with threats

Although tree species will eventually adapt to the changing climate, it will take time as individuals can only react to changes (Skrøppa et al. 2009). Breeding on the other hand, can be proactive. With well planned breeding, crosses can be performed to generate trees that are better adapted to the anticipated changes caused by climate change (Haapanen et al. 2015). Research into the phenology and adaptability of improved *P. abies* families from different breeding zones will be crucial to identify families, individuals, and traits that are well suited for the challenges climate change brings (Haapanen et al. 2015). Selecting improved trees that perform well across many breeding zones will also make it more likely that trees will be able to handle climatic changes (Haapanen et al. 2015). To meet the demand of the market for the amount of seed and provide seed that can survive a changing climate and endure disease, more genetically improved *P. abies* seed needs to be produced. This can be achieved in multiple ways, including establishing more seed orchards and breeding trees that will be adapted for future challenges.

1.5 Understanding reproductive initiation and development so breeding can be performed

As important as selecting which genotypes to use for breeding activities is, the selected genotypes need to produce seed or pollen cones to be useful. Plant breeding is based on the selection of individuals with traits that are desirable for the people making the selections. Breeding programs for specific species are developed in order to create a framework for how different parts of selection, crossing, and offspring evaluation will be performed. Producing offspring from a known parental lineage is necessary

to effectively meet the goals of a breeding program. The inability to perform crossings between selected parents hampers the development of plants that will improve the genetic material available for both the planted forests and the breeding program. Therefore, for a breeding program to be successful, the reproductive cycle of an organism must be understood so that the desired crossings can be performed.

1.6 Difficulties in *P. abies* breeding and seed production

In *P. abies*, there are many hurdles to performing the desired crossings between specific parents. *P. abies* has a long generation time, often taking 20-30 years before reaching maturity and producing any seed or pollen cones (Caudullo et al. 2016). Even when they reach maturity, they do not consistently produce cones because *P. abies* is a masting species (Broome et al. 2007). They produce an abundance of seed cones every 4-7 years if conditions are right (Broome et al. 2007). However, in spruce species not every genotype has the same capacity for seed or pollen cone production nor do cones mature in unison, providing a practical barrier to performing selected crosses with genotypes that do not produce cones very often or mature at different times, especially if they have different provenances (El-Kassaby and Reynolds 1990; Eriksson et al. 1973; Burczyk et al. 2004; Nikkanen 2001). Yearly differences in maturation date can vary by weeks depending on the weather, so calendar dates are not the optimal method to track pollen maturation and seed cone receptivity. Therefore, temperature sum is a much better predictor. (Eriksson et al. 1973; Nikkanen 2001). Seed cones often become receptive before local pollen is released making the window for pollination with pollen within a seed orchard smaller than expected (Eriksson et al. 1973; Nikkanen 2001). The differences between genotypes makes predicting and influencing the production of seed cones difficult. To try to alleviate this problem, there has been a lot of research into different methods to try to induce both seed and pollen cone production, like root pruning, supplemental light, or hormone applications (for reviews see: Owens and Blake 1985; Crain and Cregg 2017).

Other practical problems when it comes to breeding and trying to study reproductive initiation and development are the size of the trees and location of the seed and pollen cones within it. Both seed and pollen cones need to be

isolated from contamination by unwanted pollen before they emerge from dormancy. *P. abies* is wind pollinated, and *Picea* pollen can travel very far, being found even on the Svalbard archipelago which is at least 800 km from the nearest source of *Picea* pollen (Poliakova et al. 2024). Therefore, cones need to be protected from pollen coming from the south where pollen cones have matured earlier due to warmer weather (Lindgren and Lindgren 1997). Gaining access to seed cones, for both crossing and harvesting, is often done by using a machine like a sky lift or a ladder since seed cones are mainly produced in the upper third of a tree. Unlike many crop species where elite lines are produced by inbred crosses creating uniform varieties, this is not possible in *P. abies*. Outcrossing is necessary for breeding due to partial self-incompatibility, severe seed inviability from self-pollination, and inbreeding depression (Hagman 1975 and references therein; Burczyk et al. 2004; Eriksson et al. 2009).

1.7 The necessity of seed orchards

Plant breeders in Sweden need to be aware of the different climatic regions and photoperiods in the country. Genotypes adapted to a specific environment and photoperiod will not necessarily thrive in a different one (Hagman 1980; Liziniewicz et al. 2023). For *P. abies*, Sweden is divided into 22 breeding populations and 14 seed orchard zones to account for regional differences (Westin and Haapanen 2013). Breeding populations overlap and seed orchards are established using material from a few of the nearby populations (Westin and Haapanen 2013). Seed orchards then produce seed for these breeding populations. In a seed orchard, clones of genotypes that have been identified to be used for breeding are planted (Westin and Haapanen 2013; Funda and El-Kassaby 2012). The intention is for these trees to cross pollinate with each other and produce offspring that are genetically superior. The offspring will be planted by forest owners as well as provide more easily accessible material to perform breeding activities. Because *P. abies* are wind pollinated and genotypes do not produce reproductive cones at the same rate, there will be some pollen contamination and biases in genetic contribution towards certain genotypes (Heuchel et al. 2022; Eriksson et al. 1973). Seed orchards are integral for the success of the breeding program and to produce high quality improved seed for the forest owners (Westin and Haapanen 2013; Funda and El-Kassaby 2012). In order

to meet the demand for seed, more seed orchards can be planted or methods for influencing cone production can be attempted. Establishing new seed orchards is a time consuming and expensive process. The material to be used needs to be identified and produced, the right type of land needs to be found and bought, and the trees need to be planted. Then there will be a wait, for an extended amount of time, before the trees start producing cones. It takes about 10-15 years before seed cones start to be produced and 15-20 years for full seed cone production (Curt Almqvist, personal communication). Also, the seed cone production from seed orchards will be irregular and difficult to predict due to the masting behavior of *P. abies* (Broome et al. 2007). This process can take decades and the desire for more improved seed is urgent. Until new seed orchards are being established, other ways to produce more seed need to be considered.

1.8 Summary of the issues

Forest products are a large part of the Swedish economy (Forests and Forestry in Sweden 2015). *P. abies* products have traditionally been a large part of the produced forest products. In order to meet the challenges of a changing climate and the demands of the market, more *P. abies* seed needs to be produced. This is complicated by the reality of spruce reproduction and our understanding of it. Our efforts in producing more reproductive cones for breeding and seeds for planting are hampered by, among other things, infrequent cone production, size of trees, long generation time, pollination mechanism, complexity of the breeding population, and genotype variation.

What can be done to address these problems?

- Learn more about the genetic mechanisms behind seed cone initiation and development.
- Learn more about influencing cone initiation.
- Better predictions for seed cone production.
- Plant more seed orchards.

2. How did seed cones get here and where are they going?

In order to produce more seeds, a thorough understanding of the initiation and development of seed cones is needed. What genes and environmental conditions regulate seed cone initiation and development? Is it possible to influence these processes? Are there mutants that can be studied to help understand reproduction like those used in model species? With a better understanding of why *P. abies* produce seed cones, researchers and foresters will be able to better predict and increase seed cone production both to research the processes involved and to produce improved seed for planting.

2.1 Evolutionary history of *P. abies* seed cones and their similarity to angiosperm flowers

The work of Rudolf Florin has been integral in describing the evolutionary history of conifer vegetative and reproductive growth (Florin 1951). In a series of lectures, he described the anatomy of fossilized vegetative and reproductive growth in ancient conifers and cordaites from the Carboniferous period to present day. The seed-scale complex of ancient conifers contained a shoot with spirally arranged upturned sterile scales and fertile scales, each containing an ovule (Florin 1951; Serbet et al. 2010). Bracts were two-forked, and each fork harbored a seed-scale complex in its axil (Florin 1951). This combination of structures is called a dwarf shoot (Florin 1951; Serbet et al. 2010). These dwarf shoots were arranged symmetrically in a radial pattern around a central axis (Florin 1951). Ancient conifers went through reductions in the length of their dwarf shoots and the number of scales and ovules on them. Eventually, in the *Picea* lineage, only one scale remained on each of the two dwarf shoots in the axil of a bract (Florin 1951). They eventually fused into a single scale that today still contains two ovules (Florin 1951). In extant conifers, the dwarf shoots have been completely reduced in length and consist only of the seed-scale complex and a few cells of vascular tissue (Florin 1951).

Florin defines a flower as a “sporophyll carrying shoot of finite growth” and compares them to the dwarf shoots of ancestral conifers. If these dwarf

shoots can be considered as equivalent to flowers, then a group of them associating radially along a central axis would be the equivalent of an inflorescence; more commonly referred to in modern conifers as a seed cone (Florin 1951). Though the morphology of a seed cone has changed through millions of years of evolution, its central identity as an inflorescence has not. If this was true, it would be expected to see genes related to flower meristem maintenance and development expressed in the scales and inflorescence meristem genes in the apical meristem of a developing seed cone. In modern day *Picea* species, seed cones are sclerified (hardened, lingified), have ovules that sit on a predeveloped axillary structure (scale), and have a micropyle that faces the cone axis (Herting et al. 2020).

2.2 Location of different bud types

The genetic and environmental conditions necessary for a meristem to decide what type of bud it will be are not currently understood, but a meristem's location within a tree or branch plays a large role. Vegetative primordia form on apical meristems on apical branches and on any other meristem that does not become a seed or pollen cone primordia. Although some primordia may become latent or terminate. In adult trees, vegetative shoots are the "default" bud type. Branches that develop seed cones tend to be vigorous. Apical meristems of one year old second order shoots in the top ⅓ of a tree are the most likely, but not only meristems, which can become seed cones. Seed cones can also form on the second order shoots of leading branches in lower parts of the tree and commonly on 2- or 3-year-old lateral shoots. Pollen cones generally form on meristems of less vigorous branches, often in the lower ⅔ of the trees and on the hanging, curtain branches. Pollen cones can form at the base of an extending shoot as well. Both seed and pollen cones are terminal shoots, meaning no more vegetative growth is possible due to the meristem being completely consumed or dying as part of the maturation process for both cone types. Even when environmental conditions are conducive to reproductive primordia initiation, not all of the meristems that could become reproductive primordia do become reproductive primordia. Unlike seed and pollen cones, the apical meristem in vegetative buds does not terminate and will start the primordia initiation and development process over again the following spring.

2.3 Initiation and development of *P. abies* primordia

The ontogeny of primordia in different spruce species has been extensively studied from a morphological point of view and genetic studies have recently become feasible (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Hejnowicz and Obarska 1995; Wenzel et al. 2024; Sundström 2001; Carlsbecker et al. 2013). Morphological changes from a small, undifferentiated meristem at the tip of an elongating shoot in the spring all the way through dormancy in the fall have been described, but research is still in its infancy in regard to exploring what is happening genetically during primordia initiation and development.

When winter months approach, developing spruce primordia become dormant and mitosis in their apical meristem stops (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Hejnowicz and Obarska 1995; Wenzel et al. 2024). Meristematic activity restarts in the spring when mitosis in the apical meristem of a vegetative bud resumes activity about one month before the bud starts to elongate and push away the bud scales (Hejnowicz and Obarska 1995; Wenzel et al. 2024). The meristems at the tips of these newly elongating vegetative shoots start developing new bud scales which continues until the meristem differentiates into a specific bud type in the summer (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Wenzel et al. 2024). In *P. abies*, bud scales are initiated the fastest early in the spring during bud flush and shoot elongation before bud scale initiation slows down when the shoot nears full elongation (Wenzel et al. 2024).

In meristems that become vegetative primordia, bud scale-like needles that were initiated the previous fall and outer bud scales form on the outer part of the bud and become brown and lignified and some inner bud scales form that are white and soft during “fast bud scale initiation” in early spring to summer (Wenzel et al. 2024). The rest of the inner bud scales are formed during “slow bud scale initiation” and become more needle-like as the meristem gets closer to bud type differentiation in the summer (Wenzel et al. 2024). When the shoots are nearly done extending, the developing bud scales become smaller and eventually stop differentiating from the meristem (Wenzel et al. 2024). Pre-bud type differentiation could then be described as two phases;

fast and slow bud scale initiation. Bud type differentiation happens near the end of shoot elongation, so (Wenzel et al. 2024) propose that this change in bud scale type and initiation rate could indicate a change in genetic signaling from scale dominant to needle dominant genetic signaling (Wenzel et al. 2024). The order of initiation of full and intermediate types of needles and bud scales suggest this could be happening. The change to more needle-like inner bud scales could be evidence of the meristem getting genetic signals to become a vegetative primordia before physical bud differentiation happens in phase 1 (apical dome formation) of bud development. Leaving the possibility that the shape or rate of initiation of the bud scales could be different between bud types, therefore enabling earlier identification of bud types.

When the shoots are nearly done extending, the bud scales that are initiating become smaller and eventually stop differentiating from the meristem (Wenzel et al. 2024). At this point, each meristem decides if it will become a vegetative bud, seed cone, pollen cone, become latent, or abort depending on which genetic inputs they have received during bud scale initiation (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Hejnowicz and Obarska 1995; Wenzel et al. 2024; Sundström 2001). Unlike North American spruce species whose meristems differentiate simultaneously, *P. abies* has a staggered differentiation (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001).

Three main phases of development, from a meristem to a fully formed, dormant primordia, have been described (Sundström 2001; Paper II). During phase 1, an apical dome is formed which is similar for all bud types. Mitotic activity is increased which leads to the apical meristem enlarging, changing in shape, and zonation of the cells (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). Potentially reproductive meristems undergo rapid apical growth as compared to vegetative meristems (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). The size of a meristem at the time of lateral organ initiation is correlated with what type of bud a meristem will develop into. Larger, bullet shaped meristems develop into reproductive primordia, while

narrow, pointed meristems develop into vegetative primordia (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001).

Phase 2 begins when the lateral organs begin to develop; needle primordia on vegetative primordia, bracts and ovuliferous scales on seed cone primordia, and microsporophylls on pollen cone primordia (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). Lateral organs develop rapidly in a spiral pattern from the meristem on the periphery of the primordia (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). In seed cone primordia, bracts initiate first from the apical meristem and ovuliferous scales follow and initiate in the axil of the bract. The primordia widens and grows longer as more lateral organs are formed (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001).

Phase 3 marks the end of new lateral organ development (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). The already developed lateral organs undergo cell differentiation; needle primordia on vegetative primordia, two mega-mother cells on each ovuliferous scale in seed cone primordia, and two microsporangia with pollen mother cells on each microsporophyll on pollen cone primordia; and the primordia prepare for winter dormancy (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). In *P. abies*, vegetative primordia enter phase 1 in at the end of July, phase 2 in mid to late August, and phase 3 in early October; seed cone primordia enter phase 1 in mid-July, phase 2 in early August, and phase 3 in early September; pollen cone primordia enter phase 1 in early July, phase 2 in late July and phase 3 in late August (Sundström 2001; Paper II).

2.4 How can *P. abies* bud development be studied?

Currently, there is no model organism for conifers like there is in angiosperms. Due to their long generation time, and often large size,

studying growth, development, and reproduction in a controlled laboratory setting is not feasible after a few growth cycles. The inability to perform comparable studies on reproduction like in angiosperms has made the study of the initiation and development of reproductive structures in conifers extremely difficult. In angiosperm model systems like *Arabidopsis thaliana*, it is possible to have many plants of the same ecotype growing in the same conditions and collect tissue from some of them while leaving others to continue growing. This provides the opportunity to observe what genes are active at a specific stage of development and be able to collect data on how those genes affect the plant, under the assumption that different plants of the same ecotype will behave similarly.

While it is possible to clone *P. abies*, either via rooting, grafting, or somatic embryogenesis, and be able to control the genotype in a similar way as in *A. thaliana*, the size of the plant, and the inability to know how a specific meristem will develop makes it challenging to perform experiments in the same way. Collecting *P. abies* meristems before bud type differentiation is not a hard process, but due to the infrequency of reproductive initiation, especially seed cones, it is not possible to know what tissue a specific meristem will become. Even when there is heavy cone production, not every meristem that has the potential to become a seed or pollen cone will become one. Additionally, as a perennial plant, the process of vegetative to reproductive growth/reproductive initiation is different than in annuals. In annual plants, there is continuous activity within the meristem that does not stop during the life of the plant. In *Picea* species, the activity of meristems drastically changes through the year, especially when the meristem becomes dormant during the winter (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Hejnowicz and Obarska 1995).

Because it has not been possible to study spruce under the same type of laboratory conditions that *A. thaliana* has, there is much less known about which genes are active before bud type differentiation under different environmental conditions. Through comparison of genes and genomes, homologs of many genes in *A. thaliana* and other species, have been identified (Tandre et al. 1998; Sundström et al. 1999; Sundström and Engström 2002; Carlsbecker et al. 2013). Though information about them in

P. abies may be limited to sequence similarity and expression patterns, their actual function in *P. abies* are not yet established.

Even with all the difficulties in studying initiation and development of different bud types, it is not impossible. Research had to focus on techniques that were available and affordable at the time. At first this meant western blots for proteins or Southern blots, RT-qPCR, or *in situ* hybridization for RNA and then later transcriptomic studies (Uddenberg et al. 2015). Quantitative and semi-quantitative methods are limited in spatial resolution of expression and beside transcriptomic studies, are limited in the number of genes that are able to be studied at a time. *In situ* hybridization is a complicated and laborious technique limited to one or a few target genes, but images of spatial expression is produced. The use of *in situ* hybridization has been a key technique for studying the location in which a transcript is active. A protocol for effective and non-radioactive *in situ* hybridization in *P. abies* has made it easier to perform these experiments (Karlgren et al. 2009). Both radioactive and non-radioactive *in situ* hybridization have been used when studying reproductive buds. They have been useful tools in understanding the development of *P. abies* buds before and after dormancy. In whole tissue transcriptomic studies, you obtain expression information about thousands of genes, but no spatial information. Since *P. abies* buds are not uniform masses of tissue, it is not possible to know where in the tissue a gene is expressed (Figure 2A, B). Genetic studies of all kinds has been helped by the publication of the *P. abies* genome; however, its incomplete nature has meant that there are many genes missing or unannotated (Nystedt et al. 2013).

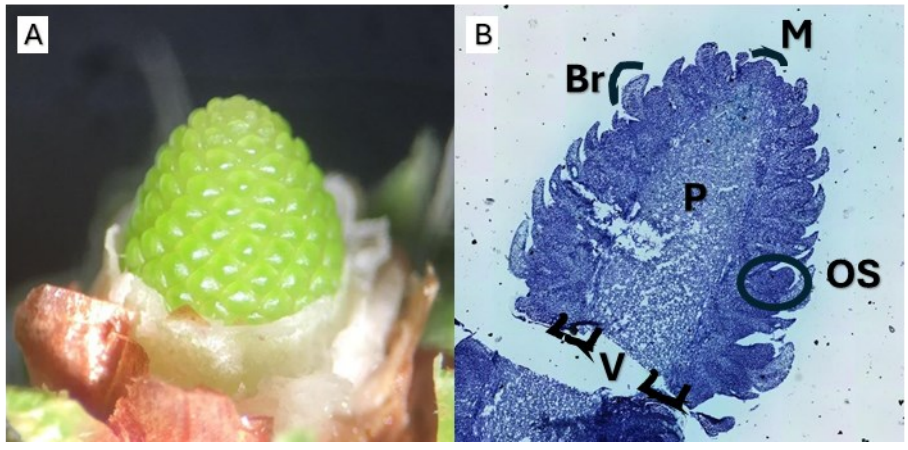

Figure 2. *P. abies* seed cone primordia in detail

(A) Seed cone primordia with bud scales removed in September. **(B)** A section of a seed cone primordia in September. M; Meristem, Br; Bract, P; Pith, OS; Ovuliferous Scale, V; Vasculature

2.5 Genetics of bud development

MADS-box (*MCM1*, *AGAMOUS*, *DEFICIENS*, *SRF*) genes are an ancient family of transcription factors present in most eukaryotic groups and are involved in many types of tissue development, especially reproductive structures and gametophytes, and some even have functions as homeotic genes that determine organ identity (Gramzow and Theissen 2010). The first MADS-box genes identified in non-angiosperms were *DAL1*, *DAL2*, and *DAL3* (*DEFICIENS*, *AGAMOUS-LIKE*) expressed in seed cones of *P. abies* (Tandre et al. 1995). All three genes were expressed in developing seed and pollen cones and *DAL1* and *DAL3* were also expressed in developing vegetative primordia (Tandre et al. 1995). Since then, a long list of genes, not only MADS-box genes, involved in reproductive bud development have been identified. *DAL1* increases in expression as a *P. abies* tree ages and has been proposed as part of the juvenile to adult regulatory mechanism (Carlsbecker et al. 2004). If a cone is considered an inflorescence and the bract-ovuliferous scale complex considered a flower, like Florin suggests, then there is the possibility to identify similar pathways of reproductive initiation and homologs from the ABCE flowering model that may have similar functions.

There are two main steps in the reproductive development process, first, a meristem changes from vegetative to reproductive growth becoming an inflorescence meristem (IM), then floral meristems (FM) are initiated from the IM and flowers develop. The IM is indeterminate and initiates many FM. According to the ABCE model, when a floral meristem is initiated, different genes and gene combinations are responsible for the development of floral organs (reviewed by: Ó'Maoiléidigh et al. 2013a). Type A genes (*APETALA1* (*AP1*) and *APETALA2* (*AP2*)) are involved in sepal and petal formation, type B genes (*PISTILLATA* (*PI*) AND *AP3*) in petal and stamen formation, and type C genes (*AGAMOUS* (*AG*)) in stamen and carpel formation (Bowman et al. 1991). With more research, type E genes (*SEPALLATA* (*SEP1/2/3/4*) were identified as integral co-factors in support of the A, B, and C genes (Pelaz et al. 2000; Ditta et al. 2004).

Although the bract-ovuliferous scale complex in *P. abies* is not quite as complicated in regard to phenotype as angiosperm flowers, if it shares common ancestry with angiosperm flowers, then there should be ABCE homologs expressed during development. In fact, this is what has been observed. Phylogenetic analysis shows *P. abies* MADS-box genes grouping with genes in class B (*DAL11*, *DAL12*, *DAL13*), class C (*DAL2*), and class E (*DAL1*, *DAL14*), and genes in the AP2/ETHYLENE RESPONSE FACTOR (ERF) family with class A (*AP2L1*, *AP2L2*, *AP2L3*) (Nilsson et al. 2006; Carlsbecker et al. 2013; Sundström et al. 1999; Tandre et al. 1995). *In situ* hybridization experiments on these genes has shown remarkable specificity in some of their expression patterns. In seed cone primordia, expression of *AP2L1*, *AP2L3*, *DAL2*, *DAL14*, and other non-ABCE genes like *DAL3*, *DAL19*, *DAL21*, and *AtLEAFY* (*LFY*) homolog *PaLFY* was almost entirely restricted to ovuliferous scale while *DAL10* was expressed in bracts and vascular tissues. A second *AtLFY* homolog, *PaNEEDLY* (*NLY*), was expressed in the ovuliferous scales and bracts (Carlsbecker et al. 2013). The specificity of expression of floral meristem maintenance and development homologs in the bract-ovuliferous scale complex support Florin's hypothesis that the complex should be considered a flower.

Most of the research into bud development has been limited to phase 2 or later of seed cone bud development, leaving other bud types and large

amounts of time unstudied. For instance, Nilsson et al. 2006 speculated that *AP2L3* might be marking the location of meristem activity due to it being expressed not only in the ovuliferous scale, but also in vegetative meristems and needle primordia. This begs the questions: are the other genes only expressed in seed cone primordia after bract and ovuliferous scale differentiation and are they female lateral organ specific? In seed cone primordia, *PaLFY* was expressed on the sides of the apical meristem, but not in the axils of developing bracts, suggesting its importance in initiating bracts from the meristem (Carlsbecker et al. 2013). The opposite expression was seen with *DAL2* and *DAL21* as they were not expressed in the apical meristem, but are expressed in the axil of the bract, initiating the development of the ovuliferous scale, while *DAL14* was not expressed in the ovuliferous scale until it had already been formed (Carlsbecker et al. 2013).

Of the genes studied and mentioned above, only *DAL21* was expressed exclusively in seed cone primordia, while *DAL2* and *DAL14* were limited to reproductive primordia and *DAL11*, *DAL12*, and *DAL13* were limited to pollen cone primordia (Carlsbecker et al. 2013; Sundström et al. 1999). The others, *DAL1*, *DAL3*, *DAL10*, *DAL19*, *PaLFY*, and *PaNLY* are expressed in vegetative and reproductive primordia, so are not seed cone specific (Carlsbecker et al. 2004; Carlsbecker et al. 2013; Uddenberg et al. 2012).

Beyond genes that are involved in reproductive development, there are many other types of genes needed for bud development in spruce species. From sensing day length and temperature in preparation for winter dormancy to the development of other tissue types like vascular tissues, there are many areas of development that can be studied (Karlgren et al. 2011; Karlgren et al. 2013a; Karlgren et al. 2013b; El Kayal et al. 2011; Asante et al. 2010; Vergara et al. 2021; Bag et al. 2021). The genes active in non-reproductive developmental events are often not studied in developing fall primordia and instead experiments are performed with needles or bark tissue. The lack of experiments that focus on bud development means that results do not reflect what processes are happening in the buds during developmental events.

3. *A. thaliana* flowering pathways and their relevance to *P. abies*

3.1 Pathways in brief

Eight flowering pathways in *A. thaliana* have been described, and a database was created to help navigate the interactions within and between them (Bouché et al. 2015). It is not currently known how many of these pathways that are evolutionary conserved between angiosperms and gymnosperms. The pathways are: Circadian clock, Photoperiod, Sugar, Temperature, Vernalization, Autonomous, Aging, and the Hormonal pathways. Direct regulation of flowering occurs when these pathways converge on "integrators." Integrators are one or a few genes that a pathway culminates in before floral meristem identity genes are activated. In short, the vernalization and autonomous pathways converge on and repress *FLOWERING LOCUS C* (*FLC*) which releases its repression of *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) expression (Michaels et al. 2005; Helliwell et al. 2006). The temperature pathway represses *SHORT VEGETATIVE PHASE* (*SVP*) at high temperatures to release repression of *FT* and *SOC1* expression (Lee et al. 2007). The circadian clock and photoperiod pathways activate *FT* expression through GIGANTEA (GI) and the protein complexes CONSTANS (CO) makes (Sawa and Kay 2011; Kumimoto et al. 2008; Kumimoto et al. 2010; Tiwari et al. 2010; Song et al. 2011). The aging pathway indirectly activates *FT* through *SQUAMOSA PROMOTER BINDING-LIKE* (*SPL*) genes. Some SPLs upregulate *miR172* which regulate *APETALA2* (*AP2*) and *AP2-like* genes through translational repression (Aukerman and Sakai 2003; Chen 2004; Wu et al. 2009; Mathieu et al. 2009). This leads to *FT* and floral meristem genes to no longer be repressed by AP2 and AP2-like proteins (Mathieu et al. 2009; Yant et al. 2010; Zhang et al. 2015a). The aging pathway also directly activates *SOC1* and floral meristem identity genes through SPLs (Yamaguchi et al. 2009; Wang et al. 2009; Yamaguchi et al. 2014). The sugar pathway indirectly activates *FT* through T6P (trehalose-6-phosphate) and indirectly activates *SOC1* through repression of miRNA156 by *HEXOKINASE 1* (*HXK1*) and sugar accumulation (Yang et al. 2013; Wahl et al. 2013). The hormone pathway indirectly activates *TWIN SISTER OF FT* (*TSF*) through cytokinins (D'Aloia

et al. 2011). Gibberellin Acid (GA) is bound by *GA INSENSITIVE DWARF 1* (*GID1*) to the degradation of DELLA proteins that are inhibiting the protein function of SPLs, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and NUCLEAR FACTOR-Y (NF-Y) (Yu et al. 2012; Hyun et al. 2016; de Lucas et al. 2008; Hou et al. 2014). The GA part of the hormone pathway indirectly activates *FT* via SPLs and PIF4 and *SOC1* via SPLs and *SOC1* directly through NF-Ys (de Lucas et al. 2008; Kumar et al. 2012; Yamaguchi et al. 2009; Wang et al. 2009; Hou et al. 2014).

3.2 *SOC1-like* genes exist in *P. abies*, but *FT-like* genes may not

Homologs in *P. abies* have potentially been found for both of the most important floral integrator genes, *AtFT* and *AtSOC1*. In a phylogenetic analysis of *FT-like* genes in (Karlgren et al. 2011; Klintenäs et al. 2012), they found the angiosperm genes to group into three different clades, *MOTHER OF FT* (*MFT*), *TERMINAL FLOWER1* (*TFL1*), and *FT*, but the *P. abies* genes did not follow this pattern. *PaMFT* genes are grouped with angiosperm *MFT* genes, but *P. abies FLOWERING LOCUS T/TERMINAL FLOWER1-Like* (*PaFTL*) and *PaTFL1* genes are grouped on their own. (Karlgren et al. 2011; Klintenäs et al. 2012). The *P. abies* genome project was not able find *AtFT* homologs nor has the recent *Pinus tabuliformis* genome (Nystedt et al. 2013; Niu et al. 2022). However, in a different analysis of many gymnosperm clades, including *P. abies*, two *FT-like* genes were potentially identified, and *PaFTL1* and *PaFTL2* were considered as *FT-like* genes (Liu et al. 2016). Whenever conifer *FT/TFL1-like* genes have been transformed into *A. thaliana*, flowering was delayed, while conifer *MFT* genes have had no influence or a weak, but statistically significant delay, in flowering time in *A. thaliana* (Karlgren et al. 2011; Klintenäs et al. 2012; Liu et al. 2016; Niu et al. 2022). This may have to do with it being a heterologous system, but further analysis in *P. abies* shows that *PaFTL1* and *PaFTL2* are more active in regulating bud set/preparation for winter dormancy and control of meristem activity (Gyllenstrand et al. 2007; Karlgren et al. 2013a). Conifer *FTL-like* genes are expressed in vegetative and reproductive primordia (Karlgren et al. 2011; Liu et al. 2016). The results from these studies suggest that conifer *FT-like* genes function more like *AtTFL* than *AtFT* (Karlgren et al. 2011; Niu et al. 2022).

In *A. thaliana*, *AtTFL1* is important in maintaining inflorescence meristem indeterminacy and helps regulate when and where a flower will begin to form by repressing the expression of *AtAP1* and *AtLFY* in the inflorescence meristem (Shannon and Meeks-Wagner 1991; Shannon and Meeks-Wagner 1993; Ratcliffe et al. 1999; Liljegren et al. 1999). However, it is possible for a single amino acid change in either *AtTFL1* or *AtFT* to swap their function in flowering from repressor to activator vice versa (Hanzawa et al. 2005). Expression of *PaFTL1*, *PaFTL2*, and *PaAP2L* have not been studied spatially in seed cone primordia, so it is not known if they have the same expression patterns as *A. thaliana* homologs of these genes. *PaLFY* has been studied spatially (Carlsbecker et al. 2013). *AtLFY* and *AtAP1* are expressed on the periphery of an inflorescence meristem denoting the location of a floral meristem (Ratcliffe et al. 1999; Carlsbecker et al. 2013). In a similar way *PaLFY* is expressed on the sides of the apical meristem in a seed cone primordia suggesting a similar function in both species (Carlsbecker et al. 2013).

Due to the lack of *FT* and *FLC* homologs with similar function in *P. abies*, flowering pathways that rely on them are less likely to behave the same way as they do in *A. thaliana* (Karlgren et al. 2011; Karlgren et al. 2013a; Liu et al. 2016; Nystedt et al. 2013). This leads to more interest in the pathways, and parts of pathways, that do not rely on FT or FLC, and instead either bypass the main floral integrators or integrate on SOC1, which does have homologs in *P. abies* (Tandre et al. 1995; Carlsbecker et al. 2013; Akhter et al. 2018; Uddenberg et al. 2012). The identified SOC1-like genes in *P. abies* are *DAL3*, *DAL4*, *DAL9*, and *DAL19* (Carlsbecker et al. 2013; Akhter et al. 2018; Uddenberg et al. 2012). *DAL19* and *DAL3* do not express in the apical meristem of a seed cone primordia, but they do express at the base of developing ovuliferous scales with *DAL19* expressed higher in more basal ovuliferous scales and *DAL3* in more apical ones (Carlsbecker et al. 2013). The gymnosperm specific *DAL21* has a similar expression pattern but is expressed higher and expresses further into the scale, and during initiation of a new ovuliferous scale in the axil of a bract (Carlsbecker et al. 2013). These *DAL* genes have an expression pattern that is similar to *AtSOC1*. *AtSOC1* is expressed in the inflorescence meristem and only in the early stages of the floral meristem and upregulates floral meristem identity genes (Immink et al. 2012; Adrian et al. 2009). *DAL2*, an *AGAMOUS* (*AG*) floral meristem

identity gene homolog, is expressed throughout a developing scale, but preferentially in the newly formed ovuliferous scales (Carlsbecker et al. 2013). It is also expressed in the axils of bracts marking location for the development of a new scale (Carlsbecker et al. 2013). In *A. thaliana*, *AG* is involved in floral meristem maintenance and termination (Gómez-Mena et al. 2005; Adrian et al. 2009; review: Prunet et al. 2009).

If the seed cone is an inflorescence and an ovuliferous scale is equivalent to a single flower as (Florin 1951) suggests, the homologs for *SOC1* and *AG* target genes could be activated by *DAL3* and *DAL19* and *DAL2* in the bract-ovuliferous scale primordia in the fall. In *A. thaliana*, SOC1 directly upregulates floral timing and flower development genes including *AGL24*, *LFY*, *SEPALLATA3* (*SEP3*), *PISTILLATA* (*PI*), *AP3*, and *SPL3/4/5* and represses *miR156*e, *AP2*, *AGL15/18*, and *CRT/DRE-BINDING FACTOR* (*CBF*) *1/2/3* (Immink et al. 2012; Jung et al. 2011). In *A. thaliana*, AG can potentially bind to regulatory regions of around 2000 genes during early flower development, though about 88% of them do not react to changes in AG activity (Ó'Maoiléidigh et al. 2013b). AG controls floral meristem identity by forming protein complexes to downregulate stem cell identity gene, *WUSCHEL* (*WUS*) and transitions the meristem to organ development through upregulating an auxin biosynthetic gene *YUCCA4* (*YUC4*) (Guo et al. 2018; Bollier et al. 2018; Sun et al. 2019; Smaczniak et al. 2012; Yamaguchi et al. 2018). During flower development AG forms protein complexes with other floral organ identity genes and regulates a wide range of genes including upregulating *AG*, *PI*, *SEP3*, *AP3*, *and UNUSUAL FLORAL ORGANS* (*UFO*) and downregulating *INNER NO OUTER* (*INO*), and *AP1* (Honma and Goto 2001; Gustafson-Brown et al. 1994; Smaczniak et al. 2012; Favaro et al. 2003; Ó'Maoiléidigh et al. 2013b; Kaufmann et al. 2009; Wuest et al. 2012).

3.3 Flowering pathways without FT interaction

Indirect initiation of flowering in *A. thaliana* can occur through most of the pathways, and it is also possible to bypass the integrators and initiate flowering. The temperature, autonomous, sugar, hormonal, photoperiod, and circadian clock pathways can indirectly initiate flowering. The aging and hormonal pathways can avoid *FT* and initiate flowering through *SOC1*. The

vernalization and aging pathways can bypass integrator genes and directly initiate flowering.

In the temperature and autonomous pathways, FLOWERING CONTROL LOCUS A (FCA), and in the circadian clock pathway, GI, converge on the aging pathway by upregulating *miR172* which represses *AP2-like* genes (Jung et al. 2007; Aukerman and Sakai 2003; Chen 2004; Wu et al. 2009; Mathieu et al. 2009). With lower expression of *AP2-like* genes, *miR156* is expressed less, allowing for the expression of the *miR156* targeted *SPLs* (Wang et al. 2009; Wu et al. 2009). The sugar pathway indirectly leads to a reproductive shift by working in conjunction with the aging pathway by repressing *miR156* expression via *HXK1* and sugar accumulation and upregulating *SPLs* thereby allowing SPLs to be expressed and upregulate *SOC1* and floral meristem identity genes (Yang et al. 2013; Wahl et al. 2013). The photoperiod and circadian pathways converge to induce flowering through CO and NF-Y protein interactions on the *SOC1* promoter in long days (Hou et al. 2014). The hormonal pathway indirectly initiates flowering by converging with the aging pathway through GA mediated degradation of DELLA proteins that inhibit protein SPLs function (Murase et al. 2008; Yu et al. 2012; Hyun et al. 2016). The vernalization pathway bypasses *SOC1* through cold induced de-repression of *AGAMOUS-LIKE 19* (*AGL19*) which activates *LFY* and *AP1* (Schönrock et al. 2006). The aging pathway can bypass *SOC1* through SPLs binding to the promoters and upregulating three floral meristem identity genes, *LFY*, *FRUITFULL* (*FUL*), and *AP1* (Yamaguchi et al. 2009; Wang et al. 2009). Though for this to happen, SPL expression needs to overcome repression by DELLA proteins and *miR156* repression. Both the aging and hormone pathways can directly initiate flowering through *SOC1* interactions. This occurs through *SPLs* in the aging pathway and through *NF-Ys* in the hormone pathway (Yamaguchi et al. 2009; Wang et al. 2009; Hou et al. 2014).

3.4 CO and NF-Y and GA interactions with *SOC1*

CO is a transcription factor that responds to the circadian clock and is active in the photoperiodic pathways of flower development. It helps mediate the signals between the circadian clock and photoperiod pathways to control flowering. *CO* is a tightly regulated gene because the plant needs to keep

track of day length and day/night cycles. It is regulated by transcription, protein stability, and binding by protein partners. *CO* transcripts fluctuate through the day and in short day conditions, its protein is degraded via a protein complex of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF PHYA-105 1 (SPA1) (Laubinger et al. 2006; Liu et al. 2008). In long days, transcripts and proteins of CO accumulate due to the GI-FKF1 (FLAVIN-BINDING, KELCH REPEAT1) complex degrading the protein of the CO repressors, CYCLING DOF FACTORS (CDFs), and positively regulating expression of CO through interactions with its promoter (Sawa et al. 2007).

The *NF-Y* family of transcription factors form a heterotrimeric protein complex from three subunits called *NF-YA*, *NF-YB*, and *NF-YC*. In *A. thaliana*, there are 10 *NF-YA* genes, 13 *NF-YB* genes, and 13 *NF-YC* genes totaling 36 genes allowing for hundreds of potential heterotrimers (Siefers et al. 2008). NF-YA can bind to the CCAAT-box in the promoters of its target genes, and most *NF-YA* genes harbor a miRNA169 binding site (Hou et al. 2014; Zhao et al. 2017; Sorin et al. 2014). *NF-Ys* have been implicated in a wide range of developmental processes and responses (for reviews see: Zanetti et al. 2017; Zhao et al. 2017). The genes within this family do not necessarily have similar functions, but genes in each subunit have been shown to be involved in the regulation of flowering time via long days (Zhao et al. 2017). Some of them promote flowering while others inhibit flowering (Kumimoto et al. 2010; Wenkel et al. 2006).

The GI-FKF1 protein complex upregulates *CO* and degrades *CO* repressor CDF1 (Sawa et al. 2007). Physical interactions between DELLA proteins and all three NF-Y complex subunit proteins (Hou et al. 2014), CO (Xu et al. 2016a), and BRAHMA (BRM) (Zhang et al. 2023) have been established. Although CO protein abundance is not influenced by GA signaling, the binding of CO and NF-Ys by DELLA proteins inhibits interactions between CO and NF-Ys resulting in *SOC1* not being upregulated (Xu et al. 2016a; Hou et al. 2014). The proteins of NY-YA, many of which are targeted by *miR169*, can interact with the promoter of *SOC1* at the NFYBE domain (Lee et al. 2010; Gyula et al. 2018; Hou et al. 2014). The expression of *miR169* is comparatively upregulated at lower temperatures (15-16C) and downregulated at higher temperatures (21, 23, 27C) (Lee et al. 2010; Gyula

et al. 2018). This difference in temperature is enough for transcripts of the *NF-YA* genes to accumulate at 21, 23, 27C (Lee et al. 2010; Gyula et al. 2018). Hou et al. 2014 propose a mechanism of how the protein interactions between CO, NF-Ys, BRM, and DELLAs eventually upregulate *SOC1*. Under short day conditions with no GA, the NF-YA-YB-YC complex is bound by a DELLA protein, NF-YC is bound by a BRM-DELLA complex, and BRM-DELLA interact with the *SOC1* promoter to stop transcription, stopping NF-YA2 from binding to the NFYBE domain in the *SOC1* promoter (Hou et al. 2014; Zhang et al. 2023). In the presence of GA, the DELLA proteins are degraded and the NF-Y proteins and the SOC1 promoter are no longer inhibited (Hou et al. 2014; Zhang et al. 2023). The NF-YA-YB-YC complex recruits RELATIVE OF EARLY FLOWERING6 (REF6) to help demethylate H3K27me3 and allow SOC1 transcription (Hou et al. 2014). In long day growth conditions, DELLA proteins are still active but are not able to stop the interactions between the NF-YA-YB-YC complex and CO (Hou et al. 2014). The NF-YA-YB-YC complex binds to the NFYBE on the *SOC1* promoter, CO binds to two CO-responsive elements flanking the NFYBE, and REF6 is recruited to the protein complex and demethylation of H3K27me3 follows, leading to the transcription of *SOC1* (Hou et al. 2014).

3.5 Drought and flowering - NF-Y and Abscisic acid (ABA) drought response induce *SOC1* expression

In response to drought conditions, a plant may induce drought-escape responses, mediated by the hormone ABA, in order to reproduce before dying (Riboni et al. 2013; Fang and Xiong 2015). A study on drought response induced flowering in *A. thaliana* found that two ABA binding factors (ABF3 and ABF4) positively regulate the transcription of *SOC1* (Hwang et al. 2019). However, there is no binding motif for these proteins in the SOC1 promoter, but ABF3 and ABF4 were active in the region where the NF-Y binding element is (Hwang et al. 2019). They showed that NF-YC directly interacts with ABF3 and ABF4 at the NFYBE in the *SOC1* promoter to positively regulate expression that lead to flowering (Hwang et al. 2019).

3.6 *CO* and *NF-Y* genes in conifers

CO homologs have been described in *P. abies* and *P. tabuliformis* (Holefors et al. 2009; Yang et al. 2024). Two *PaCOL* (*CONSTANS-LIKE*) genes group with *AtCOL3* and *AtCOL4* and *AtCOL3*, *AtCOL4*, and *AtCOL5* in a phylogenetic analysis (Holefors et al. 2009; Yang et al. 2024). *PaCOL1* and *PaCOL2* are upregulated in the shoot tips in the morning when it becomes light and down regulated when it gets dark in the evening (Holefors et al. 2009). Expression of both genes decreases when grown in short day conditions (Holefors et al. 2009). Other gymnosperm *CO* homologs may induce flowering in *Ginkgo biloba* or respond to GA by interacting with an NF-Y protein to regulate reproductive cone development in *P. tabuliformis* (Yan et al. 2017; Liu et al. 2022). There is not much research into *NF-Y* genes in *Picea* in general, but there are a few genes that have been identified in different *Picea* species. (Uddenberg et al. 2011; Yu et al. 2011; Zhang et al. 2015b). It is not currently known if *miR169* reacts to temperature in *P. abies*. If this pathway is similar to *A. thaliana*, warmer and longer days leads to lower *miR169* expression and higher *NF-YA* and *CO* expression. The PaNF-Ys could then form protein complexes with and without PaCOL that bind to the promoter of *SOC1* homologs (e.g., *DAL3*, *DAL19*) to initiate cone production.

3.7 The *miR156-SPL* module and flowering

The *SPL* family are plant specific transcription factors that are involved in many different developmental programs in flowering plants (Birkenbihl et al. 2005). They contain a conserved sequence of 76 amino acids referred to as the SBP-domain (SQUAMOSA-PROMOTER BINDING PROTEIN) that recognizes and binds to a palindromic core motif, GTAC, to regulate gene expression (Birkenbihl et al. 2005). At least 10 of the members of this family are regulated post-transcriptionally by *miR156* in *A. thaliana* (Wu et al. 2009; Xing et al. 2010; Gandikota et al. 2007; Huijser and Schmid 2011). The *miR156-SPL* module is an ancient pathway regulating the transition from juvenile to reproductive growth (Alisha et al. 2024; Tsuzuki et al. 2019). Expression of *miR156* is highest in young plants before slowly being inhibited through different processes as the plant ages (Schwarz et al. 2008; Wu and Poethig 2006; Wu et al. 2009; Guo et al. 2017; Wahl et al. 2013).

As *miR156* is downregulated, transcripts and proteins of *miR156* targeted *SPLs* begin to accumulate and some of them upregulate *miR172* (Schwarz et al. 2008; Wu and Poethig 2006; Gandikota et al. 2007; Wahl et al. 2013; Wu et al. 2009). Then, *miR172* stops translation of *AP2-like* genes that delay flowering by repressing *FT*, *SOC1*, and floral meristem genes (Aukerman and Sakai 2003; Chen 2004; Mathieu et al. 2009; Yant et al. 2010; Zhang et al. 2015a). Most of the *SPLs* targeted by *miR156* are involved in promoting aging in *A. thaliana*. Expression of *miR156* is sensitive to temperature and is expressed higher at ambient temperature and at extreme high temperatures during heat stress in *A. thaliana* (Kim et al. 2012; Lee et al. 2010; Stief et al. 2014). *SPL3/4/5*, have been shown to promote flowering and the transition to a floral meristem (Cardon et al. 1997; Gandikota et al. 2007; Wu and Poethig 2006), *SPL2/9/10/11/13/15* promote juvenile to adult phase transition and vegetative to reproductive transition (Wang et al. 2008; Wu et al. 2009; Schwarz et al. 2008; Martin et al. 2010; Xu et al. 2016b), and *SPL6* is active in the positive regulation of defense genes (Padmarabhan et al. 2013).

At least two members of the family, SPL3 and SPL9, bind to the promoters of and upregulate *SOC1* as well three floral meristem identity genes, *LFY*, *FUL*, *AP1* to promote flowering without the need of *FT* or *SOC1* as a mediator (Yamaguchi et al. 2009; Wang et al. 2009). SPL9 recruits RGA, a DELLA protein, to promote expression of *AP1* after GA concentration has gone down in the meristem (Yamaguchi et al. 2014). A mutant line harboring an *miR156* resistant and overexpressing version of *SPL3* flowers significantly earlier than the WT (Wu and Poethig 2006). Some SPLs are targeted and degraded by DELLA proteins, so GA mediated degradation of DELLAs is an important regulator of flowering though SPL activity (Galvão et al. 2012; Yu et al. 2012; Wang and Deng 2011). To further complicate things, both *miR156* and *SPL9* are upregulated by a GAMYB (MYB33) binding to their promoters and can directly upregulate floral meristem gene *LFY* (Guo et al. 2017; Gocal et al. 2001). Eleven *SPL* genes have been identified in *P. abies*, but their functions have not been explored (Nystedt et al. 2013; Zhang and Ling 2018). One difference between *P. abies* SPLs and *A. thaliana* SPLs is the existence of *miR529* and a combined *miR156/529* binding site, which has been lost in the eudicot lineage (Zhang and Ling 2018; Zhang et al. 2015c).

In a strange twist, the *miR156-SPL* module and *NF-Y* transcription factors interact in *A. thaliana*. Overexpression of one of the *NF-YA* genes (*NF-YA8*) in *A. thaliana* leads to lower expression of *FT*, *SOC1*, and *SPLs* and delayed flowering (Zhao et al. 2020). They also observed higher expression of *miR156*a, c, d, and e which was upregulated due to *NF-YA8* binding to their promoters and upregulating expression (Zhao et al. 2020). Under sugar treatment, the expression of *NF-YA8* is significantly lower which leads to lower expression of *miR156* and flowering through unrepressed SPLs (Zhao et al. 2020). In *Chrysanthemum morifolium* an NF-YB protein (CmNF-YB8) could bind to the promoter of *cmo-miR156* in yeast and that *CmNF-YB8* silenced lines had lower *cmo-miR156* expression and higher *CmSPL3/5/9* expression which led to earlier flowering (Wei et al. 2017).

4. *Picea abies* reproductive initiation and development

4.1 The *acrocona* mutant

Mutant genotypes of a species are useful tools to understand developmental processes. Most of the *A. thaliana* genes discussed previously have used mutants to help understand their function. In *A. thaliana*, mutants are identified based on phenotype and the gene or genes responsible are described or they can be generated in the lab to study a chosen gene's function under different conditions. Although there are some studies in mutant conifers, they are not widely used for research (Eklund and Tiltu 1999; Kong et al. 2021). Reasons for not using conifer mutants include yearly growth cycle, large size, missing or not fully annotated genomes, difficulties with transformation, and long generation times and inbreeding depression hampering the production of homozygotes. Although it is often not practical to use a mutant conifer in research, conifer mutants, including spruce, are popular garden plants. Some varieties come from witch's brooms (somatic mutation leading to different phenotype than the original tree) while others are seedling selections or natural mutants found in the wild. Dwarf (*P. abies* 'Pumila'), creeping (*P. abies* 'Formánek'), columnar (*P. abies* 'Cupressina'), weeping (*P. abies* 'Inversa'; *P. abies* 'Reflexa'), and non-branching (*P. abies* 'Virgata'; for more information about a 'Virgata' forest in Sweden see Gunnert 1962) are all popular growth forms with many different varieties in each form. There are also many varieties with unusual needle colors. Some colors are stable while others are only present during bud flush. Stable needle colors are normally a lighter shade of green or shades of yellow and are uncommon in the wild. There are also variegated (*P. abies* 'Glimra') varieties. The colorful needles during bud flush are short lived, and they turn green by the summer. Colors include red (*P. abies* 'Cruenta'; *P. abies* 'Rydal'), pink (*P. abies* 'Roseospicata'), yellow (*P. abies* 'Perry's Gold'), and cream/white (*P. abies* 'Argenteospica').

Mutants related to reproduction with a discernible phenotype are much rarer in spruce species than other types of spruce mutants or flowering mutants in *A. thaliana*. Despite this, one *P. abies* ('*acrocona*') and three *P. pungens* ('Early Cones', 'Hermann Naue', 'Ruby Teardrops') reproduction mutants

exist (Figure 3A, B, C, D). They all have similar mutant phenotypes, but only *P. abies* '*acrocona*' (referred to as *acrocona*) has been described. *Acrocona* is a natural mutant that was first described as "monströs" in Uppsala by Theodor Fries in 1890. Reports of trees with the same phenotype were also made around Söderköping, Orsa, and Tåsjöberget (Joneborg 1945). *Acrocona* has an unusual habit of creating "transition shoots" or TS which are leading, apical shoots that when it is time for bud type differentiation, the meristem initiates and develops vegetative tissue first, but during development, they transition to developing female tissue. The tendency to produce abundant TS with the characteristic deep red seed cone structures at a young age as well as its relatively small size due to its slow growth habit have made this variety (and its many, many derivatives) a popular garden plant as well as a convenient way to study female cone initiation and development.

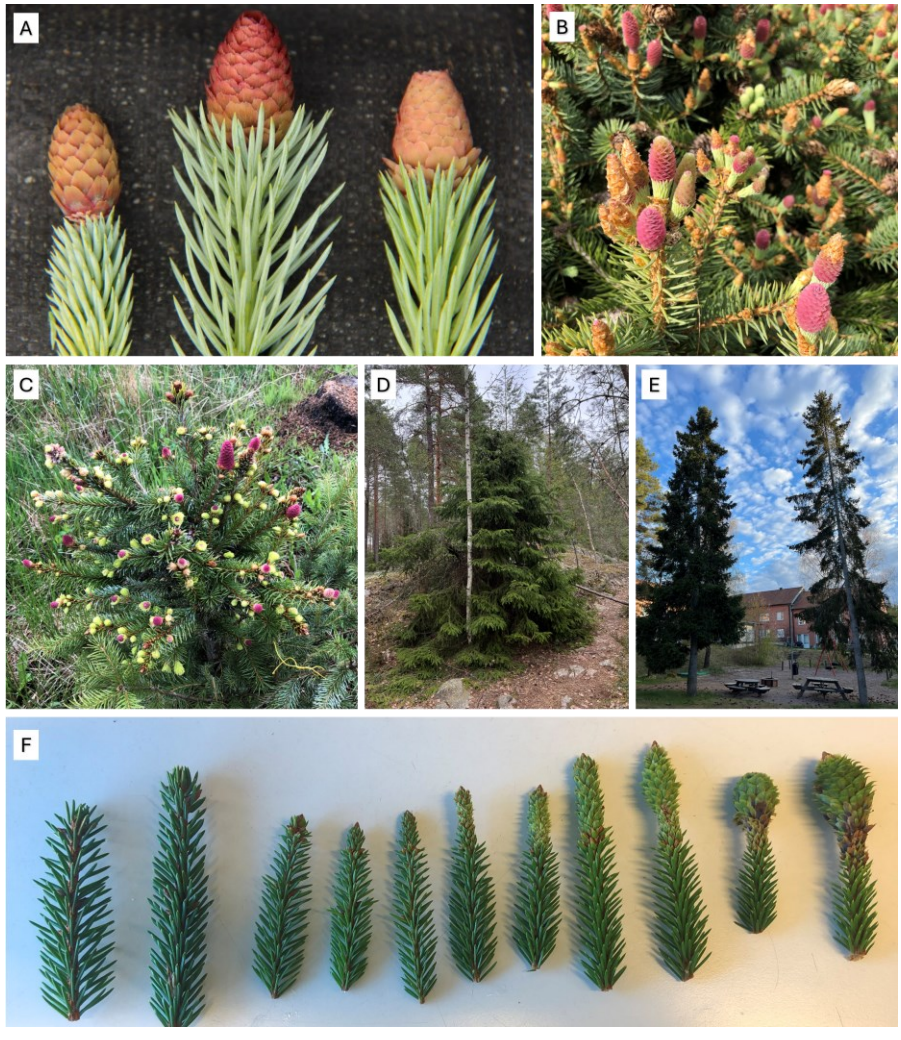

Figure 3. *Picea* reproductive mutants

(A) *P. pungens* mutants; 'Early Cones', 'Ruby Teardrops', Hermann Naue' (picture courtesy of Sam Pratt) **(B)** *P. pungens* 'Hermann Naue' **(C)** Homozygous *acrocona* **(D)** Wild growing 30+ year old *acrocona*. **(E)** Two wild type *P. abies* with very different growth habits **(F)** Two left, WT vegetative; rest; different amounts of *acrocona* "transition" to female growth

A TS is not formed on every leading meristem, and the amount a shoot transitions is variable (Uddenberg et al. 2012; Carlsbecker et al. 2013) (Figure 3F)**.** A TS can have as little as a few feminized needles at the apical end of the shoot or as much as 50% of the bud is seed cone-like with

functioning female organs capable of developing viable seed (Figure 3F). The *acrocona* trees produce more TS and more fully transitioned TS when the weather is more favorable for cone initiation. In more fully transitioned TS, needle primordia are developed at the basal end of a bud, the bract-ovuliferous scale complex is developed on the apical end, and in between them is a steady transition of lateral organs from more vegetative to more reproductive characteristics. As rows of lateral organs are initiated, needles slowly become feminized and more bract-like. Eventually ovuliferous scale-like structures with no or malformed ovules develop in the axils of needle-like bracts. Sometimes these ovuliferous scale-like structures are three lobed instead of two lobed, potentially pointing to ancestral forms that produced more than two fertile scales on a dwarf shoot (Florin 1951). And if the transition to female tissue is complete, fully formed, fertile ovuliferous scales develop in the axils of bracts. In TS like this, the meristem most often follows the seed cone developmental plan and arrests and dies, but if the transition to female tissue is not complete, the apical meristem may remain active enough to survive and initiate a new bud the next year. Since *acrocona* trees produce terminal seed cone-like tissue on the apical meristems of leading shoots, the growth of these trees is severely reduced and often bushy. Also, needles on *acrocona* trees are generally sharper than WT *P. abies*.

Like the other bud types, TS go through the three post-initiation phases of development. Even though TS start development making needle primordia, they go through the phases of development in a time frame more similar to seed cone primordia. In TS that fully transition, they enter phase 1 in mid-July. In mid-August, they develop needle primordia as part of phase 2 until mid-September where they start developing female-like lateral organs. This delays the start of phase 3, which in seed cone primordia starts in mid-September and in vegetative primordia in late September. TS continue producing female-like lateral organs until reaching phase 3 in mid-October.

The genetics behind the *acrocona* phenotype have been elusive. One study suggested that the *acrocona* phenotype is dominant and monogenic (Acheré et al. 2004). This was part of the motivation to attempt at producing viable seed from an inbred crosses to try to identify the gene or genes responsible for the *acrocona* phenotype (Uddenberg et al. 2012). Seeds obtained from the inbred cross and WT seeds were planted and grown in accelerated growth

conditions in a phytotron (Uddenberg et al. 2012). Twenty-three of the 91 inbred plants produced seed cone-like structures after only two or three growth cycles (Uddenberg et al. 2012). None of the 75 wild type plants and only two of 150 open-pollinated plants from *acrocona* mothers produced cone-like structures after four growth cycles (Uddenberg et al. 2012). Assuming Mendelian inheritance, that the parents were heterozygous, and that the plants that produced seed cone-like structures are homozygous, an extremely statistically significant result was produced (Uddenberg et al. 2012). Lending more evidence to the phenotype being dominant and monogenic. The study tried to identify a potential causal gene but was limited by having to RNA-seq needles instead of buds. They identified one transcript as being statistically significantly upregulated which corresponded to the MADS-box gene *DAL19*, an *AtSOC1* homolog, but did not find any *SPLs* or *NF-Ys* statistically significantly upregulated (Uddenberg et al. 2012).

4.2 How to influence reproductive initiation

Due to the desire for more regular seed cone production for both seed and controlled crossing, there have been many experiments to try to influence seed and pollen cone initiation. This work extends beyond *P. abies* and *Picea* in general, and into a wide variety of conifers (For reviews and summaries of different studies read: Bonnet-Masimbert and Zaerr 1987; Owens and Blake 1985; Kong and Aderkas 2004; Crain and Cregg 2017). In order to maximize the ability to induce cones, a thorough understanding of what causes cone initiation is necessary. Although it is useful to understand the genetics of flowering pathways in *A. thaliana* and how they might translate to *P. abies*, that knowledge easily applicable to the trees planted in seed orchards. Past work in seed cone initiation has covered a range of different methods and techniques, from chemical treatments of different hormones to cultural treatments like root pruning, supplemental light treatment, nutrient treatments or heat treatments. While results in conifers ranged from promising to contradictory, in spruce species, it appears that the combination of treatment with GA_4/GA_7, heat, and drought was often the most successful at initiating cones (Bonnet-Masimbert and Zaerr 1987; Kong and Aderkas 2004; Crain and Cregg 2017).

The timing of these three treatments is important and through different methods, the effects of these treatments have been shown to significantly increase seed cone initiation both in the year of treatment or a year or two after treatment. Treatments with drought had the most success when performed during early, rapid shoot elongation in the spring, treatments with heat had the most success when performed during slow shoot elongation later in the growing season, and treatments with GA_4/GA_7 (either injected or foliar spray) had the most success when performed towards the end of lateral shoot elongation, just before apical meristems on lateral branches start to differentiate (Owens and Simpson 1988; Ross 1988; Owens et al. 1992; Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Ross 1985; Marquard and Hanover 1984; Olsen 1978; Dunberg 1980; Ho 1991; Bonnet-Masimbert 1987; Luukkanen 1979; Högberg and Eriksson 1994; Johnsen et al. 1994). The combination of GA_4/GA_7, drought, and heat treatments reduced mitotic index and slowed apical meristem development which delayed bud type differentiation until the end of lateral shoot elongation in potted *Picea engelmannii* (Owens and Simpson 1988).

GA_4/GA_7 applications to *Picea* species can have other effects as well. In *Picea glauca* seedlings, individuals receiving treatment grew significantly longer than the control plants (Little and Macdonald 2003). There were similar results seen in three-year-old *P. glauca* plants (Galeano and Thomas 2020). In this study, they proposed using certain GA oxidase genes as proxies for certain growth traits as these genes were thought to be a limiting factor for families with poor growth traits (Galeano and Thomas 2020). Beyond cone induction, GA_4/GA_7 applications could help trees grow faster, which could be helpful when establishing a seed orchard or the function of GA biosynthesis genes could be used in identification and selection, or removal, of genotypes in a breeding program.

Ross 1988 and Owens et al. 1992 propose a mechanism by which seed cones are induced by these treatments in spruce species. First, an early, but moderate drought slows shoot elongation and resources are rerouted to developing terminal and axillary buds, allowing for GAs to accumulate (Pharis et al. 1987) and gives both less vigorous and more latent primordia more time to develop. This increases the amount of meristems that could

potentially become seed cones. Next, warm weather, or a polyethylene cover to mimic warm weather, before bud type differentiation encourages apical meristem enlargement and also maintains the concentration of the bioactive GAs by slowing their metabolism from bioactive to inactive forms (Chalupka et al. 1982; Dunberg et al. 1983). These treatments reduce the demand for GA for vegetative growth and GA can be diverted for reproductive growth (Pharis et al. 1987). In *Pseudotsuga menziesii*, GA has been shown to be prioritized for vegetative growth over reproductive growth and seed cones are initiated when more GA is present than is needed for vegetative growth (Ross 1983; Webber et al. 1985). Therefore, if vegetative growth is slowed during drought and GA metabolism is decreased when the weather is hot, the threshold for GA to be available for reproductive initiation is more easily met (Pharis et al. 1987). While both Ross (1988) and Owens et al. (1992) provide good explanations for how seed cone primordia are initiated for the information that was available to them at the time, their explanations were not able to provide a full picture. They did not have access to the vast amount of genetic and expression data that has been published in the past 30 years, and with that it is possible to start fleshing out seed cone primordia initiation on a genetic level.

These conclusions mesh quite nicely with the *A. thaliana* flowering pathways described earlier. Drought can induce reproduction in both *Picea* and *A. thaliana* (Ross 1988; Owens et al. 1992; Hwang et al. 2019). Reproductive initiation via a GA pathway seems to be present in *Picea* due to the increase in reproductive cones produced on GA treated trees. In *A. thaliana*, both GAs and sugars are transported to the meristem before floral initiation and help regulate flowering related genes like *SPLs*, *NF-Ys*, *SOC1*, and floral meristem genes (Eriksson et al. 2006; Hou et al. 2014; Yamaguchi et al. 2009; Wang et al. 2009; Immink et al. 2012; Jung et al. 2011). Heat during GA treatment at the ideal shoot elongation slows GA metabolism (Dunberg et al. 1983). GA is transported after treatment, some of which reaches pre-differentiated meristems (Oden et al. 1995). Trees under conditions that are inductive (hot and dry) for cones converted more GA_9 to GA_4, a bioactive GA, than trees that did were under non-inductive (cold and wet) conditions which converted more GA_9 to GA_{51}, a non-bioactive GA (Oden et al. 1995).

The slower deactivation of bioactive GA and the increased conversion of GA into bioactive GA due to heat and the transport of GA to meristems allows the bioactive GAs to exist longer in the meristems that might have the potential to become reproductive cones. Higher temperatures also lead to *miR169* and *miR156* to be downregulated in *A. thaliana* (Lee et al. 2010; Gyula et al. 2018; Kim et al. 2012; Stief et al. 2014). The relief of repression from miRNA and DELLAs could allow SPLs and NF-Ys to accumulate as well. Therefore, these conditions allow more time for the genetic process in the presence of bioactive GA to initiate a reproductive cone primordia.

Although the combination of GA_4/GA_7, drought, and heat treatments give good results in terms of reproductive cone production, other treatments should still be studied so the mechanisms behind reproductive initiation are better understood. With better clarity of how reproduction works, induction methods can be more fine-tuned, cheaper, and easier to perform. For example, it is not currently known how light influences reproductive initiation and how it interacts with the other treatments. This is a multifaceted issue since light quality, duration, and amount could all have important roles in reproduction in *Picea* species. *P. abies* has a large distribution covering many latitudes causing populations at different latitudes to need to adapt to the varying amounts of light received during the year. Photoperiod and circadian rhythm on a genetic, organism, and population level have been studied in *P. abies*, but most often in the context of bud set, bud burst, and shoot extension, not reproduction (Linde et al. 2017; Heide 1974; Clapham et al. 1998; Källman et al. 2014; Karlgren et al. 2013b; Gyllenstrand et al. 2014; Lázaro-Gimeno et al. 2024; Ekberg et al. 1979; Mølmann et al. 2005).

In 2018, a study investigated GA_4/GA_7, drought, heat, and light treatments, with a light spectrum that differed from natural light, in *P. abies* (Almqvist 2018). Potted trees were given different types of treatments; no treatment, GA_4/GA_7 and drought, GA_4/GA_7, drought, and heat, GA_4/GA_7, drought, heat, and supplemental light (Almqvist 2018). In two of the three experiments performed, the treatment of GA_4/GA_7, drought, heat, and supplemental light caused the largest proportion of trees to produce reproductive cones, while the third found no difference (Almqvist 2018). In the third experiment, natural light conditions were 15% better than in an average year which may explain why there was no difference in the proportion of trees producing

reproductive cones (Almqvist 2018). In all three experiments, trees in the treatment of GA_4/GA_7, drought, heat, and supplemental light produced significantly more seed and pollen cones than trees in other treatments (Almqvist 2018).

Understanding what treatments induce seed cones is only one part of the equation. As stated, the timing of the treatments is important. Many of the mentioned studies were not conducted in planted seed orchards, but rather with potted trees. This makes it relatively easy to control experimental conditions. Trees in pots are easy to mimic drought conditions, can be covered with polyethylene or moved into a greenhouse for heat treatments, and it is much easier to administer both foliar and injected GA. Timing treatments in these experiments was possible on a tree by tree or genotype by genotype basis because of this, and also because the experiments contained few genotypes and total number of individuals. The correct timing and dose of GA injections is important when maximizing its effect on seed cone initiation. In *Picea abies* and three species of spruce in North America, towards the end of lateral shoot elongation, potentially reproductive meristems undergo rapid apical growth as compared to vegetative meristems (Owens and Molder 1976a; Owens and Molder 1976b; Owens and Molder 1977; Harrison and Owens 1983; Sundström 2001). This is the start of physical bud type differentiation, but the genetics behind this physical change had already started. Cone induction treatments should be performed well before apical meristem dome formation.

However, due to differences in genotypes and year-to-year growth patterns, as well as lack of data on lateral shoot elongation, it is not possible to know the optimal time of GA injections on a large scale at the time when injections are effective. This becomes troublesome when trying to properly time GA treatment in seed orchards with potentially dozens of genotypes and hundreds of individuals. Therefore, a proxy for lateral shoot elongation that will give an estimate for the end of lateral shoot elongation is critical. In *Pinus sylvestris*, Almqvist (2003) explored the timing of GA injections, and the number of cones produced. In this paper, temperature sum, defined as sum of degree days over 5°C, was used to find the optimal time for GA injections for promoting female cones.

5. Results and Discussion

The initiation and development of seed cone primordia are the first steps to producing seed in *P. abies*. The reproductive ability of individuals and of the species varies and there can be years between good cone crops due to the masting behavior of seed cone production. A lack of seed and increasing disease problems have led to *P. abies* to start to fall out of favor in Swedish forestry. Additionally, climate change will impact the phenology of *P. abies* growth in difficult to predict ways. There is a need for more seeds for forest owners to plant genetically improved trees and for breeders to make the best crosses in order to meet the challenges of disease and climate change. This research into *P. abies* reproduction was performed in hopes of gaining a deeper understanding of seed cone production and development so that more seed cones can be produced.

If we consider the view of Florin, that a seed cone is equivalent to an inflorescence and a bract-ovuliferous scale complex is equivalent to a flower, and assume some evolutionary conservation of function, we would expect some floral timing, initiation, and development genes to behave similarly in both *A. thaliana* and *P. abies*. In this work those genes would include *SPLs* and *NF-Ys* and the genes they interact with.

Here are some things to consider:

- Of the flowering initiation pathways in *A. thaliana*, which are relevant to *P. abies*?
- The Aging and Hormonal pathways do to not require *FT* to function, and it is unclear if there are homologs of it in *P. abies* or other conifers.
- Both pathways directly upregulate *SOC1* (via SPLs or NF-Ys) and SPLs can directly upregulate floral meristem genes.
- GA injections have been used to induce seed cone production for decades. If the increased cone production is not due to a stress response from the application, then, in some way, *P. abies* utilizes GA to promote reproductive growth. However, the molecular mechanisms are not known.

5.1 What is expected if…

If *SPLs* function in similar ways in *P. abies* as they do in *A. thaliana* with regards to promoting reproduction, there are several expected outcomes. There should be higher expression of SPLs in adult trees, in the presence of GA, in reproductive cones, in trees that experience hot, but not stressful temperatures, in trees with higher sugar stores. Where SPLs protein is localized, there should be higher expression of *miR172*, higher expression of *SOC1*, and floral meristem orthologs. Reproductive cone primordia should have higher expression of *miR172*, *SOC1*, and floral meristem genes and lower expression of *AP2-like* genes and *miR156* than vegetative primordia.

If *NF-Ys* function similarly in *P. abies*, there should be higher expression of them in the presence of GA, more *NF-YA* expression in warm temperatures due to *miR169* down regulation, higher *SOC1* expression where reproduction associated NF-Y proteins are localized, more reproductive cones when reproduction associated NF-Y proteins are highly expressed, and interactions between NF-Ys and CO.

Reproductively competent trees with higher bioactive GA concentration in apical meristems on lateral branches will have higher *SPL* and *NF-Y* expression. Higher *SPL* and *NF-Y* expression in pre-differentiation meristems should be more likely to produce reproductive cones.

Some of these questions are in the scope of my research while others will require new projects or analysis.

5.2 Identification of genes highly upregulated in seed cone primordia and TS (Paper I)

In a previous experiment, an inbred cross was performed using an *acrocona* tree (Uddenberg et al. 2012). The result of the cross produced plants that had one of three different phenotypes: wild type (WT), *acrocona*, or extreme *acrocona* (Uddenberg et al. 2012). These results led to the interpretation that the cause of the *acrocona* phenotype was semi-dominant and monogenic, which was in agreement with a previous study by Acheré et al. (2004) (Uddenberg et al. 2012). To try to uncover the causal mutation, we collected developing WT and *acrocona* seed cone primordia, WT vegetative

primordia, and *acrocona* transition shoots (TS) in the fall at two different developmental phases. We sequenced the mRNA and miRNA fractions from all primordia.

Then, to identify mRNA transcripts that are significantly upregulated in primordia with female tissue, we performed a differential expression analysis between each of the primordia with female tissue (WT seed cone primordia, *acrocona* seed cone primordia, and TS) versus vegetative primordia at both developmental phases to produce six datasets. We compared the data sets by developmental phase to find commonly up and down regulated genes in the three bud types with female tissue. In the early primordia, there were 515 genes and in the late primordia, there were 390. Due to the fragmentary nature of the *P. abies* genome, we used a novel *de novo* transcriptome assembly tool, CLUSTRAST, to connect scaffolds that mapped to the same transcript (Westrin et al. 2024). This reduced the number of genes to 461 and 352. When comparing the two lists of genes, only 55 were upregulated at both developmental stages and of those, only 14 were upregulated in all primordia with female tissue.

Of the 14 genes, four were related to transcription factors. One was an uncharacterized *FTL* gene (MA_194736g0010) and one was *PaDAL10* (MA_86473g0010), a presumed gymnosperm specific MADS-box gene that was previously described as a marker for reproductive identity (Carlsbecker et al. 2003). In a further analysis of these two genes (Paper II; go to section 5.9 for a description of the technical processes of generating the data), the *FTL* gene showed little bud type expression preference and no tissue type preference, and *PaDAL10* was highly expressed throughout WT seed cone primordia, at the base of female lateral organs in October TS (the same result found by Carlsbecker et al. 2013), and lightly along the vasculature in September and October vegetative primordia.

The other two partial genes (MA_15381g0010 and MA_22749g0010) were the number one and two upregulated genes. By using CLUSTRAST, we were able to computationally connect these two partial genes into one complete gene. The 5' MA_15381g0010 contained an SBP domain and the 3' MA_22749g0010 contained a *miR156* binding site typical of *SPL* genes as well as a *miR529* binding site which was lost in the angiosperm lineage. We

then confirmed the combined transcript through Sanger-sequencing and named the gene *SQUAMOSA BINDING PROTEIN-LIKE1* (*PaSPL1*). Using this gene as bait, we identified and named 11 total *PaSPL* genes, four of which also have a *miR156/529* binding site. A phylogenetic analysis placed this gene with the *AtSPL2/6/9/10/11/15* clade. Genes in this clade are linked to juvenile to adult and vegetative to reproductive meristem transitions and some are able to directly upregulate *SOC1* and floral meristem genes (Xu et al. 2016b; Yamaguchi et al. 2009; Wang et al. 2009; Yamaguchi et al. 2014).

5.3 Expression of miRNA in primordia with female tissue (Paper I)

In the analysis of differentially expressed miRNA in each bud type, we found the two species of miRNA with an overlapping binding site that were predicted to target *PaSPL1*, *miR156t* and *miR529c* and *e*. We also found other miRNA with roles in flowering in *A. thaliana*, notably *miR172i*. In *A. thaliana*, *miR172* is upregulated by *AtSPL9* and *10*, and in our phylogeny, these *SPLs* are in the same clade as *PaSPL1* (Wu et al. 2009). When comparing the expression of these 3 miRNAs, we found *miR156t* and *miR529c* were essentially not expressed and *miR172i* was highly expressed in seed cone meristems and primordia. But in TS, *miR156*t and *miR529*c were highly expressed, similarly to vegetative primordia, but *miR172*i was generally higher expressed like seed cone primordia. Providing evidence that *PaSPL1* might upregulate *miR172i* like its homologs in *A. thaliana*, *AtSPL9* and *10* (Wu et al. 2009).

5.4 All *acrocona* trees have a PaSPL1 allele with a SNP in the *miR156/529* binding site (Paper I)

We investigated the sequence of the *miR156/529* binding site in *acrocona* trees. First, we *de novo* assembled the *PaSPL1* transcripts with the *miR156/529* binding site from WT and *acrocona* trees and then we Sanger sequenced them. Through this, we found a SNP (acro-SNP) in the *miR156/529* binding site of a *PaSPL1* allele in the *acrocona* tree. To get more individuals to genotype, we went into the field to identify more *acrocona* trees in the counties on the east coast of Sweden where the mutant is a native (Joneborg 1945; Fries 1890). We identified 22 wild growing *acrocona* trees

(currently 27). Then we extracted DNA from all of the wild *acrocona* trees, botanical garden trees, and trees from the inbred cross (Uddenberg et al. 2012). After Sanger sequencing, we found all wild growing and botanical garden *acrocona* trees and inbred cross trees with the *acrocona* phenotype to have one copy of the *PaSPL1* allele with the acro-SNP, all inbred cross trees with the WT phenotype to have no *PaSPL1* allele with the acro-SNP, and all inbred cross trees with an extreme *acrocona* phenotype to have two copies of the *PaSPL1* allele with the acro-SNP.

5.5 *PaSPL1* is cleaved post-transcriptionally by *miR156* and *miR529* (Paper I)

Although it is predicted that *miR156* and *miR529* will regulate *PaSPL1* transcripts, there was no direct confirmation. We performed 5' RLM RACE to investigate if the miRNAs cleaved the transcript of *PaSPL1*. We found 24 cleavage products that ended within the *miR156/529* binding site in WT vegetative samples. In TS, only three cleavage products ended within the *miR156/529* binding site. There were also a few shorter fragments and two longer degradation products. The three cleavage products and the shorter fragments from the TS were all from the WT allele, while the two longer degradation products were from the allele with the acro-SNP.

5.6 *PaSPL1* expression through time (Papers I, II, III)

We observed the proportion of PaSPL1 alleles and *miR156t* and *miR529c* and *e* counts in WT and *acrocona* seed cone primordia and in TS at two developmental phases. In the WT seed cone primordia, the miRNAs were very lowly expressed, and the allelic proportion was 50:50 at both phases. In *acrocona* seed cone primordia, the only miRNA with notable expression was *miR529* in the early developmental phase, and all miRNAs had very low expression in the later phase. The allelic proportion was about 70:30 towards the allele with the acro-SNP in the early phase. In the later phase, the proportion for both alleles was roughly 50:50. In early phase TS, *miR156t* and *miR529e* were expressed and in the later phase, *miR156t* and *miR529c* were expressed. The allelic proportion in the early phase was 70:30 towards the allele with the acro-SNP, and in the later phase it increased to 95:5. The allelic proportions combined with the miRNA cleavage data indicates that

the two miRNAs preferentially cleave the WT allele of *PaSPL1* and that the acro-SNP allele is tolerant to *miR156/529* regulation.

When investigating spatial expression of *PaSPL1* in paper II, we found it to be expressed very highly in all time points in seed cone primordia, and very low expression in all time points in vegetative primordia. In TS, there was low, but notable expression throughout the primordia in August. In September, the TS is undergoing a transition from making vegetative primordia (needles) to female primordia (bracts and ovuliferous scales). The expression of *PaSPL1* is very high in the lateral organs and vasculature. Finally, although *PaSPL1* is expressed throughout the TS in October, it is preferentially expressed in the apical portion where female lateral organs are being initiated from the meristem.

In the study on the natural cone setting ability of different *P. abies* genotypes (Paper III), material was collected from the cone setting region of three genotypes that received GA injections, both before and after treatment with GA, in June. At this time point shoot elongation is nearing completion, but meristems have not started to differentiate. It was not possible to identify bud types due to this, even if the meristems had been dissected. Meristems were collected while still covered by bud scales, but the needles were removed. Of the genotypes collected, one was classed as high cone setting, one as medium cone setting, and one as low cone setting. The mRNA was extracted from multiple meristems and sequenced from each genotype from before and after treatments and then differential expression analysis in four ways was performed. Each genotype against itself before and after treatment and all genotypes before and after treatment.

PaSPL1 was not observed to be differentially expressed in any analysis. Since it was not possible to know what bud type the meristems that were collected would eventually become, it is possible that none or only a few or all of the meristems collected would become seed cone primordia. The *miR156* and *miR529* content of these samples is not known. Under the assumption that *SPLs* in *P. abies* can bind with DELLAs like in *A. thaliana* and *P. tabuliformis*, DELLA degradation after GA injections would release SPLs in *P. abies*, and if *miR156* and *miR529* are not present, start upregulating genes (Yu et al. 2012; Guo et al. 2020; Liu et al. 2022). The

high cone setting genotype had a very good seed cone-setting year, so it is reasonable to think that some of the meristems collected would have become seed cone primordia. Looking specifically at the differentially expressed genes in the high cone setting genotype after GA treatment does not reveal any potential SPL targets as upregulated after treatment. Potential explanations range from *PaSPL1* is sufficiently down regulated by miRNA in the samples studied to *PaSPL1*, and its targets, were already expressed highly in both time points. No GA biosynthesis genes or genes regulated in the presence of GA were observed as differentially expressed in the high cone setting genotype suggesting that *PaSPL1* was not being regulated by DELLAs at either time point. A different type of expression analysis, different differential expression analysis, or sequencing the miRNA in this sample would provide a clearer picture.

5.7 Parts of the aging flowering pathway are conserved in *P. abies* (Paper I and II)

To summarize the *A. thaliana* aging pathway applicable for *P. abies*, i.e. without FT involvement, translation of *SPLs* is repressed post-transcriptionally through cleavage or translational inhibition by *miR156* and protein function is repressed by protein-protein interactions with the GA mediated DELLA proteins (Wu and Poethig 2006; Gandikota et al. 2007; Yu et al. 2012). As SPLs accumulate due to decreasing negative regulation over time, an *A. thaliana* plant can transition from juvenile growth to adult growth and gain reproductive competence (Schwarz et al. 2008; Wu and Poethig 2006; Wu et al. 2009; Guo et al. 2017; Yang et al. 2013; Wahl et al. 2013). When SPLs are active, they can drive the expression of *SOC1*, floral meristem genes, and *miR172*, which in turn represses *AP2* and *AP2-like* genes that repress floral meristem genes and upregulate *miR156* (Yamaguchi et al. 2009; Wang et al. 2009; Yamaguchi et al. 2014; Aukerman and Sakai 2003; Chen 2004; Wu et al. 2009; Mathieu et al. 2009; Yant et al. 2010).

Accumulating evidence from a wide range of conifers suggests that the juvenile to adult transition may, apart from the *miR156/529*-SPL module, also involve time keeping *AGL6-like* genes (Carlsbecker et al. 2004; Xiang et al. 2019; Katahata et al. 2014; Chen et al. 2021; Ma et al. 2021; Zhang et al. 2024). In *P. abies* and *P. tabuliformis*, *DAL1* has been identified as an age

timing gene involved in the vegetative to adult transition, and in *P. tabuliformis*, PtDAL1 has recently been shown to directly interact with the promoter of the gymnosperm specific *PtDAL10* and activate its expression in callus tissue (Ma et al. 2021; Zhang et al. 2024; Carlsbecker at al. 2004). In *P. tabuliformis*, the proteins of these two genes plus PtMADS11 can form a protein complex in heterologous systems and has been associated with adult trees becoming (Zhang et al. 2024). After reaching reproductive competence, the trees are then receptive to reproductive signals. These signals may come, in part, from SPLs.

The miRNA tolerant allele found in *acrocona* trees bypasses the age sensing/regulation pathway and directly causes cone-like structures to be produced despite the presence of *miR156a* and *miR529c* and *e*. However, *miR172i* does have higher expression in TS pointing to a potential conserved function between *P. abies* and *A. thaliana* SPLs of upregulating *miR172* (Paper I; Wu et al. 2009).

WT seed cone primordia are produced on meristems in trees that have reached reproductive competency and received reproductive signals. This is evidenced by the expression of genes related to the juvenile to adult transition and vegetative to reproductive transition. They have low expression of *miR156* and *miR529*, high expression of *miR172*, and high expression of *PaSPL1* (Paper I).

In paper II, we found expression of floral meristem genes to be only expressed in female tissue (bracts and ovuliferous scales). *DAL2* (*AG*) and *DAL14* (*AGL6/SEP*) are homologs to *A. thaliana* floral meristem genes (Carlsbecker et al. 2013). *DAL2* was highly expressed only in ovuliferous scales and *DAL14* was expressed in ovuliferous scales and young bracts, in both WT seed cones and TS in congruence with previous work (Carlsbecker et al. 2013). Neither gene was expressed in TS until after the primordia started producing ovuliferous scales in October. Expression of two *AP2*-like genes, *AP2L1* and *AP2L3* (MA_8090186g0010 and MA_2193g0020) with *miR172* binding sites, was also found (Nilsson et al. 2006). AP2L3 was expressed higher in vegetative and TS primordia. *AP2L1* was expressed throughout the vegetative and TS primordia during lateral organ formation before becoming lower expressed. In WT seed cone primordia, it is not

expressed during lateral organ formation but is expressed in the lateral organs during phase 3 of development, lateral organ cell specific differentiation. In an *in situ* experiment, expression of *AP2L1* was limited to the tips of ovuliferous scales (Nilsson et al. 2006). This may indicate that *AP2-like* genes maintain some sort of control over lateral organ development after the lateral organs have been differentiated from the meristem.

5.8 Genes co-expressed with *PaSPL1* are related to reproduction timing and development (Paper II)

We also performed a co-expression analysis of genes that were differentially expressed in the same location as *PaSPL1* to try to identify genes that function up or down stream in the same developmental pathway. This analysis identified a few MADS-box genes *DAL1*, *DAL14*, and the novel *DAL55* as significantly co-expressed. In a phylogenetic analysis, *DAL55* grouped closely with *AtAGL24* and *AtSVP*, known regulators of flowering time and floral patterning (Lee et al. 2008; Tao et al. 2012). AtSVP inhibits transcription of *SOC1* and *miR172* at low temperatures, promotes expression of *miR156* and *AP2-like* genes, and can form protein complexes with *SOC1* (Lee et al. 2013; Cho et al. 2012; Tao et al. 2012). Four *Pinus SVP-like* (*SVL*) genes have been investigated in *P. tabuliformis* (Zhou et al. 2024). They found that the proteins of two of the *PtSVL* genes formed protein complexes with *PtSOC1*-like genes in yeast, and did not bind to *PtSOC1-like* promoters (Zhou et al. 2024). *PtSVL1* was also able to bind to the promoter of an *SPL* gene, *PtSPL16*, in yeast (Zhou et al. 2024).

Other differentially expressed genes from the co-expression analysis are described as a *CYTOKININ HYDROXYLASE* (MA_10354140g0010) and *FLAVONOID 3,5-METHYLTRANSFERASE* (MA_9065834g0010). Though after doing a BLAST search, it is unclear what type of gene the *CYTOKININ HYDROXYLASE* (*CH*) is, but it does have good hits to undescribed genes in other conifers. *CH* is highly expressed in the apical meristem, pith, and lateral organs, all time points in seed cone primordia, but only becomes highly expressed in TS in October after the primordia starts initiating female tissue. It is not expressed at all in August or September vegetative primordia and there were only a few transcripts in a single section in October. *CH* was the highest differentially expressed gene in the early samples in all bud types

(WT and *acrocona* seed cone primordia and TS) as compared to vegetative primordia in Paper I. This makes CH a good marker for seed cone bud type identity. The gene described as *FLAVONOID 3,5-METHYLTRANSFERASE* has much better BLAST matches to *CAFFEOYL COENZYME A O-METHYLTRANSFERASE* (*CCOAOMT*) genes. This moves it from the anthocyanin to the lignin biosynthesis pathway. Either result is quite interesting due to its expression pattern. It is highly expressed in the vasculature of all bud types starting in September after vasculature has started to be established, but in seed cone primordia, its expression is much broader. In September and October seed cone primordia, the *CCOAOMT* gene is expressed highly throughout the pith and vasculature and extends to the tips of the bracts but does not express in the ovuliferous scales.

5.9 Genetics of primordia development revealed by Spatial Transcriptomics (Paper II)

A brief introduction to Spatial Transcriptomics (ST) will be useful (Ståhl et al. 2016; Giacomello et al. 2017). Tissue is not a uniform mass of cells, especially not seed cone primordia. All bud types in *P. abies* have shape and are continually changing and developing new tissues through the fall. Whole tissue RNA-seq techniques lose these details but provide expression information about all transcripts. *In situ* techniques are able to provide the location of expression within a tissue but are hard to quantify and the number of transcripts you can perform experiments on at one time is limited. ST combines the best parts of these techniques into one process. Below is a brief overview of the process.

First, tissue is collected, frozen, and kept at -70°C. The tissue is then embedded in Optimal Cutting Temperature compound (OCT) and left to freeze on dry ice. After the OCT has frozen, the tissue is mounted on a cryosectioner, and sections are placed on specially made microscope slides. On these slides are capture areas where tissue is placed. In each capture area, there are almost 5,000 55um spots with millions of capture probes in each. The capture probes have several important sections, a poly-T tail to hybridize with mRNA, a unique molecular identifier to allow for correct counts of transcripts, a spatial barcode to map each transcript to a specific spot in the capture area, and a sequence to aid in Next Generation Sequencing. When

the tissues are all placed in the capture area, they are fixed to the slide with methanol. Tissues are then stained and imaged for later analysis. The stain is removed, and the slide is placed in a holder. Tissues are permeabilized to allow the release of mRNA from the cell are added to the capture area for an amount of time previously optimized for the specific tissue. The mRNA then hybridizes with the probe. The permeabilization enzyme is removed and reverse transcription is performed to make the probe be a copy of the full-length transcript. Next the probe is used as a template for second strand synthesis to create a full-length copy of the probe-mRNA sequence that can be denatured and used in downstream steps. After many processing and quality control steps, a library of the denatured probe-mRNA sequences is made and sequenced. After sequencing, all reads are mapped back to the spot they came from in the capture area and analysis of expression patterns can begin.

Using ST, we investigated the expression patterns of three bud types (WT vegetative, WT female, and *acrocona* TS) at three time points (mid-August, mid-September, and mid-October). Expression and spatial data for 10s of thousands of genes was generated. With this data, we could observe the expression patterns for not only reproductive development genes, but also vegetative development, vascular development, hormone signaling and transport, light and cold sensing (aka dormancy) and many other types of genes that range extremely specific to ubiquitously broad expression patterns. To aid in visualization of the spatial expression of the genes, we developed a ShinyApp that contains bright field images of all tissue sections, all cluster locations within each tissue section, and gene expression within each tissue section.

We performed a clustering analysis over all bud types and time points and found 16 clusters. We then defined four tissue domains that 14 of these clusters represented based on location and significantly differentially expressed genes. The tissue domains are: Pith, Vasculature, Lateral Organ Primordia (LOP), and Lateral Organ Differentiated (LOD). The two that were not assigned a tissue domain did not have a discernible location or gene expression pattern. Marker genes were identified for each tissue domain based on specificity of expression of the gene within the tissue domain. Some of the genes identified were known and described genes, at least in other

species, so their expression in a specific tissue domain was not a surprise. For example, a *GLYCINE-RICH ARABINOGALACTAN3* gene (MA_381942g0010) was found highly and specifically expressed in the vascular tissue domain. Previous work on a homolog in *P. taeda* found this gene to be involved in tracheid cell wall development (Zhang et al. 2003). In *Pinus radiata*, *Picea sitchensis* and *P. abies*, arabinogalactans in general were observed to be located in secondary cell wall of tracheids (Altaner et al. 2010). In *P. abies*, this gene has also been shown to be expressed in cambium tissue (Jokipii-Likkari et al. 2017). However, what was not expected was that many of the genes did not have a match with a gene in a different species or some sort of description, and how extremely precisely some of them were expressed. For example, other undescribed genes include some of the highest expressed genes: across the dataset (ex MA_8964512g0010), in a tissue type (pith; MA_95523g0010), in a bud type (vegetative; MA_3356606g0010), or at a specific time point (October; MA_491735g0010).

5.10 NF-Y genes are expressed in vegetative, seed cone, and TS primordia (Paper I, II)

In the differential expression analysis in paper I, two *NF-Y* genes were differentially expressed. An *AtNF-YC1* homolog (MA_164507g0010) was downregulated in early female tissues, while an *AtNF-YB3* homolog (MA_96911g0010) was upregulated in early female tissues. In *A thaliana*, the proteins of *NF-Y* genes interact with DELLA proteins and CO (Hou et al. 2014). *AtNF-YB3* is upregulated by heat and its homolog in *P. wilsonii* is as well (Sato et al. 2019; Zhang et al. 2015b). When the homolog in *P. wilsonii* is transformed into *A. thaliana*, the resulting plants are early flowering. Two *P. tabuliformis* NF-YC proteins interact with PtDELLA proteins and one can interact with PtCO in yeast (Guo et al. 2020; Liu et al. 2022). No *NF-YA* genes, which are necessary in *A. thaliana* to interact with the *SOC1* promoter, were observed (Hou et al. 2014).

The Spatial Transcriptomics data found four *NF-Y* genes (Paper II). Three *AtNF-YC2* homologs (MA_10427207g0010, MA_33970g0010, and MA_9929143g0010) and the same *AtNF-YB3* homolog (MA_96911g0010). They were mostly upregulated in the pith and vascular tissues and

downregulated in the LOP and LOD. Except for MA_33970g0010 which was found to be upregulated only in the LOP. None of the genes showed preferential expression for any bud type and were widely expressed in all bud types and time points.

There are only a few *NF-Y* genes being expressed in the generated data. The genes that are expressed do not preferentially express during seed cone development. This suggests that there is no seed cone bud type specific expression of *NF-Y* genes in *P. abies*. Expression of *NF-Y* genes has been observed during reproductive development in *P. tabuliformis* (Guo et al. 2020; Liu et al. 2022). The fact that there does not seem to be any seed cone bud type specific *NF-Y* genes could be explained by *NF-Ys* being involved in either similar processes in both vegetative and reproductive primordia or the same genes are involved in different processes in different bud types, that does not explain why so few were differentially expressed during primordia development in the first place.

5.11 The spatial and temporal expression and phylogeny of *YABBY* genes in *P. abies* (Paper II)

The *YABBY* gene family are transcription factors that have a diverse range of important functions, including during vegetative and reproductive development, abaxial cell fate, phyllotaxis, vascular development, and hormone regulation and biosynthesis (Finet et al. 2016; Zhao et al. 2007; Yang et al. 2016; Sarojam et al. 2010; Siegfried et al. 1999; Goldshmidt et al. 2008). Floyd and Bowman hypothesized that *YABBY* genes are important in the origin and evolution of the leaves of seed plants because their phylogenetic analysis mapped the gene family to the last common ancestor of living seed plants and because of their importance in leaf development (Floyd and Bowman 2006; Floyd and Bowman 2009; Sarojam et al. 2010). This phylogenetic view is too narrow as there is strong evidence that a *YABBY* gene existed before seed plant divergence, and potentially as far back as the most recent common ancestor with *Micromonas*, a green algae (Evkaikina et al. 2017; Yang et al. 2017; Li et al. 2020; Worden et al. 2009).

In the *A. thaliana YABBY* gene family, there are 6 genes*: FILAMENTOUS FLOWER* (*FIL*), *YABBY2* (*YAB2*), *YABBY3* (*YAB3*), *YABBY5* (*YAB5*),

CRABS CLAW (*CRC*), *and INNER NO OUTER* (*INO*). These genes have been classified as "vegetative" (expressed in vegetative tissues and floral tissues except ovules) or "reproductive" (expressed only in carpels or ovules). *FIL/YAB3*, *YAB2*, and *YAB5* are "vegetative" while *CRC* and *INO* are "reproductive" (Bartholmes et al. 2012; Bowman and Smyth 1999; Sarojam et al. 2010; Siegfried et al. 1999; Villanueva et al. 1999; Sawa et al. 1999). In gymnosperms, four clades (A, B, C, and D) of *YABBY* genes were identified from three orders (*Gnetales*, *Ginkgoales*, and *Coniferales*) (Finet et al. 2016). Due to their impact in lateral organ development, we investigated the spatial expression of *YABBY* genes and performed a phylogenetic analysis.

In our clusters, we found 2 *YABBY* genes (MA_10432332g0010 and MA_112273g0010) to be differentially expressed. Upon further review of the *P. abies* genome, we found a third, mostly complete gene (MA_407206g0010) (Nystedt et al. 2013). The full cDNA sequence of this gene was confirmed through Sanger sequencing. We included all three genes in our phylogenetic analysis which included *YABBY* genes from a range of gymnosperms and angiosperms. The evolutionary relationship between angiosperm and gymnosperm *YABBY* genes was not clear (Finet et al. 2016). In seed plants, there is support for either one (Bartholmes et al. 2012; Evkaikina et al. 2017; Du et al. 2020) or two (Yamada et al. 2011; Finet et al. 2016, Liu et al. 2021) progenitor genes diversifying into the variation seen in living seed plants. In our analysis, we found support for one progenitor gene as the gymnosperm and angiosperm *YABBY* genes formed separate clades. We found MA_10432332g0010 to group with *YAB-C*, MA_112273g0010 to group with *YAB-A*, and MA_407206g0010 to group with *YAB-B*.

Expression of *YABBY* genes in different plant species varies wildly. In *Huperzia selago*, a lycophyte, the *HsYABBY* gene does not show polar expression and is expressed more generally than seed plant *YABBY* genes (Evkaikina et al. 2017). *HsYABBY* is expressed in the surface layer of the SAM, the surface layer of leaf primordia both abaxially and adaxially, and the procambium of cauline leaves (Evkaikina et al. 2017). In *A. thaliana*, *YABBY* genes have polar expression, and they regulate abaxial cell fate (Siegfried et al. 1999). However, in gymnosperms, polar expression is not

the rule. *P. menziesii YAB-C* does not have polar expression while *G. biloba YAB-1B*, *2B*, and *C* do have polar expression (Finet et al. 2016). Others have also found *YAB-C* in *Abies holophylla* and *Picea smithiana* to not express in a polar manner, and like *P. menziesii*, it is expressed in the provascular strand (Finet et al. 2016; Du et al. 2020). Finet et al propose that there was a loss of polarity in the expression of *YABBY* in conifers leading to the development of needle-like leaves (Finet et al. 2016). Not all polarity has been lost in gymnosperm needles, vascular tissues are arranged in a polar manner and polar genes from other families still have polar expression (Du et al. 2020). In *A. thaliana YABBY* mutants, leaf polarity is disrupted and the leaves that develop are narrow and have a more needle-like shape with less vascular venation (Sarojam et al. 2010). Change, loss, or reduction of *YABBY* expression or function can result in drastically different leaf size and shape in *A. thaliana* and other species and may partly explain the narrow/reduced leaf shape in monocots (Sarojam et al. 2010; Golz et al. 2004; Gleissberg et al. 2005; Juarez et al. 2004; Yamaguchi et al. 2010; Dai et al. 2007; Evkaikina et al. 2017).

Expression of *PaYAB-A* and *PaYAB-C* was observed in the outermost part of the primordia in all bud types and timepoints with *PaYAB-C* being higher expressed than *PaYAB-A*. Expression in female lateral organs showed some polarity as they were preferentially expressed in the bracts. Both genes were expressed higher towards the later phases of primordia development, after lateral organs had developed. *PaYAB-B* was not found in our data, but *YAB-B* homologs have been expressed in the developing leaves of *G. biloba* and the integuments of *Ephedra distachya* (Finet et al. 2016). Because integuments in *P. abies* are developed in the spring, we performed an qPCR experiment to check the expression of all three *PaYABBY* genes in vegetative and WT seed cone primordia in the spring and fall. We found all three *PaYABBY* genes to be expressed in the fall primordia, *PaYAB-A* and *PaYAB-C* were highly expressed while *PaYAB-B* was hardly expressed. In the spring seed cone bud, *PaYAB-A* was expressed lower than in the fall. PaYAB-C was similarly expressed and *PaYAB-B* continued to be hardly expressed at all. In the spring vegetative buds, the expression of *PaYAB-A* and *PaYAB-C* was much higher than in the fall. Most interestingly, *PaYAB-B* was also similarly expressed as the other two *PaYABBY* genes in spring vegetative buds. This expression pattern provides evidence that *PaYAB-B* is not expressed in

integuments. More evidence is needed to confirm this as due to the integuments being so small, there may not have been enough tissue to provide enough mRNA to be detected by qPCR. A further ST or *in situ* hybridization experiment would be able to answer this question. In any case, *PaYAB-B* expression has been found in developing spring vegetative buds after dormancy, but before vegetative bud burst.

5.12 GA injections to stimulate flowering affect genotypes differently (Paper III)

The Nässja seed orchard (60° 16' 25.5648" N, 16° 48' 5.1156" E) consists of 560 reproductively mature ramets from 18 genotypes planted in 2005. Since 2008, the seed orchard has been part of an experiment testing the cone setting response to GA_4/GA_7 injections. The experimental plot consists of 12 rows of trees flanked by wind break rows that are not part of the experiment. The 12 rows are split into four blocks of three rows. Each block contains one row with each treatment type: No treatment, GA_4/GA_7 injections in even numbered years, and GA_4/GA_7 injections every year. Injections started in 2008 and data collection the following year. In the spring, every ramet in the trial has its vitality scored (0-3), its seed cones counted, and weather permitting, is checked for the presence of pollen cones (0-1). As discussed in the introduction, decades of research has gone into the ideal time to treat trees with GA (Owens and Blake 1985; Kong and Aderkas 2004; Crain and Cregg 2017). For *P. abies* in the region of Sweden where Nässja is located, temperature sum as calculated in Almqvist (2020) gives a window for optimal treatment. The reality of an orchard with phenology differences in shoot elongation between genotypes makes timing treatments difficult. Temperature sum has been a good approximation for shoot elongation and has shown good results in regard to cone induction in treated trees. We classified cone setting in the genotypes in the orchard into four classes based on their seed cone production on untreated trees over the course of the whole trial. The classes were none, low, medium, and high cone setting.

Although there were significantly more seed cones produced on the ramets receiving GA every year vs untreated ramets, not every genotype reacted to GA treatments, regardless of what their cone setting class was. This may be due to endogenous factors specific to the genotype or incorrect timing of GA

treatments. Even so, both every year and even year GA treatment led to about twice as many seed cones produced over the course of the experiment than untreated. GA treatment also increased the percentage of genotypes that produced seed cones in every year except for one. The classes of cone setting is an interesting topic for a breeding program. Selection of genotypes for a seed orchard is important and is based on growth traits that are desirable (Curt Almqvist, personal communication). But if the genotype does not produce seed or pollen cones, it does not contribute to the seed produced and is taking up space that could be used more productively.

There is a question of competing interests in planting a seed orchard, selecting a tree for seed cone production or growth traits. Seed cone production is a resource heavy task for an individual tree. It is not currently known if heavy seed cone crops negatively affect growth or growth traits. If only high seed cone setting genotypes are planted or contribute to the seed crop, the seeds from that orchard will be unintentionally selecting for high seed cone setting. If heavy seed cone setting does negatively affect growth or growth traits, the expected genetic gain will not match the phenotypic reality. Further the expected genetic diversity of the seed crops in a seed orchard will not match with the actual seed diversity, leading to less diverse planted forests. With regards to the Nässja seed orchard, after combining all treatments, there are six genotypes that make up about 43% of the 560 ramets in the trial. But they produce just over 90% of the seed cones. So that means the other 12 genotypes make up about 57% of the trial, but only produce around 10% of the seed cones. This seed orchard is the only one that has this ramet-by-ramet seed cone production data, most do not have any data on seed cone production in each genotype. In order to know if high seed cone setting is negative for growth or growth traits, many more genotypes need their cones to be counted.

5.13 GA responsive genes and potential targets of SPLs and NF-Ys upregulated after GA treatment; genetic mechanisms of the hormonal pathway in *P. abies* (Paper III)

As detailed earlier, in the study on the natural cone setting ability of different *P. abies* genotypes (Paper III), we collected material from the cone setting

region of three genotypes that received GA injections before and after treatment and performed differential expression analyses on the mRNA extracted. A conserved function of NF-Y genes between conifers and angiosperms is the ability of their proteins to bind with DELLA proteins (Hou et al. 2014; Guo et al. 2020; Liu et al. 2022). So, it may be expected to observe more NF-Y transcripts after the GA treatment degrades DELLA proteins. However, in *A. thaliana*, it appears that DELLAs do not influence *NF-Y* or *SPL* transcription, just protein function (Yu et al. 2012; Hou et al. 2014). When NF-Ys are no longer repressed by DELLAs, they can bind to the SOC1 promoter and upregulate its expression (Hou et al. 2014). Therefore, as with PaSPL1, when looking at the differentially expressed gene lists that were generated, one should look for genes that are influenced by NF-Y protein activity. In a similar way to PaSPL1, one subunit of the NF-Y complex, NF-YA is regulated by a miRNA, *miR169* (Xu et al. 2013). NF-YA is also the subunit that can bind to the SOC1 promoter (Hou et al. 2014). The miRNA profiles of *miR169*, *miR156*, and *miR529* are important to consider as well. Especially since *miR169* and *miR156* are downregulated in warm temperatures which would lead to more NF-Y and SPL proteins being active after GA treatment (Lee et al. 2010; Gyula et al. 2018; Kim et al. 2012; Stief et al. 2014).

Neither *PaSPL1* expression nor any *NF-Ys* were differentially expressed in any of the differential expression analysis. However, if the proteins of the genes function similarly to their *A. thaliana* homologs, there are some potential targets for both of them that are upregulated after GA treatment. Two *DAL19* isoforms (MA_16120g0010 and MA_8447039g0010) were highly expressed in the combined differential expression after GA treatment (Akhter et al. 2018). These genes are *AtSOC1* homologs (Akhter et al. 2018). A qPCR experiment confirmed MA_16120g0010 to be extremely significantly upregulated in the medium cone setting genotype after treatment and MA_8447039 to be significantly expressed in the low and medium cone setting genotypes after treatment. Interestingly, two genes, a *GAMYB*, and a *Gibberellic Acid-Stimulated A. thaliana 5* (*GASA5*) were as well. *GASA5* is a negative regulator of flowering in the GA part of the hormone pathway (Zhang et al. 2009). In the qPCR analysis, the *AtAP2* homolog was highly significantly expressed in the later time point in untreated trees of all cone setting genotypes, potentially indicating inhibition

of reproductive growth. All three genotypes produced significantly fewer cones when untreated as compared to GA treated trees. One notable upregulated gene before GA treatment was ent-kaurene chloroplastic, which is an enzyme active early in the GA biosynthesis pathway and is localized to the chloroplast (Helliwell et al. 2001).

When looking at the differentially expressed genes in the medium cone setting and low cone setting genotypes, more interesting patterns emerge. In the medium cone setting genotype a *GASA5-like* gene was expressed highly before GA treatment. After GA treatment, two *AP2-like* genes and a *GA2ox* gene. In *A. thaliana*, *GA2ox* genes are strongly upregulated by the presence of GA_4 which leads to GA_4 being deactivated by *GA2ox* and turned into GA_{34} (Katyayini et al. 2020). All together these genes point to a tree trying not to reproduce. In the low cone setting genotype, ent-kaurene chloroplastic is highly expressed before GA treatment indicating it is trying to make its own GA. However, after GA treatment, *A. thaliana* GA responsive homolog genes like *GASA5*, *GASA7*, *GASA2*, and *GASA11* become active suggesting that this genotype is reacting to the treatment and its endogenous GA biosynthesis is not working properly or is being interfered with. Even a *GA3ox* gene becomes active after GA treatment. These genes turn inactive precursor GAs into bioactive GAs (Katyayini et al. 2020). If its GA biosynthesis was working, these genes would have already been active in the presence of endogenous GA made through ent-kaurene chloroplastic being expressed before treatment. Further evidence that something in the GA biosynthesis pathway is not working correctly in the low cone setting genotype, the concentration of GA_4 after treatment in the low cone setting genotype was half of that of the other genotypes. This could be due to an overactive or extremely efficient version of *GA2ox* protein converting bioactive GA into inactive GA.

6. Conclusions

Support for evolutionary conservation of at least part of the age dependent flowering pathway between flowering plants and the conifer *P. abies* was presented. We discovered a SNP (acro-SNP) in the *miR156/529* binding site of an *SPL* transcription factor (*PaSPL1*) of a *P. abies* reproduction mutant, *acrocona*, that is highly correlated with its phenotype. We provide evidence that *PaSPL1* is negatively regulated post-transcriptionally by *miR156* and *miR529*, but that the allele with the acro-SNP is resistant to miR156/529 mediated cleavage. By avoiding cleavage, the transcript of the acro-SNP allele accumulates in tissue where it should not, eventually leading to transition shoots (TS), shoots with basal needles and apical seed cone-like structures, in apical positions, and juvenile trees. We show evidence that *miR172i* is upregulated in the tissues where *PaSPL1* is also expressed. Homologs of *A. thaliana* genes active during flowering were upregulated in seed cone primordia and some are co-expressed with *PaSPL1*. These include *SOC1* (*DAL19*), *AG* (*DAL2*), *AGL6/SEP* (*DAL1* and *DAL14*), and *SVP* (*DAL55*) homologs.

Only a few *NF-Y* genes were found in any analysis. When they were, there was no specific pattern of expression for bud type, time point, or tissue type.

A spatial atlas of gene expression in vegetative, seed cone, and TS primordia at three time points during fall development was produced using Spatial Transcriptomics. In those experiments, we analyzed the expression and developmental patterns of the three bud types. Four tissue domains were identified based on location within a primordia and gene expression. Genes expressed in different tissues, bud types, and time points were identified. A ShinyApp was developed to easily see the expression of genes detected in the experiment.

We analyzed the *YABBY* genes in *P. abies*. In our phylogenetic analysis, we found support for only one ancestral *YABBY* gene at the split between gymnosperms and angiosperms. We found high expression for *PaYAB-A* and *PaYAB-C* in the lateral organs of all bud types supporting that there is conserved function with angiosperms as an abaxial identity gene. Expression of *PaYAB-B* was not observed in the fall. A qPCR experiment was performed

using spring seed cone and vegetative buds and found *PaYAB-B* expressed only in vegetative buds, while *PaYAB-A* and *PaYAB-C* were expressed in both. Suggesting, but not ruling out, that the Pa*YABBY* genes do not have reproductive function.

Hormonal treatments to induce reproductive cone production have been successfully used in *P. abies* for decades, suggesting that elements in the GA dependent flowering pathway are evolutionarily conserved between angiosperms and gymnosperms. We provide more evidence of this and start to investigate the genetics behind it. The ability to produce seed cones differs between genotypes. At an experimental seed orchard, we show that stem injection of GA_4/GA_7 leads to more genotypes producing seed cones and significantly higher seed cone production as compared to no treatment. We also observed that some genotypes do not react to GA_4/GA_7, but it is unclear if that is due to an innate property of the genotypes or improper injection timing or both. In a medium and a low seed cone producing genotypes, we found higher expression of genes (i.e., *AP2-like*, *GASA5*) associated with stopping reproduction. Further, in the low seed cone producing genotype, hormone quantification showed a much lower concentration of GA_4 after injection with GA_4/GA_7, and differentially higher expression of bioactive GA deactivator, *GA2ox*. Neither *PaSPL1* nor any *NF-Ys* were observed in a differential expression analysis of GA_4/GA_7 treated trees, but two potential targets were. Two isoforms of *DAL19*, an *AtSOC1* homolog that previously have been associated with cone setting shoots in the *acrocona* mutant.

7. Future perspectives

For how vast and overwhelming the research into flowering timing and development in *A. thaliana* is, the realities of the actual genetics involved is even more grand. The gulf in knowledge of the processes between *P. abies* and *A. thaliana* feels similar to that. A long generation time, infrequent and difficult to predict cone production, hard to access reproductive cone locations, and a lack of reproductive mutants are just some of the factors that have limited our ability to study *Picea* reproduction. This has led to a lack of knowledge with real world implications for industry, breeding programs, and research.

The identification of the acro-SNP has opened the door to more effectively use the *acrocona* mutant to study *P. abies* reproductive initiation and development. Clarification of which reproductive pathways are relevant to P. *abies* will allow for more focused research in the areas that are more likely to be involved.

The development of a spatial atlas of expression using the ST data will provide an invaluable resource for researchers interested not only in reproductive development, but also any sort of tissue specific, time specific, or primordia type specific development.

The research into the natural variation of cone setting and GA injections provides another way to study seed cone initiation and development in a more “real life” situation.

7.1 Open questions

When is *PaSPL1* expressed? A TS is supposed to be a vegetative shoot, so why is *PaSPL1* expressed there in the first place? Is it always on and miRNA, or something else, is required to properly regulate it?

What regulates *PaSPL1* (other than miRNA) and what does it regulate?

Why do heterozygous *acrocona* trees typically only produce transition shoots on their leading meristems and not also on lateral? Homozygous

acrocona trees produce TS on lateral and leading meristems. Is PaSPL1 lower expressed in lateral meristems so having 2 acro-SNP copies can accumulate enough? Why would PaSPL1 be expressed differently in lateral meristems?

Following this same line of thought, what is going on in the three *Picea pungens* mutants? Their phenotypes are similar to each other and to *acrocona*.

What are the genetic mechanisms at play before bud type differentiation that lead to reproductive buds?

Do high seed cone setting genotypes have worse growth rates or traits? Either due to associated traits/genes or because producing so many cones is a toll on the tree.

Larix and *Pinus* are more likely to produce at least a few seed cones every year, what are the reasons for this?

Pinus reaches sexual maturity earlier than *Picea*, can SPLs or another gene (*DAL1*) be the cause of this?

Larix, *Pseudotsuga*, and *Keteleeria* have basal needles that transition into bracts before ovuliferous scales start to form in their axils (Figure 4A, B, C). For *Pseudotsuga* and *Keteleeria*, this seems to be a rather quick process with only a few needles or needle like bracts (Figure 4C). For *Larix*, the transition zone can be just as long as the cone (Figure 4A, B). The seed cones from these genera are similar to *acrocona* transition shoots (Figure 4D). Are there more similarities than just phenotype? Are there similar genetic mechanisms at play?

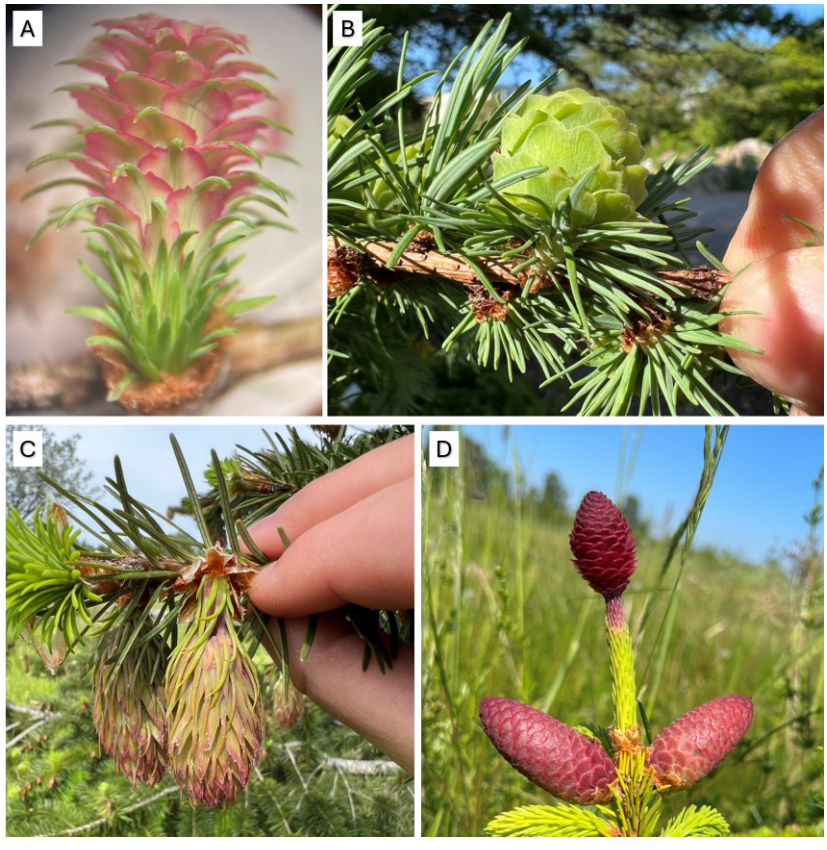

Figure 4. *Larix* cones, *Pseudotsuga* cones, and *acrocona* transition shoot

(A) Receptive *Larix* seed cone with basal needles, needles transitioning into bracts, and bracts. **(B)** *Larix* seed cone after pollination. Ovuliferous scale has extended past most of the bracts, but the tips of the bracts are still visible. Basal needles remain until fall when all needles senesce. **(C)** *Pseudotsuga* seed cone after pollination with basal needles, needles transitioning into bracts, and bracts. Ovuliferous scales have not fully extended yet. **(D)** Transition shoot on a heterozygous *acrocona* with basal needles, needles transitioning into bracts, and bracts. Ovuliferous scales have extended past all bracts. Two full seed cones flank the transition shoot.

I place my stone at the top of the mountain

References

Acheré, V., Faivre-Rampant, P., Jeandroz, S., Besnard, G., Markussen, T., Aragones, A., Fladung, M., Ritter, E., Favre, J.-M., 2004. A full saturated linkage map of *Picea abies* including AFLP, SSR, ESTP, 5S rDNA and morphological markers. Theoretical and Applied Genetics 108, 1602–1613. https://doi.org/10.1007/s00122-004-1587-y

Adrian, J., Torti, S., Turck, F., 2009. From Decision to Commitment: The Molecular Memory of Flowering. Molecular Plant 2, 628–642. https://doi.org/10.1093/mp/ssp031

Akhter, S., Kretzschmar, W.W., Nordal, V., Delhomme, N., Street, N.R., Nilsson, O., Emanuelsson, O., Sundström, J.F., 2018. Integrative Analysis of Three RNA Sequencing Methods Identifies Mutually Exclusive Exons of MADS-Box Isoforms During Early Bud Development in *Picea abies*. Frontiers in Plant Science 9. https://doi.org/10.3389/fpls.2018.01625

Alisha, A., Szweykowska-Kulinska, Z., Sierocka, I., 2024. Comparative analysis of SPL transcription factors from streptophyte algae and embryophytes reveals evolutionary trajectories of SPL family in streptophytes. Scientific Reports 14. https://doi.org/10.1038/s41598-024-51626-2

Almqvist, C., 2003. Timing of GA4/7 application and the flowering of *Pinus sylvestris* grafts in the greenhouse. Tree Physiology 23, 413–418. https://doi.org/10.1093/treephys/23.6.413

Almqvist, C., 2018. Improving floral initiation in potted *Picea abies* by supplemental light treatment. Silva Fennica 52. https://doi.org/10.14214/sf.7772

Almqvist, C. and Wennström, U., 2020. Förädlat skogsodlingsmaterial 2020–2064. Skogforsk Arbetsrapport, pp.1066-2020.

Almqvist, C., 2020. Optimal tidpunkt för gibberellinbehandling (GA4/7) i granfröplantager. Skogforsk.

Almqvist, C., Personal Communication, n.d.

Altaner, C.M., Tokareva, E.N., Jarvis, M.C., Harris, P.J., 2010. Distribution of (1→4)-β-galactans, arabinogalactan proteins, xylans and (1→3)-β-glucans in tracheid cell walls of softwoods. Tree Physiology 30, 782–793. https://doi.org/10.1093/treephys/tpq021

Arctic Climate Impact Assessment, 2005. Arctic Climate Impact Assessment - Scientific Report. Cambridge University Press.

Asante, D.K.A., Yakovlev, I.A., Fossdal, C.G., Holefors, A., Opseth, L., Olsen, J.E., Junttila, O., Johnsen, Ø., 2010. Gene expression changes during short day induced terminal bud formation in Norway spruce. Plant, Cell & Environment 34, 332–346. https://doi.org/10.1111/j.1365-3040.2010.02247.x

Aukerman, M.J., Sakai, H., 2003. Regulation of Flowering Time and Floral Organ Identity by a MicroRNA and Its APETALA2-Like Target Genes. The Plant Cell 15, 2730–2741. https://doi.org/10.1105/tpc.016238

Bag, P., Lihavainen, J., Delhomme, N., Riquelme, T., Robinson, K.M., Jansson, S., 2021. An atlas of the Norway spruce needle seasonal transcriptome. The Plant Journal 108, 1815–1829. https://doi.org/10.1111/tpj.15530

Bartholmes, C., Hidalgo, O., Gleissberg, S., 2011. Evolution of the YABBY gene family with emphasis on the basal eudicot *Eschscholzia californica* (Papaveraceae). Plant Biology 14, 11–23. https://doi.org/10.1111/j.1438-8677.2011.00486.x

Birkenbihl, R.P., Jach, G., Saedler, H., Huijser, P., 2005. Functional Dissection of the Plant-specific SBP-Domain: Overlap of the DNA-binding and Nuclear Localization Domains. Journal of Molecular Biology 352, 585–596. https://doi.org/10.1016/j.jmb.2005.07.013

Bollier, N., Sicard, A., Leblond, J., Latrasse, D., Gonzalez, N., Gévaudant, F., Benhamed, M., Raynaud, C., Lenhard, M., Chevalier, C., Hernould, M., Delmas, F., 2018. At-MINI ZINC FINGER2 and sl-inhibitor OF MERISTEM ACTIVITY, a conserved missing link in the regulation of floral meristem termination in *Arabidopsis* and tomato. The Plant Cell 30, 83–100. https://doi.org/10.1105/tpc.17.00653

Bonnet-Masimbert, M., 1987. Preliminary results on gibberellin induction of flowering of seedlings and cuttings of Norway spruce indicate some carry-over effects. Forest Ecology and Management 19, 163–171. https://doi.org/10.1016/0378-1127(87)90022-3

Bonnet-Masimbert, M., Zaerr, J.B., 1987. 2. The role of plant growth regulators in promotion of flowering. Plant Growth Regulation 6, 13–35. https://doi.org/10.1007/bf00043948

Bouché, F., Lobet, G., Tocquin, P., Périlleux, C., 2015. FLOR-ID: An interactive database of flowering-time gene networks in *Arabidopsis thaliana*. Nucleic Acids Research 44, D1167–D1171. https://doi.org/10.1093/nar/gkv1054

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M., 1991. Genetic interactions among floral homeotic genes of *Arabidopsis*. Development 112, 1–20. https://doi.org/10.1242/dev.112.1.1

Bowman, J.L., Smyth, D.R., 1999. CRABS CLAW, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. Development 126, 2387–2396. https://doi.org/10.1242/dev.126.11.2387

Broome, A., Hendry, S., Peace, A., 2007. Annual and spatial variation in coning shown by the Forest Condition Monitoring programme data for Norway spruce, Sitka spruce and Scots pine in Britain. Forestry 80, 17–28. https://doi.org/10.1093/forestry/cpl046

Burczyk, J., Lewandowski, A., Chalupka, W., 2004. Local pollen dispersal and distant gene flow in Norway spruce (*Picea abies* [L.] Karst.). Forest Ecology and Management 197, 39–48. https://doi.org/10.1016/j.foreco.2004.05.003

Cardon, G.H., Höhmann, S., Nettesheim, K., Saedler, H., Huijser, P., 1997. Functional analysis of the *Arabidopsis thaliana* SBP-box gene SPL3: a novel gene involved in the floral transition. The Plant Journal 12, 367–377. https://doi.org/10.1046/j.1365-313x.1997.12020367.x

Carlsbecker, A., Sundström, J., Tandre, K., Englund, M., Kvarnheden, A., Johanson, U., Engström, P., 2003. The DAL10 gene from Norway spruce (*Picea abies*) belongs to a potentially gymnosperm-specific subclass of MADS-box genes and is specifically active in seed cones and pollen cones. Evolution & Development 5, 551–561. https://doi.org/10.1046/j.1525-142x.2003.03060.x

Carlsbecker, A., Tandre, K., Johanson, U., Englund, M., Engström, P., 2004. The MADS-box gene DAL1 is a potential mediator of the juvenile-to-adult transition in Norway spruce (*Picea abies*). The Plant Journal 40, 546–557. https://doi.org/10.1111/j.1365-313x.2004.02226.x

Carlsbecker, A., Sundström, J.F., Englund, M., Uddenberg, D., Izquierdo, L., Kvarnheden, A., Vergara-Silva, F., Engström, P., 2013. Molecular control of normal and *acrocona* mutant seed cone development in Norway spruce (*Picea abies*) and the evolution of conifer ovule-bearing organs. New Phytologist 200, 261–275. https://doi.org/10.1111/nph.12360

Caudullo, G., Tinner, W., De Rigo, D., 2016. *Picea abies* in Europe: distribution, habitat, usage and threats. Publication Office of the European Union 114–116.

Chalupka, W., Giertych, M., Kopcewicz, J., 1982. Effect of polythene covers, a flower inducing treatment, on the content of endogenous gibberellin-like substances in grafts of Norway spruce. Physiologia Plantarum 54, 79–82. https://doi.org/10.1111/j.1399-3054.1982.tb00581.x

Chen, X., 2004. A MicroRNA as a Translational Repressor of APETALA2 in *Arabidopsis* Flower Development. Science 303, 2022–2025. https://doi.org/10.1126/science.1088060

Chen, X., Zhu, Q., Nie, Y., Han, F., Li, Y., Wu, H.X., Niu, S., 2021. Determination of conifer age biomarker DAL1 interactome using Y2H-seq. Forestry Research 1, 1–12. https://doi.org/10.48130/fr-2021-0012

Cho, H.J., Kim, J.J., Lee, J.H., Kim, W., Jung, J.-H., Park, C.-M., Ahn, J.H., 2012. SHORT VEGETATIVE PHASE (SVP) protein negatively regulates miR172 transcription via direct binding to the pri-miR172a promoter in *Arabidopsis*. FEBS Letters 586, 2332–2337. https://doi.org/10.1016/j.febslet.2012.05.035

Clapham, D.H., Dormling, I., Ekberg, L., Eriksson, G., Qamaruddin, M., Vince-Prue, D., 1998. Latitudinal cline of requirement for far-red light for the photoperiodic control of budset and extension growth in *Picea abies* (Norway spruce). Physiologia Plantarum 102, 71–78. https://doi.org/10.1034/j.1399-3054.1998.1020110.x

Crain, B.A., Cregg, B.M., 2017. Regulation and Management of Cone Induction in Temperate Conifers. Forest Science 64, 82–101. https://doi.org/10.5849/FS-20l6-131

D'Aloia, M., Bonhomme, D., Bouché, F., Tamseddak, K., Ormenese, S., Torti, S., Coupland, G., Périlleux, C., 2011. Cytokinin promotes flowering of *Arabidopsis* via transcriptional activation of the FT paralogue TSF. The Plant Journal 65, 972–979. https://doi.org/10.1111/j.1365-313x.2011.04482.x

Dai, M., Hu, Y., Zhao, Y., Liu, H., Zhou, D.-X., 2007. A WUSCHEL-LIKE HOMEOBOX Gene Represses a YABBY Gene Expression Required for Rice Leaf Development. Plant Physiology 144, 380–390. https://doi.org/10.1104/pp.107.095737

de Lucas, M., Davière, J.-M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blázquez, M.A., Titarenko, E., Prat, S., 2008. A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480–484. https://doi.org/10.1038/nature06520

Ditta, G., Pinyopich, A., Robles, P., Pelaz, S., Yanofsky, M.F., 2004. The SEP4 Gene of *Arabidopsis thaliana* Functions in Floral Organ and Meristem Identity. Current Biology 14, 1935–1940. https://doi.org/10.1016/j.cub.2004.10.028

Du, H., Ran, J.-H., Feng, Y.-Y., Wang, X.-Q., 2020. The flattened and needlelike leaves of the pine family (Pinaceae) share a conserved genetic network for adaxial-abaxial polarity but have diverged for photosynthetic adaptation. BMC Evolutionary Biology 20. https://doi.org/10.1186/s12862-020-01694-5

Dunberg, A., 1980. Stimulation of flowering in *Picea abies* by gibberellins. Silvae Genetica 29, 51–53.

Dunberg, A., Malmberg, G., Sassa, T., Pharis, R.P., 1983. Metabolism of Tritiated Gibberellins A4 and A9 in Norway Spruce, *Picea abies* (L.) Karst. Plant Physiology 71, 257–262. https://doi.org/10.1104/pp.71.2.257

Ekberg, I., Eriksson, G., Dormling, I., 1979. Photoperiodic reactions in conifer species. Ecography 2, 255–263. https://doi.org/10.1111/j.1600-0587.1979.tb01297.x

Eklund, L., Tiltu, A., 1999. Cambial activity in "normal" spruce *Picea abies* Karst (L.) and snake spruce *Picea abies* (L.) Karst f. virgata (Jacq.) Rehd in response to ethylene. Journal of Experimental Botany 50, 1489–1493. https://doi.org/10.1093/jxb/50.338.1489

El Kayal, W., Allen, C.C.G., Ju, C.J., Adams, E., King-Jones, S., Zaharia, L.I., Abrams, S.R., Cooke, J.E.K., 2011. Molecular events of apical bud formation in white spruce, *Picea glauca*. Plant, Cell & Environment 34, 480–500. https://doi.org/10.1111/j.1365-3040.2010.02257.x

El-Kassaby, Y.A., Reynolds, S., 1990. Reproductive phenology, parental balance, and supplemental mass pollination in a sitka-spruce seed-orchard. Forest Ecology and Management 31, 45–54. https://doi.org/10.1016/0378-1127(90)90110-w

Eriksson, G., Jonsson, A., Lindgren, D., 1973. Flowering in a clone trial of *Picea abies* Karst. Studia Forestalia Suecica.

Eriksson, S., Böhlenius, H., Moritz, T., Nilsson, O., 2006. GA4 is the active gibberellin in the regulation of LEAFY transcription and *Arabidopsis* floral initiation. The Plant Cell 18, 2172–2181. https://doi.org/10.1105/tpc.106.042317

Eriksson, G., Schelander, B., Åkebrand, V., 2009. Inbreeding depression in an old experimental plantation of *Picea abies*. Hereditas 73, 185–193. https://doi.org/10.1111/j.1601-5223.1973.tb01081.x

European Commission: Joint Research Centre, Caudullo, G., De Rigo, D., Mauri, A., Houston Durrant, T., San-Miguel-Ayanz, J., 2016. European atlas of forest tree species.

European Environment Agency, 2006. European Forest Types. Categories and types for sustainable forest management reporting and policy. EEA, Report No.9/2006. https://doi.org/10.3832/efor0425-003

Evkaikina, A.I., Berke, L., Romanova, M.A., Proux-Wéra, E., Ivanova, A.N., Rydin, C., Pawlowski, K., Voitsekhovskaja, O.V., 2017. The *Huperzia selago* Shoot Tip Transcriptome Sheds New Light on the Evolution of Leaves. Genome Biology and Evolution 9, 2444–2460. https://doi.org/10.1093/gbe/evx169

Fang, Y., Xiong, L., 2014. General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular and Molecular Life Sciences 72, 673–689. https://doi.org/10.1007/s00018-014-1767-0

Favaro, R., Pinyopich, A., Battaglia, R., Kooiker, M., Borghi, L., Ditta, G., Yanofsky, M.F., Kater, M.M., Colombo, L., 2003. MADS-Box protein complexes control carpel and ovule development in *Arabidopsis*. The Plant Cell 15, 2603–2611. https://doi.org/10.1105/tpc.015123

Felton, A., Petersson, L., Nilsson, O., Witzell, J., Cleary, M., Felton, A.M., Björkman, C., Sang, Å.O., Jonsell, M., Holmström, E., Nilsson, U., Rönnberg, J., Kalén, C., Lindbladh, M., 2019. The tree species matters: Biodiversity and ecosystem service implications of replacing Scots pine production stands with Norway spruce. Ambio 49, 1035–1049. https://doi.org/10.1007/s13280-019-01259-x

Finet, C., Floyd, S.K., Conway, S.J., Zhong, B., Scutt, C.P., Bowman, J.L., 2016. Evolution of the YABBY gene family in seed plants. Evolution & Development 18, 116–126. https://doi.org/10.1111/ede.12173

Florin, R., 1951. Evolution in cordaites and conifers. Acta Horti Bergiani 15, 285–388.

Floyd, S.K., Bowman, J.L., 2006. Distinct Developmental Mechanisms Reflect the Independent Origins of Leaves in Vascular Plants. Current Biology 16, 1911–1917. https://doi.org/10.1016/j.cub.2006.07.067

Floyd, S.K., Bowman, J.L., 2009. Gene expression patterns in seed plant shoot meristems and leaves: homoplasy or homology? Journal of Plant Research 123, 43–55. https://doi.org/10.1007/s10265-009-0256-2

Forests and Forestry in Sweden, 2015. Royal Swedish Academy of Agriculture and Forestry, Stockholm.

Fries, T., 1890. Strödda bidrag till kännedom om Skandinaviens barrträd. Botaniska Notiser 1, 250–260.

Funda, T., El-Kassaby, Y.A., 2012. Seed orchard genetics. CABI Reviews 1–23. https://doi.org/10.1079/pavsnnr20127013

Galeano, E., Thomas, B.R., 2020. Effect of elevated gibberellic acid application on growth and gene expression patterns in white spruce families from a tree improvement program in Alberta, Canada. Tree Physiology 41, 472–490. https://doi.org/10.1093/treephys/tpaa133

Galvão, V.C., Horrer, D., Küttner, F., Schmid, M., 2012. Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*. Development 139, 4072–4082. https://doi.org/10.1242/dev.080879

Gandikota, M., Birkenbihl, R.P., Höhmann, S., Cardon, G.H., Saedler, H., Huijser, P., 2007. The miRNA156/157 recognition element in the 3′ UTR of the *Arabidopsis* SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. The Plant Journal 49, 683–693. https://doi.org/10.1111/j.1365-313x.2006.02983.x

Giacomello, S., Salmén, F., Terebieniec, B.K., Vickovic, S., Navarro, J.F., Alexeyenko, A., Reimegård, J., McKee, L.S., Mannapperuma, C., Bulone, V., Ståhl, P.L., Sundström, J.F., Street, N.R., Lundeberg, J., 2017. Spatially resolved transcriptome profiling in model plant species. Nature Plants 3. https://doi.org/10.1038/nplants.2017.61

Gleissberg, S., Groot, E.P., Schmalz, M., Eichert, M., Kölsch, A., Hutter, S., 2005. Developmental events leading to peltate leaf structure in *Tropaeolum majus* (*Tropaeolaceae*) are associated with expression domain changes of a YABBY gene. Development Genes and Evolution 215, 313–319. https://doi.org/10.1007/s00427-005-0479-8

Gocal, G.F.W., Sheldon, C.C., Gubler, F., Moritz, T., Bagnall, D.J., MacMillan, C.P., Li, S.F., Parish, R.W., Dennis, E.S., Weigel, D., King, R.W., 2001.

GAMYB-like Genes, Flowering, and Gibberellin Signaling in *Arabidopsis*. Plant Physiology 127, 1682–1693. https://doi.org/10.1104/pp.010442

Goldshmidt, A., Alvarez, J.P., Bowman, J.L., Eshed, Y., 2008. Signals Derived from YABBY Gene Activities in Organ Primordia Regulate Growth and Partitioning of *Arabidopsis* Shoot Apical Meristems. The Plant Cell 20, 1217–1230. https://doi.org/10.1105/tpc.107.057877

Golz, J.F., Roccaro, M., Kuzoff, R., Hudson, A., 2004. GRAMINIFOLIA promotes growth and polarity of *Antirrhinum* leaves. Development 131, 3661–3670. https://doi.org/10.1242/dev.01221

Gómez-Mena, C., de Folter, S., Costa, M.M.R., Angenent, G.C., Sablowski, R., 2005. Transcriptional program controlled by the floral homeotic gene AGAMOUS during early organogenesis. Development 132, 429–438. https://doi.org/10.1242/dev.01600

Gramzow, L., Theissen, G., 2010. A hitchhiker's guide to the MADS world of plants. Genome Biology 11, 214. https://doi.org/10.1186/gb-2010-11-6-214

Gunnert, L., 1962. Undersökning av ormgransbeståndet i Lekeryd (*Picea abies* (L.) H. Karst f. virgata (Jacques) Rehd.) Investigation of the virgate spruce stand at Lekeryd. Statens Skogsforskningsinstitut, Stockholm.

Guo, C., Xu, Y., Shi, M., Lai, Y., Wu, X., Wang, H., Zhu, Z., Poethig, R.S., Wu, G., 2017. Repression of miR156 by miR159 Regulates the Timing of the Juvenile-to-Adult Transition in *Arabidopsis*. The Plant Cell 29, 1293–1304. https://doi.org/10.1105/tpc.16.00975

Guo, L., Cao, X., Liu, Y., Li, J., Li, Y., Li, D., Zhang, K., Gao, C., Dong, A., Liu, X., 2018. A chromatin loop represses WUSCHEL expression in *Arabidopsis*. The Plant Journal 94, 1083–1097. https://doi.org/10.1111/tpj.13921

Guo, Y., Niu, S., El-Kassaby, Y.A., Li, W., 2020. Transcriptome-wide isolation and expression of NF-Y gene family in male cone development and hormonal treatment of *Pinus tabuliformis*. Physiologia Plantarum 171, 34–47. https://doi.org/10.1111/ppl.13183

Gustafson-Brown, C., Savidge, B., Yanofsky, M.F., 1994. Regulation of the *Arabidopsis* floral homeotic gene APETALA1. Cell 76, 131–143. https://doi.org/10.1016/0092-8674(94)90178-3

Gyllenstrand, N., Clapham, D., Källman, T., Lagercrantz, U., 2007. A Norway Spruce FLOWERING LOCUS T Homolog Is Implicated in Control of Growth Rhythm in Conifers. Plant Physiology 144, 248–257. https://doi.org/10.1104/pp.107.095802

Gyllenstrand, N., Karlgren, A., Clapham, D., Holm, K., Hall, A., Gould, P.D., Källman, T., Lagercrantz, U., 2014. No Time for Spruce: Rapid Dampening of Circadian Rhythms in *Picea abies* (L. Karst). Plant and Cell Physiology 55, 535–550. https://doi.org/10.1093/pcp/pct199

Gyula, P., Baksa, I., Tóth, T., Mohorianu, I., Dalmay, T., Szittya, G., 2018. Ambient temperature regulates the expression of a small set of sRNAs influencing plant development through NF-YA2 and YUC2. Plant, Cell & Environment 41, 2404–2417. https://doi.org/10.1111/pce.13355

Haapanen, M., Jansson, G., Nielsen, U.B., Steffenrem, A., Stener, L.-G., 2015. The status of tree breeding and its potential for improving biomass production – A review of breeding activities and genetic gains in Scandinavia and Finland. Skogforsk.

Hagman, M., 1975. Incompatibility in forest trees. Proceedings of the Royal Society of London. Series B. Biological Sciences 188, 313–326. https://doi.org/10.1098/rspb.1975.0022

Hagman, M., 1980. Experiences with Norway spruce provenances in Finland. Silva Fennica 14. https://doi.org/10.14214/sf.a15002

Hanzawa, Y., Money, T., Bradley, D., 2005. A single amino acid converts a repressor to an activator of flowering. Proceedings of the National Academy of Sciences 102, 7748–7753. https://doi.org/10.1073/pnas.0500932102

Harrison, D.L.S., Owens, J.N., 1983. Bud development in *Picea engelmannii*. I. Vegetative bud development, differentiation, and early development of reproductive buds. Canadian Journal of Botany 61, 2291–2301. https://doi.org/10.1139/b83-251

Heide, O.M., 1974. Growth and Dormancy in Norway Spruce Ecotypes (*Picea abies*) I. Interaction of Photoperiod and Temperature. Physiologia Plantarum 30, 1–12. https://doi.org/10.1111/j.1399-3054.1974.tb04983.x

Hejnowicz, A., Obarska, E., 1995. Structure and development of vegetative buds, from the lower crown of *Picea abies*. Annales des Sciences Forestières 52, 433–447. https://doi.org/10.1051/forest:19950504

Helliwell, C.A., Sullivan, J.A., Mould, R.M., Gray, J.C., Peacock, W.J., Dennis, E.S., 2001. A plastid envelope location of *Arabidopsis* ent-kaurene oxidase links the plastid and endoplasmic reticulum steps of the gibberellin biosynthesis pathway. The Plant Journal 28, 201–208. https://doi.org/10.1046/j.1365-313x.2001.01150.x

Helliwell, C.A., Wood, C.C., Robertson, M., James Peacock, W., Dennis, E.S., 2006. The *Arabidopsis* FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. The Plant Journal 46, 183–192. https://doi.org/10.1111/j.1365-313x.2006.02686.x

Herting, J., Stützel, T., Klaus, K.V., 2020. The Ancestral Conifer Cone: What Did It Look Like? A Modern Trait-Evolution Approach. International Journal of Plant Sciences 181, 871–886. https://doi.org/10.1086/710489

Heuchel, A., Hall, D., Zhao, W., Gao, J., Wennström, U., Wang, X.-R., 2022. Genetic diversity and background pollen contamination in Norway spruce

and Scots pine seed orchard crops. Forestry Research 2, 0–0. https://doi.org/10.48130/fr-2022-0008

Ho, R.H., 1991. Timing of gibberellin A4/7 application for cone production in potted black spruce grafts. Canadian Journal of Forest Research 21, 1137–1140. https://doi.org/10.1139/x91-158

Högberg, K., Eriksson, U., 1994. Effects of root pruning and stem injections with Gibberellin a 4/7 on flowering and cone harvest in three *Picea abies* seed orchards. Scandinavian Journal of Forest Research 9, 323–328. https://doi.org/10.1080/02827589409382847

Holefors, A., Opseth, L., Ree Rosnes, A.K., Ripel, L., Snipen, L., Fossdal, C.G., Olsen, J.E., 2009. Identification of PaCOL1 and PaCOL2, two CONSTANS-like genes showing decreased transcript levels preceding short day induced growth cessation in Norway spruce. Plant Physiology and Biochemistry 47, 105–115. https://doi.org/10.1016/j.plaphy.2008.11.003

Honma, T., Goto, K., 2001. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409, 525–529. https://doi.org/10.1038/35054083

Hou, X., Zhou, J., Liu, C., Liu, L., Shen, L., Yu, H., 2014. Nuclear factor Y-mediated H3K27me3 demethylation of the SOC1 locus orchestrates flowering responses of *Arabidopsis*. Nature Communications 5. https://doi.org/10.1038/ncomms5601

Huijser, P., Schmid, M., 2011. The control of developmental phase transitions in plants. Development 138, 4117–4129. https://doi.org/10.1242/dev.063511

Hwang, K., Susila, H., Nasim, Z., Jung, J.-Y., Ahn, J.H., 2019. *Arabidopsis* ABF3 and ABF4 Transcription Factors Act with the NF-YC Complex to Regulate SOC1 Expression and Mediate Drought-Accelerated Flowering. Molecular Plant 12, 489–505. https://doi.org/10.1016/j.molp.2019.01.002

Hyun, Y., Richter, R., Vincent, C., Martinez-Gallegos, R., Porri, A., Coupland, G., 2016. Multi-layered regulation of SPL15 and cooperation with SOC1 integrate endogenous flowering pathways at the *Arabidopsis* shoot meristem. Developmental Cell 37, 254–266. https://doi.org/10.1016/j.devcel.2016.04.001

Immink, R.G.H., Posé, D., Ferrario, S., Ott, F., Kaufmann, K., Valentim, F.L., de Folter, S., van der Wal, F., van Dijk, A.D.J., Schmid, M., Angenent, G.C., 2012. Characterization of SOC1's Central Role in Flowering by the Identification of Its Upstream and Downstream Regulators. Plant Physiology 160, 433–449. https://doi.org/10.1104/pp.112.202614

IPCC, 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T.

Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2391 pp. doi:10.1017/9781009157896.

Jactel, H., Koricheva, J., Castagneyrol, B., 2019. Responses of forest insect pests to climate change: Not so simple. Current Opinion in Insect Science 35, 103–108. https://doi.org/10.1016/j.cois.2019.07.010

Johnsen, Ø., Haug, G., Dæhlen, O.G., Grønstad, B.S., Rognstad, A.T., 1994. Effects of heat treatment, timing of heat treatment, and gibberellin A4/7 on flowering in potted *Picea abies* grafts. Scandinavian Journal of Forest Research 9, 333–340. https://doi.org/10.1080/02827589409382849

Jokipii-Lukkari, S., Sundell, D., Nilsson, O., Hvidsten, T.R., Street, N.R., Tuominen, H., 2017. NorWood: a gene expression resource for evo-devo studies of conifer wood development. New Phytologist 216, 482–494. https://doi.org/10.1111/nph.14458

Joneborg, S., 1945. Montros kotte-bildning hos granen. Vegetativt skott eller kotte? Svenska skogsvårdsföreningens tidskrift.

Jönsson, A.M., Bärring, L., 2011. Ensemble analysis of frost damage on vegetation caused by spring backlashes in a warmer Europe. Natural Hazards and Earth System Sciences 11, 401–418. https://doi.org/10.5194/nhess-11-401-2011

Juarez, M.T., Twigg, R.W., Timmermans, M.C.P., 2004. Specification of adaxial cell fate during maize leaf development. Development 131, 4533–4544. https://doi.org/10.1242/dev.01328

Jung, J.-H., Seo, Y.-H., Seo, P.J., Reyes, J.L., Yun, J., Chua, N.-H., Park, C.-M., 2007. The GIGANTEA-Regulated MicroRNA172 Mediates Photoperiodic Flowering Independent of CONSTANS in *Arabidopsis*. The Plant Cell 19, 2736–2748. https://doi.org/10.1105/tpc.107.054528

Jung, J., Ju, Y., Seo, P.J., Lee, J., Park, C., 2011. The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in *Arabidopsis*. The Plant Journal 69, 577–588. https://doi.org/10.1111/j.1365-313x.2011.04813.x

Källman, T., De Mita, S., Larsson, H., Gyllenstrand, N., Heuertz, M., Parducci, L., Suyama, Y., Lagercrantz, U., Lascoux, M., 2014. Patterns of Nucleotide Diversity at Photoperiod Related Genes in Norway Spruce [*Picea abies* (L.) Karst.]. PLoS ONE 9, e95306. https://doi.org/10.1371/journal.pone.0095306

Karlgren, A., Carlsson, J., Gyllenstrand, N., Lagercrantz, U., Sundström, J.F., 2009. Non-radioactive in situ hybridization protocol applicable for Norway spruce and a range of plant species. Journal of Visualized Experiments. https://doi.org/10.3791/1205

Karlgren, A., Gyllenstrand, N., Källman, T., Sundström, J.F., Moore, D., Lascoux, M., Lagercrantz, U., 2011. Evolution of the PEBP gene family in plants:

Functional diversification in seed plant evolution. Plant Physiology 156, 1967–1977. https://doi.org/10.1104/pp.111.176206

Karlgren, A., Gyllenstrand, N., Clapham, D., Lagercrantz, U., 2013a. FLOWERING LOCUS T/TERMINAL flower1-like genes affect growth rhythm and bud set in Norway Spruce. PLANT PHYSIOLOGY 163, 792–803. https://doi.org/10.1104/pp.113.224139

Karlgren, A., Gyllenstrand, N., Källman, T., Lagercrantz, U., 2013b. Conserved Function of Core Clock Proteins in the Gymnosperm Norway Spruce (*Picea abies* L. Karst). PLoS ONE 8, e60110. https://doi.org/10.1371/journal.pone.0060110

Katahata, S.-I., Futamura, N., Igasaki, T., Shinohara, K., 2014. Functional analysis of SOC1-like and AGL6-like MADS-box genes of the gymnosperm *Cryptomeria japonica*. Tree Genetics & Genomes 10, 317–327. https://doi.org/10.1007/s11295-013-0686-9

Katyayini, N.U., Rinne, P.L.H., Tarkowská, D., Strnad, M., van der Schoot, C., 2020. Dual Role of Gibberellin in Perennial Shoot Branching: Inhibition and Activation. Frontiers in Plant Science 11. https://doi.org/10.3389/fpls.2020.00736

Kaufmann, K., Muiño, J.M., Jauregui, R., Airoldi, C.A., Smaczniak, C., Krajewski, P., Angenent, G.C., 2009. Target genes of the MADS transcription factor SEPALLATA3: Integration of developmental and hormonal pathways in the *Arabidopsis* flower. PLoS Biology 7, e1000090. https://doi.org/10.1371/journal.pbio.1000090

Kilpeläinen, A., Kellomäki, S., Strandman, H., Venäläinen, A., 2010. Climate change impacts on forest fire potential in boreal conditions in Finland. Climatic Change 103, 383–398. https://doi.org/10.1007/s10584-009-9788-7

Kim, J.J., Lee, J.H., Kim, W., Jung, H.S., Huijser, P., Ahn, J.H., 2012. The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 Module Regulates Ambient Temperature-Responsive Flowering via FLOWERING LOCUS T in *Arabidopsis*. Plant Physiology 159, 461–478. https://doi.org/10.1104/pp.111.192369

Klintenäs, M., Pin, P.A., Benlloch, R., Ingvarsson, P.K., Nilsson, O., 2012. Analysis of conifer FLOWERING LOCUS T/TERMINAL FLOWER1-like genes provides evidence for dramatic biochemical evolution in the angiosperm FT lineage. New Phytologist 196, 1260–1273. https://doi.org/10.1111/j.1469-8137.2012.04332.x

Kong, L., von Aderkas, P., 2004. Plant growth regulators and cone induction in Pinaceae. Published on-line by the Forest Genetics Council of British Columbia. https://doi.org/http://www.fgcouncil.bc.ca/Pinaceae-cone-ind-lit-rev.pdf

Kong, L., Jaquish, B., Zaharia, L.I., von Aderkas, P., 2021. Phytohormone profiles of sterile Douglas-fir mutants and the responses to stem-injected gibberellins. Trees 35, 1961–1969. https://doi.org/10.1007/s00468-021-02163-2

Kumar, S.V., Lucyshyn, D., Jaeger, K.E., Alós, E., Alvey, E., Harberd, N.P., Wigge, P.A., 2012. Transcription factor PIF4 controls the thermosensory activation of flowering. Nature 484, 242–245. https://doi.org/10.1038/nature10928

Kumimoto, R.W., Adam, L., Hymus, G.J., Repetti, P.P., Reuber, T.L., Marion, C.M., Hempel, F.D., Ratcliffe, O.J., 2008. The Nuclear Factor Y subunits NF-YB2 and NF-YB3 play additive roles in the promotion of flowering by inductive long-day photoperiods in *Arabidopsis*. Planta 228, 709–723. https://doi.org/10.1007/s00425-008-0773-6

Kumimoto, R.W., Zhang, Y., Siefers, N., Holt III, B.F., 2010. NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated, photoperiod-dependent flowering in *Arabidopsis thaliana*. The Plant Journal 63, 379–391. https://doi.org/10.1111/j.1365-313x.2010.04247.x

Langvall, O., 2011. Impact of climate change, seedling type and provenance on the risk of damage to Norway spruce (*Picea abies* (L.) Karst.) seedlings in Sweden due to early summer frosts. Scandinavian Journal of Forest Research 26, 56–63. https://doi.org/10.1080/02827581.2011.564399

Laubinger, S., Marchal, V., Gentilhomme, J., Wenkel, S., Adrian, J., Jang, S., Kulajta, C., Braun, H., Coupland, G., Hoecker, U., 2006. *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development 133, 3213–3222. https://doi.org/10.1242/dev.02481

Lázaro-Gimeno, D., Ferrari, C., Delhomme, N., Johansson, M., Sjölander, J., Singh, R.K., Mutwil, M., Eriksson, M.E., 2024. The circadian clock participates in seasonal growth in Norway spruce (*Picea abies*). Tree Physiology 44. https://doi.org/10.1093/treephys/tpae139

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., Ahn, J.H., 2007. Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. Genes & Development 21, 397–402. https://doi.org/10.1101/gad.1518407

Lee, J., Oh, M., Park, H., Lee, I., 2008. SOC1 translocated to the nucleus by interaction with AGL24 directly regulates LEAFY. The Plant Journal 55, 832–843. https://doi.org/10.1111/j.1365-313x.2008.03552.x

Lee, H., Yoo, S.J., Lee, J.H., Kim, W., Yoo, S.K., Fitzgerald, H., Carrington, J.C., Ahn, J.H., 2010. Genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs in *Arabidopsis*. Nucleic Acids Research 38, 3081–3093. https://doi.org/10.1093/nar/gkp1240

Lee, J.H., Ryu, H.-S., Chung, K.S., Posé, D., Kim, S., Schmid, M., Ahn, J.H., 2013. Regulation of Temperature-Responsive Flowering by MADS-Box

Transcription Factor Repressors. Science 342, 628–632. https://doi.org/10.1126/science.1241097

Li, F.-W., Nishiyama, T., Waller, M., Frangedakis, E., Keller, J., Li, Z., Fernandez-Pozo, N., Barker, M.S., Bennett, T., Blázquez, M.A., Cheng, S., Cuming, A.C., de Vries, J., de Vries, S., Delaux, P.-M., Diop, I.S., Harrison, C.J., Hauser, D., Hernández-García, J., Kirbis, A., Meeks, J.C., Monte, I., Mutte, S.K., Neubauer, A., Quandt, D., Robison, T., Shimamura, M., Rensing, S.A., Villarreal, J.C., Weijers, D., Wicke, S., Wong, G.K.-S., Sakakibara, K., Szövényi, P., 2020. *Anthoceros* genomes illuminate the origin of land plants and the unique biology of hornworts. Nature Plants 6, 259–272. https://doi.org/10.1038/s41477-020-0618-2

Liljegren, S.J., Gustafson-Brown, C., Pinyopich, A., Ditta, G.S., Yanofsky, M.F., 1999. Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 Specify Meristem Fate. The Plant Cell 11, 1007–1018. https://doi.org/10.1105/tpc.11.6.1007

Linde, A., Eklund, D.M., Kubota, A., Pederson, E.R.A., Holm, K., Gyllenstrand, N., Nishihama, R., Cronberg, N., Muranaka, T., Oyama, T., Kohchi, T., Lagercrantz, U., 2017. Early evolution of the land plant circadian clock. New Phytologist 216, 576–590. https://doi.org/10.1111/nph.14487

Lindgren, D., Lindgren, K., 1997. Long distance pollen transfer may make gene conservation difficult, in: Nordic Group for Forest Genetics and Tree Breeding Meeting in Estonia June 3-7, 1996. Estonian Agricultural University, Tartu.

Little, C.H.A., MacDonald, J.E., 2003. Effects of exogenous gibberellin and auxin on shoot elongation and vegetative bud development in seedlings of *Pinus* sylvestris and *Picea glauca*. Tree Physiology 23, 73–83. https://doi.org/10.1093/treephys/23.2.73

Liu, L.-J., Zhang, Y.-C., Li, Q.-H., Sang, Y., Mao, J., Lian, H.-L., Wang, L., Yang, H.-Q., 2008. COP1-Mediated Ubiquitination of CONSTANS Is Implicated in Cryptochrome Regulation of Flowering in *Arabidopsis*. The Plant Cell 20, 292–306. https://doi.org/10.1105/tpc.107.057281

Liu, Y., Yang, K., Wei, X., Wang, X., 2016. Revisiting the phosphatidylethanolamine-binding protein (PEBP) gene family reveals cryptic FLOWERING LOCUS T gene homologs in gymnosperms and sheds new light on functional evolution. New Phytologist 212, 730–744. https://doi.org/10.1111/nph.14066

Liu, H., Wang, X., Wang, G., Cui, P., Wu, S., Ai, C., Hu, N., Li, A., He, B., Shao, X., Wu, Z., Feng, H., Chang, Y., Mu, D., Hou, J., Dai, X., Yin, T., Ruan, J., Cao, F., 2021. The nearly complete genome of *Ginkgo biloba* illuminates gymnosperm evolution. Nature Plants 7, 748–756. https://doi.org/10.1038/s41477-021-00933-x

Liu, H., Guo, Y., Wang, H., Yang, W., Yang, J., Zhang, J., Liu, D., El-Kassaby, Y.A., Li, W., 2022. Involvement of PtCOL5-PtNF-YC4 in reproductive cone development and gibberellin signaling in Chinese pine. Plant Science 323, 111383. https://doi.org/10.1016/j.plantsci.2022.111383

Liziniewicz, M., Berlin, M., Solvin, T., Hallingbäck, H.R., Haapanen, M., Ruotsalainen, S., Steffenrem, A., 2023. Development of a universal height response model for transfer of Norway spruce (*Picea abies* L. Karst) in Fennoscandia. Forest Ecology and Management 528, 120628. https://doi.org/10.1016/j.foreco.2022.120628

Luukkanen, O., 1979. Hormonal treatment increases flowering in Norway spruce grafts grown in a plastic greenhouse. Finnish Foundation for Forest Tree Breeding Annual Reports 22–26.

Ma, J.-J., Chen, X., Song, Y.-T., Zhang, G.-F., Zhou, X.-Q., Que, S.-P., Mao, F., Pervaiz, T., Lin, J.-X., Li, Y., Li, W., Wu, H.X., Niu, S.-H., 2021. MADS-box transcription factors MADS11 and DAL1 interact to mediate the vegetative-to-reproductive transition in pine. Plant Physiology 187, 247–262. https://doi.org/10.1093/plphys/kiab250

Marquard, R.D., Hanover, J.W., 1984. Relationship between gibberellin A4/7 concentration, time of treatment, and crown position on flowering of *Picea glauca*. Canadian Journal of Forest Research 14, 547–553. https://doi.org/10.1139/x84-101

Martin, R.C., Asahina, M., Liu, P.-P., Kristof, J.R., Coppersmith, J.L., Pluskota, W.E., Bassel, G.W., Goloviznina, N.A., Nguyen, T.T., Martínez-Andújar, C., Arun Kumar, M.B., Pupel, P., Nonogaki, H., 2010. The regulation of post-germinative transition from the cotyledon- to vegetative-leaf stages by microRNA-targeted SQUAMOSA PROMOTER-BINDING PROTEIN LIKE13 in *Arabidopsis*. Seed Science Research 20, 89–96. https://doi.org/10.1017/s0960258510000073

Mathieu, J., Yant, L.J., Mürdter, F., Küttner, F., Schmid, M., 2009. Repression of Flowering by the miR172 Target SMZ. PLoS Biology 7, e1000148. https://doi.org/10.1371/journal.pbio.1000148

Michaels, S.D., Himelblau, E., Kim, S.Y., Schomburg, F.M., Amasino, R.M., 2005. Integration of flowering signals in winter-annual *Arabidopsis*. Plant Physiology 137, 149–156. https://doi.org/10.1104/pp.104.052811

Mølmann, J.A., Junttila, O., Johnsen, Ø., Olsen, J.E., 2005. Effects of red, far-red and blue light in maintaining growth in latitudinal populations of Norway spruce (*Picea abies*). Plant, Cell & Environment 29, 166–172. https://doi.org/10.1111/j.1365-3040.2005.01408.x

Murase, K., Hirano, Y., Sun, T., Hakoshima, T., 2008. Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456, 459–463. https://doi.org/10.1038/nature07519

Nikkanen, T., 2001. Reproductive phenology in a Norway spruce seed orchard. Silva Fennica 35. https://doi.org/10.14214/sf.602

Nilsson, L., Carlsbecker, A., Sundås-Larsson, A., Vahala, T., 2006. APETALA2 like genes from *Picea abies* show functional similarities to their *Arabidopsis* homologues. Planta 225, 589–602. https://doi.org/10.1007/s00425-006-0374-1

Niu, S., Li, J., Bo, W., Yang, W., Zuccolo, A., Giacomello, S., Chen, X., Han, F., Yang, J., Song, Y., Nie, Y., Zhou, B., Wang, P., Zuo, Q., Zhang, H., Ma, J., Wang, J., Wang, L., Zhu, Q., Zhao, H., Liu, Z., Zhang, X., Liu, T., Pei, S., Li, Z., Hu, Y., Yang, Y., Li, Wenzhao, Zan, Y., Zhou, L., Lin, J., Yuan, T., Li, Wei, Li, Y., Wei, H., Wu, H.X., 2022. The Chinese pine genome and methylome unveil key features of conifer evolution. Cell 185, 204-217.e14. https://doi.org/10.1016/j.cell.2021.12.006

Nystedt, B., Street, N.R., Wetterbom, A., Zuccolo, A., Lin, Y.-C., Scofield, D.G., Vezzi, F., Delhomme, N., Giacomello, S., Alexeyenko, A., Vicedomini, R., Sahlin, K., Sherwood, E., Elfstrand, M., Gramzow, L., Holmberg, K., Hällman, J., Keech, O., Klasson, L., Koriabine, M., Kucukoglu, M., Käller, M., Luthman, J., Lysholm, F., Niittylä, T., Olson, Å., Rilakovic, N., Ritland, C., Rosselló, J.A., Sena, J., Svensson, T., Talavera-López, C., Theißen, G., Tuominen, H., Vanneste, K., Wu, Z.-Q., Zhang, B., Zerbe, P., Arvestad, L., Bhalerao, R., Bohlmann, J., Bousquet, J., Garcia Gil, R., Hvidsten, T.R., de Jong, P., MacKay, J., Morgante, M., Ritland, K., Sundberg, B., Lee Thompson, S., Van de Peer, Y., Andersson, B., Nilsson, O., Ingvarsson, P.K., Lundeberg, J., Jansson, S., 2013. The Norway spruce genome sequence and conifer genome evolution. Nature 497, 579–584. https://doi.org/10.1038/nature12211

Ó'Maoiléidigh, Diarmuid Seosamh, Graciet, E., Wellmer, F., 2013a. Gene networks controlling *Arabidopsis thaliana* flower development. New Phytologist 201, 16–30. https://doi.org/10.1111/nph.12444

Ó'Maoiléidigh, Diarmuid S., Wuest, S.E., Rae, L., Raganelli, A., Ryan, P.T., Kwaśniewska, K., Das, P., Lohan, A.J., Loftus, B., Graciet, E., Wellmer, F., 2013b. Control of Reproductive Floral Organ Identity Specification in *Arabidopsis* by the C Function Regulator AGAMOUS. The Plant Cell 25, 2482–2503. https://doi.org/10.1105/tpc.113.113209

Oden, P.C., Wang, Q., Hogberg, K.-A., Werner, M., 1995. Transport and metabolism of gibberellins in relation to flower bud differentiation in Norway spruce (*Picea abies*). Tree Physiology 15, 451–456. https://doi.org/10.1093/treephys/15.7-8.451

Olsen, H.C., 1978. Blomstringsinduktion hos roedgran (Induction of flowering in Norway spruce). Det Forstlige Forsøgsvæsen I Danmark 36, 230–266.

Owens, J.N., Molder, M., 1976a. Bud development in Sitka spruce. II. Cone differentiation and early development. Canadian Journal of Botany 54, 766–779. https://doi.org/10.1139/b76-083

Owens, J.N., Molder, M., 1976b. Bud development in Sitka spruce. I. Annual growth cycle of vegetative buds and shoots. Canadian Journal of Botany 54, 313–325. https://doi.org/10.1139/b76-029

Owens, J.N., Molder, M., 1977. Bud development in *Picea glauca*. II. Cone differentiation and early development. Canadian Journal of Botany 55, 2746–2760. https://doi.org/10.1139/b77-313

Owens, J.N., Blake, M.D., 1985. Forest tree seed production. A review of the literature and recommendations for future research. Canadian Forestry Service, Petawawa National Forestry Institute.

Owens, J.N., Simpson, S.J., 1988. Bud and shoot development in *Picea engelmannii* in response to cone induction treatments. Canadian Journal of Forest Research 18, 231–241. https://doi.org/10.1139/x88-034

Owens, J.N., Philipson, J.J., Harrison, D.L.S., 1992. The effects of the duration and timing of drought plus heat plus gibberellin A4/7 on apical meristem development and coning in Sitka spruce [*Picea* sitchensis (Bong.) Carr]. New Phytologist 122, 515–528. https://doi.org/10.1111/j.1469-8137.1992.tb00081.x

Padmanabhan, M.S., Ma, S., Burch-Smith, T.M., Czymmek, K., Huijser, P., Dinesh-Kumar, S.P., 2013. Novel Positive Regulatory Role for the SPL6 Transcription Factor in the N TIR-NB-LRR Receptor-Mediated Plant Innate Immunity. PLoS Pathogens 9, e1003235. https://doi.org/10.1371/journal.ppat.1003235

Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E., Yanofsky, M.F., 2000. B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature 405, 200–203. https://doi.org/10.1038/35012103

Pharis, R.P., Webber, J.E., Ross, S.D., 1987. The promotion of flowering in forest trees by gibberellin and cultural treatments: A review of the possible mechanisms. Forest Ecology and Management 19, 65–84. https://doi.org/10.1016/0378-1127(87)90012-0

Poliakova, A., Brown, A.G., Alsos, I.G., 2024. Exotic pollen in sediments from the high Arctic Lake Tenndammen, Svalbard archipelago: diversity, sources, and transport pathways. Palynology 48. https://doi.org/10.1080/01916122.2023.2287005

Prunet, N., Morel, P., Negrutiu, I., Trehin, C., 2009. Time to Stop: Flower Meristem Termination. Plant Physiology 150, 1764–1772. https://doi.org/10.1104/pp.109.141812

Pureswaran, D.S., Roques, A., Battisti, A., 2018. Forest insects and climate change. Current Forestry Reports 4, 35–50. https://doi.org/10.1007/s40725-018-0075-6

Ratcliffe, O.J., Bradley, D.J., Coen, E.S., 1999. Separation of shoot and floral identity in *Arabidopsis*. Development 126, 1109–1120. https://doi.org/10.1242/dev.126.6.1109

Riboni, M., Galbiati, M., Tonelli, C., Conti, L., 2013. GIGANTEA Enables Drought Escape Response via Abscisic Acid-Dependent Activation of the Florigens and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1. PLANT PHYSIOLOGY 162, 1706–1719. https://doi.org/10.1104/pp.113.217729

Ross, S.D., 1983. Enhancement of shoot elongation in Douglas-fir by gibberellin A4/7 and its relation to the hormonal promotion of flowering. Canadian Journal of Forest Research 13, 986–994. https://doi.org/10.1139/x83-131

Ross, S.D., 1985. Promotion of flowering in potted *Picea engelmannii* (Perry) grafts: effects of heat, drought, gibberellin A4/7, and their timing. Canadian Journal of Forest Research 15, 618–624. https://doi.org/10.1139/x85-101

Ross, S.D., 1988. Effects of temperature, drought, and gibberellin A4/7, and timing of treatment, on flowering in potted *Picea engelmannii* and *Picea glauca* grafts. Canadian Journal of Forest Research 18, 163–171. https://doi.org/10.1139/x88-026

Rytter, L., Ingerslev, M., Kilpeläinen, A., Torssonen, P., Lazdina, D., Löf, M., Madsen, P., Muiste, P., Stener, L.-G., 2016. Increased forest biomass production in the Nordic and Baltic countries – a review on current and future opportunities. Silva Fennica 50. https://doi.org/10.14214/sf.1660

Sarojam, R., Sappl, P.G., Goldshmidt, A., Efroni, I., Floyd, S.K., Eshed, Y., Bowman, J.L., 2010. Differentiating *Arabidopsis* Shoots from Leaves by Combined YABBY Activities. The Plant Cell 22, 2113–2130. https://doi.org/10.1105/tpc.110.075853

Sato, H., Suzuki, T., Takahashi, F., Shinozaki, K., Yamaguchi-Shinozaki, K., 2019. NF-YB2 and NF-YB3 Have Functionally Diverged and Differentially Induce Drought and Heat Stress-Specific Genes. Plant Physiology 180, 1677–1690. https://doi.org/10.1104/pp.19.00391

Sawa, S., Ito, T., Shimura, Y., Okada, K., 1999. FILAMENTOUS FLOWER Controls the Formation and Development of *Arabidopsis* Inflorescences and Floral Meristems. The Plant Cell 11, 69. https://doi.org/10.2307/3870839

Sawa, M., Nusinow, D.A., Kay, S.A., Imaizumi, T., 2007. FKF1 and GIGANTEA Complex Formation Is Required for Day-Length Measurement in *Arabidopsis*. Science 318, 261–265. https://doi.org/10.1126/science.1146994

Sawa, M., Kay, S.A., 2011. GIGANTEA directly activates Flowering Locus T in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences 108, 11698–11703. https://doi.org/10.1073/pnas.1106771108

Schönrock, N., Bouveret, R., Leroy, O., Borghi, L., Köhler, C., Gruissem, W., Hennig, L., 2006. Polycomb-group proteins repress the floral activator

AGL19 in the FLC-independent vernalization pathway. Genes & Development 20, 1667–1678. https://doi.org/10.1101/gad.377206

Schwarz, S., Grande, A.V., Bujdoso, N., Saedler, H., Huijser, P., 2008. The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in *Arabidopsis*. Plant Molecular Biology 67, 183–195. https://doi.org/10.1007/s11103-008-9310-z

Serbet, R., Escapa, I., Taylor, T.N., Taylor, E.L., Cúneo, N.R., 2010. Additional observations on the enigmatic Permian plant Buriadia and implications on early coniferophyte evolution. Review of Palaeobotany and Palynology 161, 168–178. https://doi.org/10.1016/j.revpalbo.2010.03.011

Shannon, S., Meeks-Wagner, D.R., 1991. A Mutation in the *Arabidopsis* TFL1 Gene Affects Inflorescence Meristem Development. The Plant Cell 877–892. https://doi.org/10.1105/tpc.3.9.877

Shannon, S., Meeks-Wagner, D.R., 1993. Genetic Interactions That Regulate Inflorescence Development in *Arabidopsis*. The Plant Cell 639–655. https://doi.org/10.1105/tpc.5.6.639

Siefers, N., Dang, K.K., Kumimoto, R.W., Bynum IV, W.E., Tayrose, G., Holt III, B.F., 2008. Tissue-Specific Expression Patterns of *Arabidopsis* NF-Y Transcription Factors Suggest Potential for Extensive Combinatorial Complexity. Plant Physiology 149, 625–641. https://doi.org/10.1104/pp.108.130591

Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N., Bowman, J.L., 1999. Members of the YABBY gene family specify abaxial cell fate in *Arabidopsis*. Development 126, 4117–4128. https://doi.org/10.1242/dev.126.18.4117

Skrøppa, T., Tollefsrud, M.M., Sperisen, C., Johnsen, Ø., 2009. Rapid change in adaptive performance from one generation to the next in *Picea abies*—Central European trees in a Nordic environment. Tree Genetics & Genomes 6, 93–99. https://doi.org/10.1007/s11295-009-0231-z

SLU, Roberge, C., Dahlgren, J., Nilsson, P., Wikberg, P.-E., Fridman, J., 2024. Skogsdata 2024. Aktuella uppgifter om de svenska skogarna från SLU Riksskogstaxeringen. SLU Institutionen för skoglig resurshushållning, Umeå.

Smaczniak, C., Immink, R.G.H., Muiño, J.M., Blanvillain, R., Busscher, M., Busscher-Lange, J., Dinh, Q.D. (Peter), Liu, S., Westphal, A.H., Boeren, S., Parcy, F., Xu, L., Carles, C.C., Angenent, G.C., Kaufmann, K., 2012. Characterization of MADS-domain transcription factor complexes in *Arabidopsis* flower development. Proceedings of the National Academy of Sciences 109, 1560–1565. https://doi.org/10.1073/pnas.1112871109

Song, Y.H., Lee, I., Lee, S.Y., Imaizumi, T., Hong, J.C., 2011. CONSTANS and ASYMMETRIC LEAVES 1 complex is involved in the induction of FLOWERING LOCUS T in photoperiodic flowering in *Arabidopsis*. The

Plant Journal 69, 332–342. https://doi.org/10.1111/j.1365-313x.2011.04793.x

Sorin, C., Declerck, M., Christ, A., Blein, T., Ma, L., Lelandais-Brière, C., Njo, M.F., Beeckman, T., Crespi, M., Hartmann, C., 2014. A miR169 isoform regulates specific NF-YA targets and root architecture in *Arabidopsis*. New Phytologist 202, 1197–1211. https://doi.org/10.1111/nph.12735

Ståhl, P.L., Salmén, F., Vickovic, S., Lundmark, A., Navarro, J.F., Magnusson, J., Giacomello, S., Asp, M., Westholm, J.O., Huss, M., Mollbrink, A., Linnarsson, S., Codeluppi, S., Borg, Å., Pontén, F., Costea, P.I., Sahlén, P., Mulder, J., Bergmann, O., Lundeberg, J., Frisén, J., 2016. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 353, 78–82. https://doi.org/10.1126/science.aaf2403

Stief, A., Altmann, S., Hoffmann, K., Pant, B.D., Scheible, W.-R., Bäurle, I., 2014. *Arabidopsis* miR156 Regulates Tolerance to Recurring Environmental Stress through SPL Transcription Factors. The Plant Cell 26, 1792–1807. https://doi.org/10.1105/tpc.114.123851

Sturrock, R.N., Frankel, S.J., Brown, A.V., Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., Woods, A.J., 2011. Climate change and forest diseases. Plant Pathology 60, 133–149. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Sun, B., Zhou, Y., Cai, J., Shang, E., Yamaguchi, N., Xiao, J., Looi, L.-S., Wee, W.-Y., Gao, X., Wagner, D., Ito, T., 2019. Integration of transcriptional repression and polycomb-mediated silencing of WUSCHEL in floral meristems. The Plant Cell 31, 1488–1505. https://doi.org/10.1105/tpc.18.00450

Sundström, J., Carlsbecker, A., Svensson, M.E., Svenson, M., Johanson, U., Theißen, G., Engström, P., 1999. MADS-box genes active in developing pollen cones of Norway spruce (*Picea abies*) are homologous to the B-class floral homeotic genes in angiosperms. Developmental Genetics 25, 253–266. https://doi.org/10.1002/(sici)1520-6408(1999)25:3

Sundström, J., 2001. Evolution of genetic mechanism regulating reproductive development in plants (Doctoral dissertation, PhD thesis. Uppsala University, Acta Universitatis Upsaliensis, Uppsala, Sweden).

Sundström, J., Engström, P., 2002. Conifer reproductive development involves B-type MADS-box genes with distinct and different activities in male organ primordia. The Plant Journal 31, 161–169. https://doi.org/10.1046/j.1365-313x.2002.01343.x

Tandre, K., Albert, V.A., Sundås, A., Engström, P., 1995. Conifer homologues to genes that control floral development in angiosperms. Plant Molecular Biology 27, 69–78. https://doi.org/10.1007/bf00019179

Tandre, K., Svenson, M., Svensson, M.E., Engström, P., 1998. Conservation of gene structure and activity in the regulation of reproductive organ development

of conifers and angiosperms. The Plant Journal 15, 615–623. https://doi.org/10.1046/j.1365-313x.1998.00236.x

Tao, Z., Shen, L., Liu, C., Liu, L., Yan, Y., Yu, H., 2012. Genome-wide identification of SOC1 and SVP targets during the floral transition in *Arabidopsis*. The Plant Journal 70, 549–561. https://doi.org/10.1111/j.1365-313x.2012.04919.x

Tiwari, S.B., Shen, Y., Chang, H., Hou, Y., Harris, A., Ma, S.F., McPartland, M., Hymus, G.J., Adam, L., Marion, C., Belachew, A., Repetti, P.P., Reuber, T.L., Ratcliffe, O.J., 2010. The flowering time regulator CONSTANS is recruited to the FLOWERING LOCUS T promoter via a unique cis-element. New Phytologist 187, 57–66. https://doi.org/10.1111/j.1469-8137.2010.03251.x

Tsuzuki, M., Futagami, K., Shimamura, M., Inoue, C., Kunimoto, K., Oogami, T., Tomita, Y., Inoue, K., Kohchi, T., Yamaoka, S., Araki, T., Hamada, T., Watanabe, Y., 2019. An Early Arising Role of the MicroRNA156/529-SPL Module in Reproductive Development Revealed by the Liverwort *Marchantia polymorpha*. Current Biology 29, 3307-3314.e5. https://doi.org/10.1016/j.cub.2019.07.084

Uddenberg, D., Valladares, S., Abrahamsson, M., Sundström, J.F., Sundås-Larsson, A., von Arnold, S., 2011. Embryogenic potential and expression of embryogenesis-related genes in conifers are affected by treatment with a histone deacetylase inhibitor. Planta 234, 527–539. https://doi.org/10.1007/s00425-011-1418-8

Uddenberg, D., Reimegård, J., Clapham, D., Almqvist, C., von Arnold, S., Emanuelsson, O., Sundström, J.F., 2012. Early Cone Setting in *Picea abies acrocona* Is Associated with Increased Transcriptional Activity of a MADS Box Transcription Factor. Plant Physiology 161, 813–823. https://doi.org/10.1104/pp.112.207746

Uddenberg, D., Akhter, S., Ramachandran, P., Sundström, J.F., Carlsbecker, A., 2015. Sequenced genomes and rapidly emerging technologies pave the way for conifer evolutionary developmental biology. Frontiers in Plant Science 6. https://doi.org/10.3389/fpls.2015.00970

Vergara, A., Haas, J.C., Aro, T., Stachula, P., Street, N.R., Hurry, V., 2021. Norway spruce deploys tissue-specific responses during acclimation to cold. Plant, Cell & Environment 45, 427–445. https://doi.org/10.1111/pce.14241

Villanueva, J.M., Broadhvest, J., Hauser, B.A., Meister, R.J., Schneitz, K., Gasser, C.S., 1999. INNER NO OUTER regulates abaxial- adaxial patterning in *Arabidopsis* ovules. Genes & Development 13, 3160–3169. https://doi.org/10.1101/gad.13.23.3160

Wahl, V., Ponnu, J., Schlereth, A., Arrivault, S., Langenecker, T., Franke, A., Feil, R., Lunn, J.E., Stitt, M., Schmid, M., 2013. Regulation of Flowering by

Trehalose-6-Phosphate Signaling in *Arabidopsis thaliana*. Science 339, 704–707. https://doi.org/10.1126/science.1230406

Wang, J.-W., Schwab, R., Czech, B., Mica, E., Weigel, D., 2008. Dual Effects of miR156-Targeted SPL Genes and CYP78A5/KLUH on Plastochron Length and Organ Size in *Arabidopsis thaliana*. The Plant Cell 20, 1231–1243. https://doi.org/10.1105/tpc.108.058180

Wang, J.-W., Czech, B., Weigel, D., 2009. miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in *Arabidopsis thaliana*. Cell 138, 738–749. https://doi.org/10.1016/j.cell.2009.06.014

Wang, F., Deng, X.W., 2011. Plant ubiquitin-proteasome pathway and its role in gibberellin signaling. Cell Research 21, 1286–1294. https://doi.org/10.1038/cr.2011.118

Webber, J.E., Ross, S.D., Pharis, R.P., Owens, J.N., 1985. Interaction between gibberellin A4/7 and root-pruning on the reproductive and vegetative process in Douglas-fir. II. Effects on shoot elongation and its relationship to flowering. Canadian Journal of Forest Research 15, 348–353. https://doi.org/10.1139/x85-056

Wei, Q., Ma, C., Xu, Y., Wang, T., Chen, Y., Lü, J., Zhang, L., Jiang, C.-Z., Hong, B., Gao, J., 2017. Control of chrysanthemum flowering through integration with an aging pathway. Nature Communications 8. https://doi.org/10.1038/s41467-017-00812-0

Wenkel, S., Turck, F., Singer, K., Gissot, L., Le Gourrierec, J., Samach, A., Coupland, G., 2006. CONSTANS and the CCAAT Box Binding Complex Share a Functionally Important Domain and Interact to Regulate Flowering of *Arabidopsis*. The Plant Cell 18, 2971–2984. https://doi.org/10.1105/tpc.106.043299

Wenzel, C.L., Xia, A.Y., Saunders, R., Ly, H.H., Mo, A., Holloway, D.M., 2024. Needle and bud scale development in *Picea abies*. Trees 38, 777–792. https://doi.org/10.1007/s00468-024-02518-5

Westin, J., Haapanen, M., 2013. Norway spruce – *Picea abies* (L.) Karst, in: Mullin, T., Lee, S. (Eds.), Best Practice for Tree Breeding in Europe. Gävle.

Westrin, K.J., Kretzschmar, W.W., Emanuelsson, O., 2024. ClusTrast: a short read de novo transcript isoform assembler guided by clustered contigs. BMC Bioinformatics 25. https://doi.org/10.1186/s12859-024-05663-3

Worden, A.Z., Lee, J.-H., Mock, T., Rouzé, P., Simmons, M.P., Aerts, A.L., Allen, A.E., Cuvelier, M.L., Derelle, E., Everett, M.V., Foulon, E., Grimwood, J., Gundlach, H., Henrissat, B., Napoli, C., McDonald, S.M., Parker, M.S., Rombauts, S., Salamov, A., Von Dassow, P., Badger, J.H., Coutinho, P.M., Demir, E., Dubchak, I., Gentemann, C., Eikrem, W., Gready, J.E., John, U., Lanier, W., Lindquist, E.A., Lucas, S., Mayer, K.F.X., Moreau, H., Not, F., Otillar, R., Panaud, O., Pangilinan, J., Paulsen, I., Piegu, B., Poliakov, A., Robbens, S., Schmutz, J., Toulza, E., Wyss, T., Zelensky, A., Zhou, K.,

Armbrust, E.V., Bhattacharya, D., Goodenough, U.W., Van de Peer, Y., Grigoriev, I.V., 2009. Green Evolution and Dynamic Adaptations Revealed by Genomes of the Marine Picoeukaryotes *Micromonas*. Science 324, 268–272. https://doi.org/10.1126/science.1167222

Wu, G., Poethig, R.S., 2006. Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. Development 133, 3539–3547. https://doi.org/10.1242/dev.02521

Wu, G., Park, M.Y., Conway, S.R., Wang, J.-W., Weigel, D., Poethig, R.S., 2009. The Sequential Action of miR156 and miR172 Regulates Developmental Timing in *Arabidopsis*. Cell 138, 750–759. https://doi.org/10.1016/j.cell.2009.06.031

Wuest, S.E., Ó'Maoiléidigh, D.S., Rae, L., Kwasniewska, K., Raganelli, A., Hanczaryk, K., Lohan, A.J., Loftus, B., Graciet, E., Wellmer, F., 2012. Molecular basis for the specification of floral organs by APETALA3 and PISTILLATA. Proceedings of the National Academy of Sciences 109, 13452–13457. https://doi.org/10.1073/pnas.1207075109

Xiang, W.-B., Li, W.-F., Zhang, S.-G., Qi, L.-W., 2019. Transcriptome-wide analysis to dissect the transcription factors orchestrating the phase change from vegetative to reproductive development in *Larix kaempferi*. Tree Genetics & Genomes 15. https://doi.org/10.1007/s11295-019-1376-z

Xing, S., Salinas, M., Höhmann, S., Berndtgen, R., Huijser, P., 2010. miR156-Targeted and Nontargeted SBP-Box Transcription Factors Act in Concert to Secure Male Fertility in *Arabidopsis*. The Plant Cell 22, 3935–3950. https://doi.org/10.1105/tpc.110.079343

Xu, M.Y., Zhang, L., Li, W.W., Hu, X.L., Wang, M.-B., Fan, Y.L., Zhang, C.Y., Wang, L., 2013. Stress-induced early flowering is mediated by miR169 in *Arabidopsis thaliana*. Journal of Experimental Botany 65, 89–101. https://doi.org/10.1093/jxb/ert353

Xu, F., Li, T., Xu, P., Li, L., Du, S., Lian, H., Yang, H., 2016a. DELLA proteins physically interact with CONSTANS to regulate flowering under long days in *Arabidopsis*. FEBS Letters 590, 541–549. https://doi.org/10.1002/1873-3468.12076

Xu, M., Hu, T., Zhao, J., Park, M.-Y., Earley, K.W., Wu, G., Yang, L., Poethig, R.S., 2016b. Developmental Functions of miR156-Regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) Genes in *Arabidopsis thaliana*. PLOS Genetics 12, e1006263. https://doi.org/10.1371/journal.pgen.1006263

Yamada, T., Yokota, S., Hirayama, Y., Imaichi, R., Kato, M., Gasser, C.S., 2011. Ancestral expression patterns and evolutionary diversification of YABBY genes in angiosperms. The Plant Journal 67, 26–36. https://doi.org/10.1111/j.1365-313x.2011.04570.x

Yamaguchi, A., Wu, M.-F., Yang, L., Wu, G., Poethig, R.S., Wagner, D., 2009. The MicroRNA-Regulated SBP-Box Transcription Factor SPL3 Is a Direct Upstream Activator of LEAFY, FRUITFULL, and APETALA1. Developmental Cell 17, 268–278. https://doi.org/10.1016/j.devcel.2009.06.007

Yamaguchi, T., Yano, S., Tsukaya, H., 2010. Genetic Framework for Flattened Leaf Blade Formation in Unifacial Leaves of *Juncus prismatocarpus*. The Plant Cell 22, 2141–2155. https://doi.org/10.1105/tpc.110.076927

Yamaguchi, N., Winter, C.M., Wu, M.-F., Kanno, Y., Yamaguchi, A., Seo, M., Wagner, D., 2014. Gibberellin Acts Positively Then Negatively to Control Onset of Flower Formation in *Arabidopsis*. Science 344, 638–641. https://doi.org/10.1126/science.1250498

Yamaguchi, N., Huang, J., Tatsumi, Y., Abe, M., Sugano, S.S., Kojima, M., Takebayashi, Y., Kiba, T., Yokoyama, R., Nishitani, K., Sakakibara, H., Ito, T., 2018. Chromatin-mediated feed-forward auxin biosynthesis in floral meristem determinacy. Nature Communications 9. https://doi.org/10.1038/s41467-018-07763-0

Yan, J., Mao, D., Liu, X., Wang, L., Xu, F., Wang, G., Zhang, W., Liao, Y., 2017. Isolation and functional characterization of a circadian-regulated CONSTANS homolog (GbCO) from *Ginkgo biloba*. Plant Cell Reports 36, 1387–1399. https://doi.org/10.1007/s00299-017-2162-8

Yang, L., Xu, M., Koo, Y., He, J., Poethig, R.S., 2013. Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of MIR156A and MIR156C. eLife 2. https://doi.org/10.7554/elife.00260

Yang, C., Ma, Y., Li, J., 2016. The rice YABBY4 gene regulates plant growth and development through modulating the gibberellin pathway. Journal of Experimental Botany 67, 5545–5556. https://doi.org/10.1093/jxb/erw319

Yang, M., You, W., Wu, S., Fan, Z., Xu, B., Zhu, M., Li, X., Xiao, Y., 2017. Global transcriptome analysis of *Huperzia serrata* and identification of critical genes involved in the biosynthesis of huperzine A. BMC Genomics 18. https://doi.org/10.1186/s12864-017-3615-8

Yang, W., Zhou, C., Guo, Y., Niu, S., El-Kassaby, Y.A., Li, W., 2024. Genome-wide identification of the *Pinus tabuliformis* CONSTANS-like gene family and their potential roles in reproductive cone development. International Journal of Biological Macromolecules 254, 127621. https://doi.org/10.1016/j.ijbiomac.2023.127621

Yant, L., Mathieu, J., Dinh, T.T., Ott, F., Lanz, C., Wollmann, H., Chen, X., Schmid, M., 2010. Orchestration of the Floral Transition and Floral Development in *Arabidopsis* by the Bifunctional Transcription Factor APETALA2. The Plant Cell 22, 2156–2170. https://doi.org/10.1105/tpc.110.075606

Yu, Y., Li, Y., Huang, G., Meng, Z., Zhang, D., Wei, J., Yan, K., Zheng, C., Zhang, L., 2011. PwHAP5, a CCAAT-binding transcription factor, interacts with

PwFKBP12 and plays a role in pollen tube growth orientation in *Picea wilsonii*. Journal of Experimental Botany 62, 4805–4817. https://doi.org/10.1093/jxb/err120

Yu, S., Galvão, V.C., Zhang, Y.-C., Horrer, D., Zhang, T.-Q., Hao, Y.-H., Feng, Y.-Q., Wang, S., Schmid, M., Wang, J.-W., 2012. Gibberellin Regulates the *Arabidopsis* Floral Transition through miR156-Targeted SQUAMOSA PROMOTER BINDING–LIKE Transcription Factors. The Plant Cell 24, 3320–3332. https://doi.org/10.1105/tpc.112.101014

Zanetti, M.E., Rípodas, C., Niebel, A., 2017. Plant NF-Y transcription factors: Key players in plant-microbe interactions, root development and adaptation to stress. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 1860, 645–654. https://doi.org/10.1016/j.bbagrm.2016.11.007

Zhang, Y., Brown, G., Whetten, R., Loopstra, C., Neale, D., Kieliszewski, M., Sederoff, R., 2003. An arabinogalactan protein associated with secondary cell wall formation in differentiating xylem of loblolly pine. Plant Molecular Biology 52, 91–102. https://doi.org/10.1023/a:1023978210001

Zhang, S., Yang, C., Peng, J., Sun, S., Wang, X., 2009. GASA5, a regulator of flowering time and stem growth in *Arabidopsis thaliana*. Plant Molecular Biology 69, 745–759. https://doi.org/10.1007/s11103-009-9452-7

Zhang, B., Wang, L., Zeng, L., Zhang, C., Ma, H., 2015a. *Arabidopsis* TOE proteins convey a photoperiodic signal to antagonize CONSTANS and regulate flowering time. Genes & Development 29, 975–987. https://doi.org/10.1101/gad.251520.114

Zhang, T., Zhang, D., Liu, Y., Luo, C., Zhou, Y., Zhang, L., 2015b. Overexpression of a NF-YB3 transcription factor from *Picea wilsonii* confers tolerance to salinity and drought stress in transformed *Arabidopsis thaliana*. Plant Physiology and Biochemistry 94, 153–164. https://doi.org/10.1016/j.plaphy.2015.05.001

Zhang, S.-D., Ling, L.-Z., Zhang, Q.-F., Xu, J.-D., Cheng, L., 2015c. Evolutionary Comparison of Two Combinatorial Regulators of SBP-Box Genes, MiR156 and MiR529, in Plants. PLOS ONE 10, e0124621. https://doi.org/10.1371/journal.pone.0124621

Zhang, S.-D., Ling, L.-Z., 2018. Diversification of SQUAMOSA promoter binding protein-like (SPL) genes by changes of miR156/529 binding sites in land plants. Plant Gene 14, 55–63. https://doi.org/10.1016/j.plgene.2018.04.007

Zhang, C., Jian, M., Li, W., Yao, X., Tan, C., Qian, Q., Hu, Y., Liu, X., Hou, X., 2023. Gibberellin signaling modulates flowering via the DELLA–BRAHMA–NF-YC module in *Arabidopsis*. The Plant Cell 35, 3470–3484. https://doi.org/10.1093/plcell/koad166

Zhang, H., Wang, P., Song, Y., Zhao, H., Zuo, Q., Chen, X., Han, F., Liu, H., Nie, Y., Liu, M., Guo, M., Niu, S., 2024. The MADS-domain transcription factor DAL10 is a direct target of putative DAL1-mediated age pathway in

conifers. Journal of Experimental Botany 75, 6462–6475. https://doi.org/10.1093/jxb/erae329

Zhao, H., Wu, D., Kong, F., Lin, K., Zhang, H., Li, G., 2017. The *Arabidopsis thaliana* Nuclear Factor Y Transcription Factors. Frontiers in Plant Science 07. https://doi.org/10.3389/fpls.2016.02045

Zhao, H., Lin, K., Ma, L., Chen, Q., Gan, S., Li, G., 2020. *Arabidopsis* NUCLEAR FACTOR Y A8 inhibits the juvenile-to-adult transition by activating transcription of MIR156s. Journal of Experimental Botany 71, 4890–4902. https://doi.org/10.1093/jxb/eraa197

Zhou, C., Liu, H., Wang, H., Niu, S., El-Kassaby, Y.A., Li, W., 2024. Deciphering the Role of SVP-Like Genes and Their Key Regulation Networks During Reproductive Cone Development in *Pinus tabuliformis*. Plant, Cell & Environment. https://doi.org/10.1111/pce.15129

Popular science summary

Norway spruce (*Picea abies*) is one of the most common trees in Sweden. It dominates forests, both natural and planted across the country. Its economic importance has meant that millions of trees are planted each year. To make sure the best trees are available for forest owners to plant, a breeding program was started in the mid-1900s. Every growing season, trees prepare new vegetative shoots, and if conditions are right, seed cones and pollen cones. These will emerge the following spring. Pollen is released and floats in the wind until it lands in a seed cone and fertilizes it. Hundreds of seeds can be produced from a single seed cone.

Although pollen cones are common, seed cones are not. Every tree has its own, natural ability to make seed cones. Some trees produce a lot of cones, while others hardly produce any. It is common for all spruce trees within a large area to produce a massive amount of cones at the same time, and then not make any for a few years. This tendency can become a problem when industry runs out of seed to plant or when the breeding program is unable to do the crosses it wants to.

In order to understand why and how Norway spruce produces cones, we used a variety of approaches.

First, we identified a mutant tree that is native to Uppsala. This mutant, called '*acrocona*,' produces seed cones and seed cone-like growth much earlier in life than wild spruce and on branches it should not. We identified a mutation in *acrocona's* DNA that causes a gene (*PaSPL1*) to not be regulated correctly. This gene is part of the process of a tree deciding when and where to make a seed cone. This gene is normally stopped from telling the tree to make seed cones, but the mutant tree is unable to stop it. This means *acrocona* trees produce many seed cones and seed cone-like growth. We have used the knowledge gained from discovering this gene to identify other genes that are important in the process of producing cones.

Second, we performed a series of experiments (Spatial Transcriptomics) to see where in a developing vegetative shoot, seed cone, and *acrocona* shoot genes are being expressed. We compared expression between these three

shoots at three time points to create a map of expression that can be used by the scientific community to identify important genes in many different processes of development.

Third, we analyzed the natural ability of different trees to produce seed cones. Both under their own power and with the help of hormones that stimulate seed cone production. We observed the natural hormone concentration in the trees and how they processed the applied hormones. In general, trees that received hormone treatments produced twice as many seed cones.

Populärvetenskaplig sammanfattning

Gran (*Picea abies*) är ett av de vanligaste barrträden i Sverige. Den är naturligt förekommande men planteras även frekvent, vilket gör att den dominerar både gammelskogar och nyplanterade skogar över stora delar av landet. Dess ekonomiska betydelse har bidragit till att miljontals granar planteras varje år. För att säkerställa att de bästa träden finns tillgängliga för landets skogsägare att plantera, startades förädlingsprogram för gran redan i mitten av 1900-talet. Trots detta råder det idag en brist på förädlat planteringsmaterial. Till stor del beror denna brist på granens långa generationstid och oregelbundna kottsättning, men även på att skadegörare som angriper granens honkottar har en negativ inverkan på fröproduktionen

Under växtsäsongen förbereder träden knoppar för nästa års vegetativa skott, och om förhållandena är de rätta, han- och honkottar. Dessa kommer att växa fram följande vår. Pollen frigörs från hankottarna och sprids sedan med vinden tills det landar på en honkotte och befruktar dess fröanlag. Varje honkotte kan då ge upphov till ett hundratal frön.

Även om hankottar är vanliga, är granens honkottar inte det. Dessutom har varje träd sin egen, inneboende förmåga att bilda kottar. Vissa träd producerar många kottar, medan andra knappt producerar några kottar alls. Det är vanligt att kottsättningen inom ett geografiskt område är synkroniserad så att de flesta granarna producerar kottar samtidigt under ett visst år; för att därefter inte producera några kottar alls på några år. Detta mönster är ett problem för industrin eftersom de i perioder med utebliven kottsättning kan få brist på frömaterial. För förädlingsprogrammen utgör den ojämna kottsättningen också ett problem eftersom man inte enkelt kan göra de korsningar man vill varje år.

I mitt avhandlingsarbete har jag strävat efter att ökakunskapen om de genetiska faktorer som reglerar kottsättningen i gran för att på sikt kunna öka fröproduktionen och säkerställa en ökad tillgång till förädlat planteringsmaterial för landets skogsägare. För att förstå varför och hur granen producerar kottar använde vi oss av flera olika metoder.

I avhandlingens första studie använde jag mig av en naturlig varietet av vanlig gran som kallas *acrocona*. *Acrocona-granen* härstammar från Uppsala och beskrevs redan 1891 av dåvarande botanikprofessorn Theodor Fries vid Uppsala Universitet. *Acrocona*-granen bär på en mutation, dvs en förändring i ett arvsanlag som gör att den producerar honkottar vid en lägre ålder än vanliga granar. Redan efter tre tillväxtperioder istället för 20-25 år som vanliga granar gör. Den producerar även honkottsliknande strukturer på toppskotten; vilka vanligtvis alltid är vegetativa. Därav namnet *acrocona* som betyder "toppkotte".

I mina studier av *acrocona*-granen har jag identifierat en förändring i ett arvsanlag (en gen) som är kopplat till *acrocona*-granens tidiga kottsättning och produktion av toppkottar. Förändringen gör att regleringen av arvsanlaget (som vi kan kalla *acrocona*-genen) inte sker på ett korrekt sätt och att *acrocona*-genens protein därmed kan verka i väldigt unga träd och i skott som normalt hade varit vegetativa. De resultat som jag presenterar i min avhandling tyder på att *acrocona*-genens protein inducerar den tidiga kottsättningen i dessa plantor, vilket i sin tur innebär att *acrocona*-genens protein är en del av processen som bestämmer när och var en gran ska bilda kottar. I uppföljande experiment har jag därefter använt mig av kunskapen från upptäckten av *acrocona*-genen för att identifiera andra arvsanlag som är viktiga i processen för att producera kottar.

I avhandlingens andra studie har jag utfört experiment med en metod som kallas Spatial Transcriptomics. Det är en storskalig metod där man kan studera aktiviteten av en stor antal arvsanlag samtidigt och dessutom koppla aktiviteten till olika celler inom en vävnad. I mina försök har jag studerat gen aktiviteten under tidiga utvecklingsstadier av honkottar, vegetativa skott och *acrocona*-granens speciella toppkottar. Jag har därefter jämfört aktiviteten av olika arvsanlag mellan dessa skottyper för att skapa en karta över arvsanlagens aktivitet under den tidiga skottutvecklingen. Denna karta kan nu användas av forskarvärlden för att identifiera arvsanlag som är aktiva i många olika utvecklingsprocesser under kottutvecklingen.

I avhandlingens tredje studie har jag studerat den naturliga variation som finns i granens förmåga att sätta kottar. I denna studie har jag använt mig att ett fältförsök med granar som representerar den genetiska diversiteten i

Mellansverige. I försöket ingår både kloner av träd som får sätta kottar naturligt och kloner där man stimulerar kottsättningen med hjälp av ett växthormon. I mina experiment har jag räknat på hur klonerna svarar på hormonbehandlingarna och även studerat hur uttrycket av olika arvsanlag förändras efter en hormonbehandling. Jag har då kunnat visa att det finns en variation i hur olika kloner av träd svarar på hormonbehandlingen samt att antalet arvsanlag som uppvisar en förändrad aktivitet skiljer sig åt mellan kloner som har olika genetisk bakgrund. I allmänhet producerar dock träd som får hormonbehandlingar dubbelt så många honkottar som obehandlade träd.

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I

New Phytologist

Research

Cone-setting in spruce is regulated by conserved elements of the age-dependent flowering pathway

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Summary

- Reproductive phase change is well characterized in angiosperm model species, but less studied in gymnosperms. We utilize the early cone-setting *acrocona* mutant to study reproductive phase change in the conifer *Picea abies* (Norway spruce), a gymnosperm. The *acrocona* mutant frequently initiates cone-like structures, called transition shoots, in positions where wild-type *P. abies* always produces vegetative shoots.
- We collect *acrocona* and wild-type samples, and RNA-sequence their messenger RNA (mRNA) and microRNA (miRNA) fractions. We establish gene expression patterns and then use allele-specific transcript assembly to identify mutations in *acrocona*. We genotype a segregating population of inbred *acrocona* trees.
- A member of the *SQUAMOSA BINDING PROTEIN-LIKE (SPL)* gene family, *PaSPL1*, is active in reproductive meristems, whereas two putative negative regulators of *PaSPL1*, *miRNA156* and the conifer specific *miRNA529*, are upregulated in vegetative and transition shoot meristems. We identify a mutation in a putative miRNA156/529 binding site of the *acrocona PaSPL1* allele and show that the mutation renders the *acrocona* allele tolerant to these miRNAs. We show co-segregation between the early cone-setting phenotype and trees homozygous for the *acrocona* mutation.
- In conclusion, we demonstrate evolutionary conservation of the age-dependent flowering pathway and involvement of this pathway in regulating reproductive phase change in the conifer *P. abies*.

Introduction

Molecular clock-based studies, calibrated using fossil data, suggest that the gymnosperm and angiosperm lineages of extant seed plants separated *c.* 300 million years ago (Smith *et al.*, 2010). Although the lineages share a common feature in the seed, their seed-bearing structures differ. Gymnosperms form seed- and pollen-bearing structures from separate shoot meristems, commonly referred to as cones (Florin, 1951) whereas angiosperm flowers, in their ancestral state, are bisexual (Sauquet *et al.*, 2017). Cones and flowers also differ in their branching order (Florin, 1951), where at least seed cones can be viewed as reproductive shoots analogous to angiosperm inflorescences, rather than flowers.

Comparative studies indicate that the genetic mechanisms that determine male or female organ identity are conserved between the two lineages (Rutledge *et al.*, 1998; Tandre *et al.*, 1998; Mouradov *et al.*, 1999; Sundstrom *et al.*, 1999; Winter *et al.*, 1999). However, it is currently disputed if the mechanisms that regulate the on-set of cone-setting in gymnosperms and flowering in angiosperms are homologous (Karlgren *et al.*, 2011; Klintenas *et al.*, 2012; Liu *et al.*, 2016).

Angiosperm flowering is regulated by several independent pathways that act in parallel, and converge on common floral integrators (O'Maoileidigh *et al.*, 2014). The pathways are often referred to as the Age-dependent pathway, the Day-Length pathway, the Hormonal pathway, and the Vernalization pathway (Blazquez & Weigel, 2000). The transition from vegetative growth to flowering occurs once in annual plants but can occur repeatedly in perennials (Albani & Coupland, 2010). The repeated flowering of the perennial herb *Arabis alpina* can be explained by the regulation of transcription factor proteins belonging to the SQUAMOSA BINDING PROTEIN-LIKE (SPL) family (Hyun *et al.*, 2019). SQUAMOSA BINDING PROTEIN-LIKE proteins act as activators of flowering through the regulation of flower meristem identity genes (Wang

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et al., 2009). *Arabis alpina SPL15* transcript levels are negatively regulated in vegetative meristems through the joint activity of factors involved in the Vernalization pathway and the age-dependent pathway. In response to winter temperatures and vernalization, the repression of *SPL15* is temporarily lifted. Flowering is allowed to occur, but only in meristems that have reached a certain age, since *SPL15* is also negatively regulated by the Age-dependent pathway through the activity of *micro-RNA156* (*miR156*; Hyun *et al.*, 2019).

Like many conifers, wild-type *Picea abies* trees go through a long juvenile period of 20–25 yr before initiating cones. Thereafter, cone-setting occurs every third to fifth year (Lindgren *et al.*, 1977). To study cone-setting, we use a naturally occurring *P. abies* mutant called *acrocona*. Homozygous *acrocona* plants display a recessive early cone-setting phenotype and initiate cones already in their second growth period (Uddenberg *et al.*, 2013). After the first cone-setting, *acrocona* trees also initiate cones frequently, almost every year. This frequent cone-setting phenotype is semi-dominant and can to a degree also be observed in adult heterozygous *acrocona* mutants. In addition, heterozygous *acrocona* mutants commonly form cone-like structures, called transition shoots, on leading vegetative branches (Carlsbecker *et al.*, 2013; Uddenberg *et al.*, 2013).

Massively parallel DNA sequencing has been employed to study different aspects of reproductive development in conifers by us (Uddenberg *et al.*, 2013; Giacomello *et al.*, 2017) and others (Niu *et al.*, 2014, 2016; Futamura *et al.*, 2019). Previously, we have studied inbred siblings of young *acrocona* trees (Uddenberg *et al.*, 2013) and identified the MADS-box gene *DEFICIENS AGAMOUS LIKE 19 (DAL19)* as being upregulated in needle samples of early cone-setting shoots. Later we have shown that distinct *DAL19* isoforms are expressed in male and female cones, and in vegetative shoots (Akhter *et al.*, 2018).

In the present study, we take advantage of the transition shoots and the numerous female cones that regularly form on adult heterozygous *acrocona* trees, and during cone-years also in the upper one-third of adult wild-type *P. abies* trees. We use massively parallel DNA sequencing to analyse both the messenger RNA (mRNA) and microRNA (miRNA) fractions of early meristems and transition shoot primordia from *acrocona* and compare those to corresponding samples from wild-type vegetative shoots and female cones. We hypothesize that candidate genes active in these early meristems are important not only for the *acrocona* phenotype but also for the regulation of cone-setting in wild-type *P. abies*. In line with this hypothesis, we identify candidates for a cone-setting regulatory circuit consisting of a conifer SPL-gene family member and two miRNAs. Furthermore, by genotyping a segregating sibling population of inbred *acrocona* trees, we provide evidence for a functional link between a mutation in a candidate gene, *PaSPL1*, and the early cone-setting *acrocona* phenotype.

Materials and Methods

Plant materials and morphological conditions

Plant material was collected from an *acrocona* tree located in Uppsala, Sweden and from a wild-type Norway spruce (*P. abies* (L.) H. Karst.) at the Rörby seed orchard (latitude 59°54′290″N) near Uppsala, Sweden. Both trees were estimated to be at least 50 yr. Samples representing two developmental stages were collected from both genotypes. In the first developmental stage, the samples consisted of meristematic tissue. Samples in the second developmental stage harboured bud primordia with differentiating lateral organs. The *acrocona* samples consisted of transition shoots collected from apical positions on leading branches and female cones collected from apical positions on lateral branches. The *acrocona* samples used in RNA-sequencing (RNA-Seq) experiments were collected at two dates in 2016, 1 August and 18 October. Whereas the corresponding wild-type samples consisted of vegetative shoots collected from apical positions on leading branches and female cones collected from apical positions on lateral branches. Wild-type samples were collected in 2016, on 1 August, 16 September, and 25 October. Independent control samples of female cones and vegetative shoots were also collected from four additional wild-type genotypes on 8 October 2013. All plant materials used for RNA preparations were snap-frozen in liquid nitrogen and stored at −70°C. For a summary of the sample information and a detailed description of the sampling procedure, see Table S1.

RNA preparation

Tissue homogenization, extraction, CHISAM (chloroform/isoamylalcohol, 24 : 1) purification and isopropanol precipitation were performed as described by Azevedo *et al.* (2003). Harvested RNA pellets were dissolved in 350 µl RLT buffer (Qiagen RNeasy Kit; Qiagen, Carlsbad, CA, USA). Separate miRNA-enriched fractions (<200 nt) and total RNA fractions were purified from each RNA sample using the RNeasy MinElute Cleanup Kit (74204; Qiagen) following manufacturer's instructions. RNA integrity was assessed via Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and NanoDrop ND-1000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). All RNA samples used for sequencing and subsequent molecular analyses had an RNA Integrity Number (RIN) between seven and nine.

Library preparation and RNA-sequencing (mRNA)

Sequencing libraries were prepared from 500 ng total RNA using the TruSeq stranded mRNA library preparation kit (RS-122-2101/2102; Illumina Inc., San Diego, CA, USA) including polyA selection. The library preparation was performed according to the manufacturer's protocol (#5031047). A 2 × 125 bp short-read paired-end RNA-Seq of all bud samples was performed using a HiSeq2500 with v4-sequencing chemistry by The SNP & SEQ Technology Platform in Uppsala, Sweden.

Pre-processing of RNA-sequencing data: quality control, gene quantification (mRNA)

The data pre-processing was performed following the guidelines described in http://www.epigenesys.eu/en/protocols/bio-informatics/1283-guidelines-for-rna-seq-data-analysis. Briefly, the quality of

the raw sequence data was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), v.0.11.4. Residual ribosomal RNA (rRNA) contamination was assessed and filtered using SortMeRNA (v.2.1; Kopylova & No, 2012). Data were then filtered to remove adapters and trimmed for quality using Trimmomatic (v.0.36; Bolger *et al.*, 2014). After both filtering steps, FastQC was run again to ensure that no technical artefacts were introduced. Read counts were obtained using kallisto (v.0.43.0; Bray *et al.*, 2016) using the *P. abies* v.1.0 complementary DNA (cDNA) sequences as a reference (retrieved from the PlantGenIE resource (Sundell *et al.*, 2015)). The kallisto abundance values were imported into R (v.3.4.0; R_Core_Team, 2013) using the Bioconductor (v.3.4; Gentleman *et al.*, 2004) tximport package (v.1.4.0; Soneson *et al.*, 2015). For the data quality assessment (QA) and visualization, the read counts were normalized using a variance stabilizing transformation as implemented in the Bioconductor DESeq2 package (v.1.16.1; Love *et al.*, 2014).

Principal component analysis and differential gene expression analysis

Principal component analysis (PCA) was conducted in R 3.5.0 using the built-in R function prcomp on normalized read count data. The PCA was performed on the complete set of expressed genes in the sequenced materials to check biological relevance of the data. We plotted the first three PCA three dimensions using cran package scatterplot3d v.0.3-41. We performed differential expression analysis on the normalized read counts using a negative binomial distribution as implemented in DESeq2 v.1.16.1. (Love *et al.*, 2014). The threshold to judge the significance of gene expression differences was false discovery rate (FDR) ≤ 0.01 and the absolute value of $\log_2$FoldChange ($\log_2$FC) ≥ 0.5 as per the recommendation from Schurch *et al.* (2016). The package vennDiagram was used to create a venn-diagram of differentially expressed genes (DEGs).

Cloning of full-length complementary DNA clones

To verify the presence of full-length transcripts we synthesized cDNA libraries using M-MLV Reverse Transcriptase (28025013; Invitrogen) and 500 ng of total RNA derived from female cones as template. The cDNA was used in a PCR-reaction (1 × 98°C for 3 min, 35× (98°C for 10 s, 61°C for 30 s, 72°C for 2 min), 72°C for 12 min) to amplify the sequence corresponding to the open reading frame of PaSPL1 using primers listed in Table S2a and Phusion High-Fidelity DNA Polymerase (F530L; ThermoFisher Scientific). The amplified PCR-product was subsequently cloned into a Zero Blunt TOPO (K2800J10; Invitrogen) cloning vector and sent to Eurofins Genomics (Ebersberg, Germany) for Sanger *et al.* (1977) sequencing.

Reverse transcription quantitative polymerase chain reaction

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) amplifications were performed as described in Akhter *et al.* (2018). Gene specific primers were designed using the Primer3 algorithm implemented in Geneious pro v.10.2.3 created by Biomatters Ltd, Auckland, New Zealand; http://www.geneious.com. All primers used in this study amplified with an efficiency between 85 and 110% (Table S2b). Gene expression was measured in three biological samples of each tissue type. All biological samples were analysed in duplicate. The expression data of each gene were normalized against the expression of three reference genes, *ACTIN*, *POLYUBIQUITIN*, and *HISTONE2A*. Calculations and normalizations were done using the CFX software based on the ΔC_t or $\Delta\Delta C_t$ methods (Bio-Rad, Hercules, CA, USA). Statistical analyses were performed using R v.3.4.2.

Phylogenetic analysis

Annotated *SPL* genes from *P. abies*, *Arabidopsis thaliana* and *Populus trichocarpa* were included in the analysis. For each gene, the coding sequences were translationally aligned using the Mafft module in Geneious (Geneious v.10.2.3; Biometters Ltd, Auckland, New Zealand) and the resulting alignments were curated using the Bmge software with default settings (Criscuolo & Gribaldo, 2010). Phylogenetic analysis was carried out using MrBayes v.3.2.6 (Huelsenbeck & Ronquist, 2001).

Library preparation and RNA-sequencing (miRNA)

Sequencing libraries were prepared from the fraction of small (< 200 nt) RNAs resulting from the RNA preparation using the TruSeq small RNA library preparation kit (RS-200-0012; Illumina Inc.) according to the manufacturer's protocol. A 2 × 50 bp short-read paired-end RNA-Seq of all bud samples were performed using a NovaSeq SP-100 by the SNP & SEQ Technology Platform in Uppsala, Sweden.

Pre-processing of RNA-sequencing data: quality control, gene quantification (miRNA)

Small RNA fraction RNA-Seq reads were pre-processed by means of quality pruning and adapter trimming using Fastp (Chen *et al.*, 2018) with the default settings, resulting in a set of high-quality reads.

We used the miRNA database miRBase release 22.1 to match high-quality reads with known miRNAs (Kozomara *et al.*, 2019) originating from *P. abies*, *A. thaliana* and *Populus trichocarpa*. We used kallisto to estimate miRNA abundance levels (both estimated counts and transcript per million (TPM)) by creating an index of 19-mers and 100 bootstrap samples during the actual quantification (Bray *et al.*, 2016). We used sleuth to perform differential expression analysis of miRNAs (Pimentel *et al.*, 2017). In sleuth, we used the likelihood ratio test (LRT) to detect differential expression and excluded miRNAs with a *q*-value larger than 0.05 from further analyses.

Transcriptome reconstruction

We used ClusTrast (Westrin *et al.*, 2022) with default settings (and using the built-in approach to generate the so-called guiding

contigs it requires) to generate a *de novo* assembly of the entire transcriptome. We included all the samples from 1 August in the assembly and aligned the *de novo* assembled transcripts to the *P. abies* reference genome (*P. abies* v.1.0) using MINIMAP2 (Li, 2018), with the preset option 'splice:hq'.

Since several of the *de novo* assembled transcripts mapped sequentially to several ConGenIE scaffolds (MAs) we used this information to connect the exon sequences of genes that mapped to multiple scaffolds in the current genome assembly. Each reference sequence was counted for only once.

Allele specific assembly

To identify single nucleotide polymorphisms (SNPs) in candidate genes, we performed separate *de novo* assemblies using linked De Bruijn graphs (Turner *et al.*, 2018) combined with KALLISTO (Bray *et al.*, 2016), as outlined in Akhter *et al.* (2018). The method has since been named Abeona and is available at: https://github.com/winni2k/abeona.

Genotyping

Genomic DNA was extracted from tissue samples using a CTAB protocol (Kim *et al.*, 1997). PCR-fragments covering *acrocona* specific SNPs were PCR-amplified using 100 ng of genomic DNA as template, Phusion DNA Polymerase (F-530; ThermoFisher Scientific) and primers listed in Table S2a. The resulting PCR-products were purified using the QIAquick PCR Purification Kit according to manufacturer's instructions (28104; Qiagen) and sent to Eurofins Genomics for Sanger *et al.* (1977) sequencing. The presence of a polymorphism was detected as double peaks in the resulting ab1-files (Fig. S1).

Allele specific expression analysis

The *PaSPL1* alleles were used to generate an index for KALLISTO, which we used to run each mRNA sample on. In the output, we could detect the allele frequencies from the TPM values, presented as average allele frequency across three biological samples.

PaSPL1 transcript degradation estimated by 5′ RLM RACE

RNA Ligase-Mediated 5′ Rapid Amplification of cDNA Ends (RLM RACE) was performed essentially as described by Llave *et al.* (2011). Samples used in the RLM RACE experiments are listed in Table S3. Briefly, RNA oligonucleotide adaptors were ligated to the 5′ terminus of cleaved transcripts using T4 RNA ligase (EL0021; ThermoFisher Scientific). The ligated RNA samples were subsequently reverse transcribed into first-strand cDNA using Superscript IV Reverse Transcriptase (18090010; Invitrogen). To amplify *PaSPL1* degradation products, we performed a primary touch-down PCR, followed by a nested secondary PCR using Phusion DNA Polymerase (F-530, ThermoFisher Scientific) and primers listed in Table S2c. The resulting PCR products were size separated on an agarose gel, cloned into Zero Blunt TOPO cloning vector (K22800J10; Invitrogen) and transformed into chemically competent OneShot TOP10 *Escherichia coli* cells (C404010; Invitrogen). Transformed cells were pre-screened for the presence of the *PaSPL1* sequence using colony PCR, and selected clones were sent to Eurofins Genomics for Sanger sequencing (Sanger *et al.*, 1977).

To quantify the *PaSPL1* degradation products, we performed qPCR experiments using Maxima SYBR Green qPCR Master Mix (KO221; ThermoFisher Scientific) and *PaSPL1* specific primer pairs (Table S2c). PCR fragments were quantified on a CFX Connect Real-Time PCR Detection System (Bio-Rad). The relative abundance of *PaSPL1* degradation products was normalized against the C_t value of the 5′-fragment in each sample, as outlined by Muller *et al.* (2002).

Results

Apical buds on leading branches formed *acrocona* transition shoots

In order to identify genes important to the *acrocona* transition shoot phenotype we sequenced the mRNA fraction of samples collected from leading shoots and lateral shoots (Fig. 1a) on branches situated in the cone-setting region of two *P. abies* genotypes: an *acrocona* mutant and a wild-type comparator. Lateral shoot meristems produced female cones in both wild-type *P. abies* and in the *acrocona* mutant (Fig. 1b,d) whereas leading shoot meristems produced vegetative shoots in the wild-type and transition shoots in the *acrocona* mutant (Fig. 1c,d).

We collected samples at the initiation of bud development and when the buds had started to differentiate. The buds harboured enlarged shoot apical meristems with only a few or no lateral organs at the early time-point. The later samples bore bud primordia with differentiating lateral organs while only a small meristem remained. Wild-type vegetative shoots initiated needles, whereas female shoots from both genotypes produced bracts and ovuliferous scales. In the *acrocona* transition shoots, needles had been formed in the basal part of the shoot, and bracts and ovuliferous scale-like structures had been formed in the apical part of the shoot (Fig. 1d).

Fourteen genes were commonly upregulated in *acrocona* transition shoots and female shoots

A PCA was carried out to analyse the relationships between samples and mRNA transcription profiles (Fig. 2a). The first principal component (PC1) explained 48% of the total variation, and samples grouped according to collection dates along this axis (Fig. 2a). The second principal component (PC2) explained 15% of the variation in the samples. Notably, all *acrocona* samples grouped close to each other along this axis and were distinct from wild-type bud samples (Fig. 2a). Thus, the samples formed distinct groups and the grouping could be attributed to collection date (i.e. growth phase) and genotype.

We identified mRNA transcripts with a significant difference in expression levels in at least one of three sample types (*acrocona* transition shoots, *acrocona* and wild-type female shoots) as

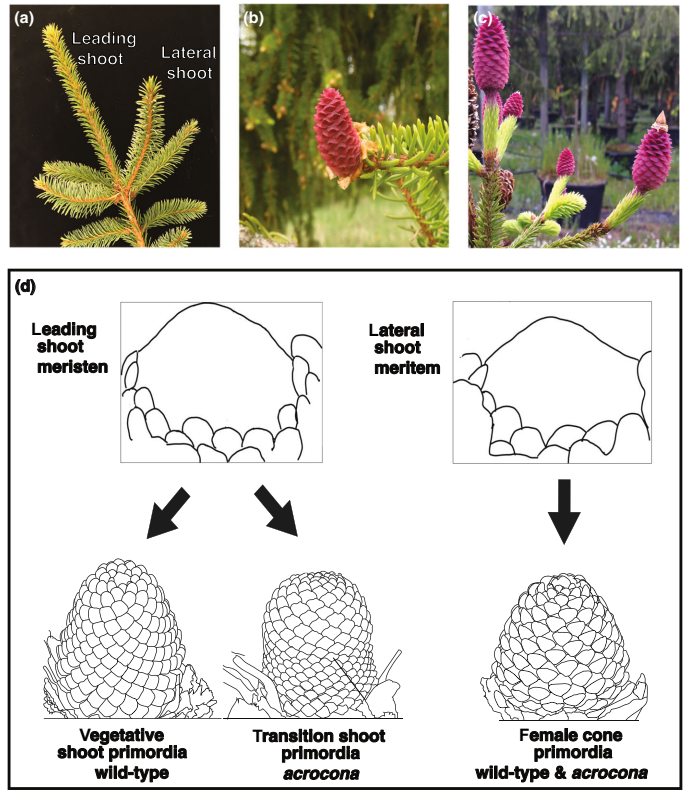

Fig. 1 Illustration of female cones, vegetative shoots and *acrocona* transition shoots. Shown in (a) is a branch of *Picea abies*, with the leading shoot and lateral shoots indicated. The pictures show a mature female cone in (b) and mature *acrocona* transition shoots in (c). The drawings in (d) illustrate the tissue types sampled in this study. Leading shoot meristems develop into vegetative (Veg) shoot primordia in wild-type, and into transition shoot (TS) primordia in the *acrocona* mutant. Lateral shoot meristems develop into female (F) cones primordia in both wild-type *Picea abies* and the *acrocona* mutant.

compared to the base-line sample (wild-type vegetative leading shoots). We did this separately for both meristem (early) and primordia (late) samples. In total 8407 genes were significantly differentially expressed between buds collected in the early developmental stages (Fig. 2b). Among those, 515 genes were either upregulated or downregulated in all three comparisons (Figs 2b, S2; Dataset S1). Similarly, a total of 10 542 genes were significantly differentially expressed between bud samples collected in the late developmental stages (Fig. 2c). In those samples, 390 genes were differentially expressed in all three comparisons (Figs 2c, S3; Dataset S1).

In the differential expression analysis, we used the *P. abies* v.1.0 cDNAs as a reference (Sundell *et al.*, 2015). In this assembly, known transcripts frequently map to several scaffolds due to assembly fragmentation. For example, the transcript of the MADS-box gene *DAL10* (Carlsbecker *et al.*, 2003) (GenBank accession no. AF064080) maps in 5′ to 3′ direction to four ConGenIE scaffolds: MA_15122, MA_18073, MA_121040, and MA_86473g0010 (Sundell *et al.*, 2015). To connect different ConGenIE scaffolds we performed a *de novo* transcriptome assembly using a novel assembly tool, CLUSTRAST (Westrin *et al.*, 2022). We used the assembly to connect scaffolds that mapped to the same transcript. This reduced the list of differentially expressed transcripts to 461 in the meristem samples and to 352 in the primordia samples (Dataset S2a,b). Fifty-five genes were differentially expressed in both meristem and primordia samples. Out of these, 14 genes were upregulated in *acrocona* transition shoots, female *acrocona*, and female wild-type shoots, as compared wild-type vegetative leading shoots (Table 1). We verified the upregulation of these 14 genes in female cones from four additional wild-type genotypes (Fig. S4).

Three transcription factors were commonly upregulated in *acrocona* transition shoots and female shoots

We were primarily interested in transcription factors that may influence the shift from vegetative to reproductive shoot identity. The most significantly differentially expressed candidate among the 14 upregulated genes, MA_15381g0010, encoded a

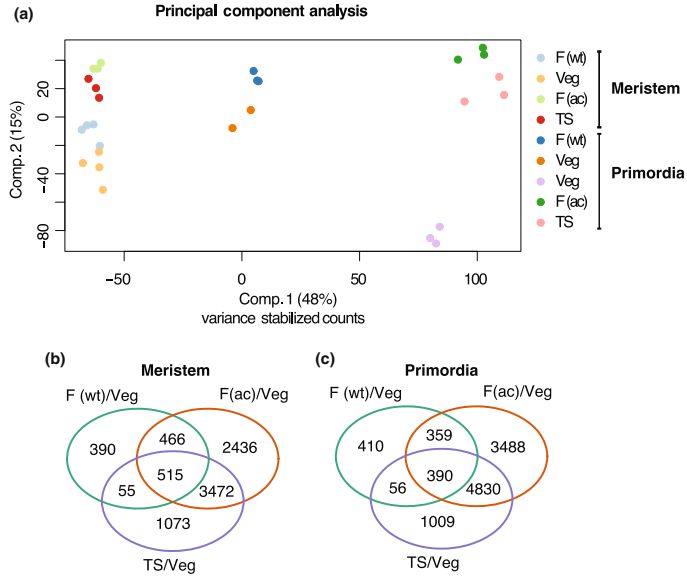

Fig. 2 Transcriptome sequencing of *acrocona* transition shoots. (a) Principal component analysis of RNA-sequencing data from 27 samples. The first and second principal components (PC1 and PC2) define the *x*- and *y*-axes of the two-dimensional space, respectively. PC1 represents 48%, and PC2 represents 15% of the total variation in the samples. Coloured dots represent wild-type and *acrocona* samples of different bud types, in bud meristem and primordia samples. F(ac), *acrocona* female sample; F(wt), wild-type female sample; TS, *acrocona* transition shoot sample; Veg, wild-type vegetative sample. (b) Venn diagram representing differentially expressed genes (DEGs) in meristem samples collected during the early growth phase in summer, whereas the Venn diagram in (c) shows the distribution of DEGs in primordia samples collected during the late growth phase in the autumn. The green circles represent DEGs between female wild-type samples and vegetative samples (F(wt)/Veg). The orange circles represent DEGs between female *acrocona* samples and vegetative samples (F(ac)/Veg). The violet circles represent DEGs between *acrocona* transition shoot samples and vegetative samples (TS/Veg). The threshold to judge the significance of gene expression differences was false discovery rate (FDR) ≤ 0.01 and the absolute value of $\log_2$FoldChange ($\log_2$FC) ≥ 0.5.

Table 1 Commonly upregulated transcripts in *Picea abies* reproductive meristems and primordia.

Scaffold ID	Common name	Pfam-domains/gene family	*P*-value adj. (F (wt)/Veg)
MA_15381	*PaSPL1*	PF03110-SBP domain/*SPL*-gene family	0
MA_22749	*PaSPL1*	*SPL*-gene family (C-terminal)	6.4e-260
MA_381942		Unknown	1.8e-59
MA_10430758		PF03330-Rare lipoprotein A	4.8e-53
MA_10428213		PF00135-Carboxylesterase family	2.0e-38
MA_63231		PF00044-Glyceraldehyde 3-phosphate dehydrogenase	3.7e-35
MA_65113		PF12481-Aluminium induced protein	8.9e-33
MA_86473	*DAL10*	PF00319-MADS-domain transcription factor (C-terminal)	2.0e-32
MA_194736	*FT-like*	PF01161-Phosphatidylethanolamine-binding protein	2.3e-12
MA_210262		PF00538-linker histone H1 and H5 family	1.0e-11
MA_10427625		PF01658-Myo-inositol-1-phosphate synthase	6.2e-11
MA_10436587		PF06200-tify domain	1.7e-10
MA_210555		PF00485-Phosphoribulokinase / Uridine kinase family	9.9e-08
MA_941055		PF00397-WW domain	0.0007
MA_10197498		PF00578-AhpC/TSA family	0.003

F, female cone; Veg, vegetative shoot.

transcription factor belonging to the *SPL*-gene family (Table 1). Notably, in our *de novo* transcriptome-wide assembly we were able to connect MA_15381g0010 (on ConGenIE scaffold MA_15381) and the second most significant gene, MA_22749g0010 (on ConGenIE scaffold MA_22749), into a single transcript (ClusTrast ID 9986_s_0_0) suggesting that

they are in fact part of the same gene. We named this gene *P. abies SQUAMOSA BINDING PROTEIN-LIKE1* (*PaSPL1*). MA_15381g0010 harbours the signature domain of the *SPL*-gene family (Pfam domain PF03110) and MA_22749g0010 harbours a conserved *miRNA156* binding site commonly found in *SPL*-genes, as well as a binding site for *miRNA529* (Fig. 3a). To find independent proof of the connection between MA_15381g0010 and MA_22749 g0010 we PCR-amplified and Sanger-sequenced the corresponding full-length cDNA clone (Fig. S5). Next, we confirmed the upregulation of *PaSPL1* in female shoots and *acrocona* transition shoots as compared to wild-type vegetative shoots using independent RT-qPCR experiments (Fig. 3b). Among the upregulated genes we detected two additional transcription factors that both belong to gene families important for flowering and floral meristem identity in angiosperms: (1) The MADS-box gene *DAL10* (MA_86473g0010) (Fig. 3c; Table 1), suggested to be a marker for reproductive shoot identity in *P. abies* (Carlsbecker *et al.*, 2003), and (2) a previously uncharacterized *FLOWERING LOCUS T-LIKE* gene (MA_194736g0010) belonging to the PEBP-family (Karlgren *et al.*, 2011; Klintenas *et al.*, 2012; Liu *et al.*, 2016) of transcription factors (Table 1).

PaSPL1 is homologous to angiosperm *SPL*-genes involved in reproductive phase change

Several publications have reported phylogenetic reconstructions of the MADS-box gene family (e.g. Carlsbecker *et al.*, 2003; Gramzow *et al.*, 2014; Akhter *et al.*, 2018). In those analyses, the *DAL10* gene commonly grouped into a gymnosperm specific sub-clade, which appears to be lost in the angiosperm lineage. In order to analyse the evolutionary relationship between conifer and angiosperm *SPL*-genes, we used the conserved SBP-domain of *PaSPL1* as bait to search for additional members of this gene family in the *P. abies* genome v.1.0 (Sundell *et al.*, 2015). In total we retrieved 10 additional members of the *SPL*-gene family from *P. abies*, here named *PaSPL2-11*. Among those, *PaSPL1*, *PaSPL2*, *PaSPL10* and *PaSPL11* harbour the conserved *miR156/529* binding site (Table S4). The evolutionary relationship between the *P. abies* SPL-genes and genes from the model species *A. thaliana* and *Populus trichocarpa* was analysed using Bayesian phylogenetics (Fig. S6). In this phylogeny, *PaSPL1*, *PaSPL10* and *PaSPL11* formed a clade that grouped basal to the *Arabidopsis thaliana* genes *AtSPL2*, *AtSPL6*, *AtSPL9*, *AtSPL10*, *AtSPL11* and *AtSPL15*, which all contain the *miR156* binding site, and

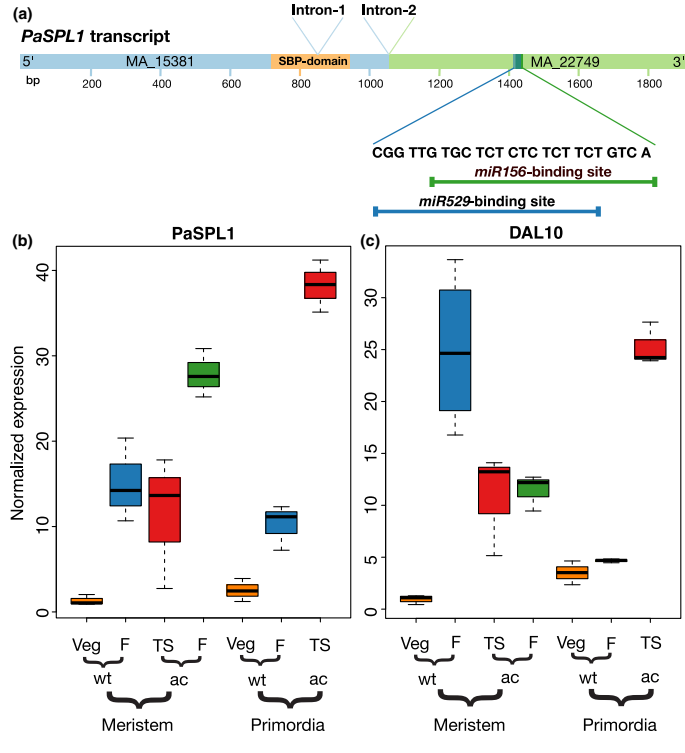

Fig. 3 Verification of expression of *PaSPL1* and *DAL10* using reverse transcription quantitative polymerase chain reaction (RT-qPCR). (a) Graphical representation of the *PaSPL1* transcript assembled by CLUSTRAST (Westrin *et al.*, 2022) with the coverage of the ConGenIE scaffolds MA_15381 and MA_22749 indicated in blue and green colour, respectively. Indicated are also the positions of the two introns present in the *PaSPL1* open reading frame, the signature SBP-domain and the overlapping binding sites of *miR156* and *miR529*. The boxplots in (b) and (c) show the normalized expression of *PaSPL1* and *DAL10* assayed by RT-qPCR. Veg, vegetative; F, female; TS, transition shoot; wt, wild-type; ac, acrocona. Box-plot elements: Line, median; box limits, upper and lower quartiles; whiskers, points.

have implicated roles in reproductive phase change (Preston & Hileman, 2013). The other *P. abies SPL*-genes included in the analysis grouped with other *A. thaliana* and *Populus trichocarpa* genes, e.g. *PaSPL3*, *PaSPL4* and *PaSPL5* grouped together with *AtSPL8* and *PtSPL8* (Fig. S6).

MicroRNA156 and *miR529* were upregulated in vegetative shoots and *acrocona* transition shoots

Next, we analysed the expression of miRNAs in the meristematic samples (Table S1). Illumina sequencing reads of the small RNA fraction were mapped against previously known miRNAs present in miRBase (Kozomara *et al.*, 2019). The read length of the RNA-Seq reactions allowed us to identify both precursor and mature miRNAs. We identified miRNAs with a significant difference in expression levels in at least one sample type (*acrocona* transition shoots, *acrocona* and wild-type female shoots) as compared to the same base-line sample as used in mRNA analysis (wild-type vegetative). Next, we performed hierarchical clustering of 1231 precursor and 966 mature miRNAs based on their estimated abundance levels (Figs S7, S8). Among the differentially expressed miRNAs, we identified *miR156t* and *miR529c*, which both have the capacity to bind the *PaSPL1* mRNA in a partly overlapping manner (Fig. 3a). Both *miR156t* and *miR529c* were upregulated in wild-type vegetative shoots and *acrocona* transition shoots, compared to female shoots from both genotypes (Fig. S9a,b; $P < 0{,}001$; Dataset S3). The estimated expression levels of *miR156t* and *miR529c* were negatively correlated to the expression level of *PaSPL1* in wild-type *P. abies*, whereas *PaSPL1* and the miRNAs *miR156t* and *miR529c* were all upregulated in *acrocona* transition shoot meristems. A similar expression pattern of *miR156t* and *miR529c* was also detected in the late primordia samples (Fig. S10).

Apart from *miR156t* we also detected expression of other miRNAs that have implicated roles in the regulation of flowering or floral patterning in angiosperms (Spanudakis & Jackson, 2014), e.g. *miR159*, *miR172*, *miR167*, *miR319* and *miR390* (Figs S7, S8). Among those, *miR172* stood out as it, like *miR156t*, had a clear differential expression pattern between samples of different bud-types. In contrast to *miR156t*, *miR172i* was upregulated in female shoot meristems instead of wild-type vegetative meristems (Fig. S9c). In *acrocona* leading shoot meristems, *miR172i* had a more variable expression.

The *acrocona* mutant harbours a SNP in the *miR156/529* binding-site of *PaSPL1*

A point mutation or a SNP in the *miR156/529* binding-site could explain the simultaneous expression of *PaSPL1*, and the miRNAs *miR156* and *miR529*, in *acrocona* transition shoots. To address this notion, we performed a separate *de novo* assembly of *PaSPL1* transcripts from either wild-type samples, or *acrocona* samples using Abeona assembly. This method can be used to identify alleles and individual SNPs in short read transcriptome datasets (Akhter *et al.*, 2018). As a reference, we performed similar allele-specific assemblies of the remaining candidate genes listed in Table 1 and four additional genes that also harbour the *miR156/529* binding-site. Thirteen of the assembled genes had SNPs in the *acrocona* mutant background (Table S5; Dataset S4). Next, we compared SNPs present in the assembled *acrocona*-transcripts with SNPs present in the Swedish breeding population of *P. abies* (Wang *et al.*, 2020). In this comparison, only four genes had *acrocona* specific SNPs, and among those, only *PaSPL1* also had a *miR156/529* binding site (Table S5). *PaSPL1* had two *acrocona* specific SNPs, one located 256 nucleotides from the assumed start codon, and a second in the *miR156/529* binding-site at nt 1421 (Fig. 4a). Sanger sequencing of full-length cDNA

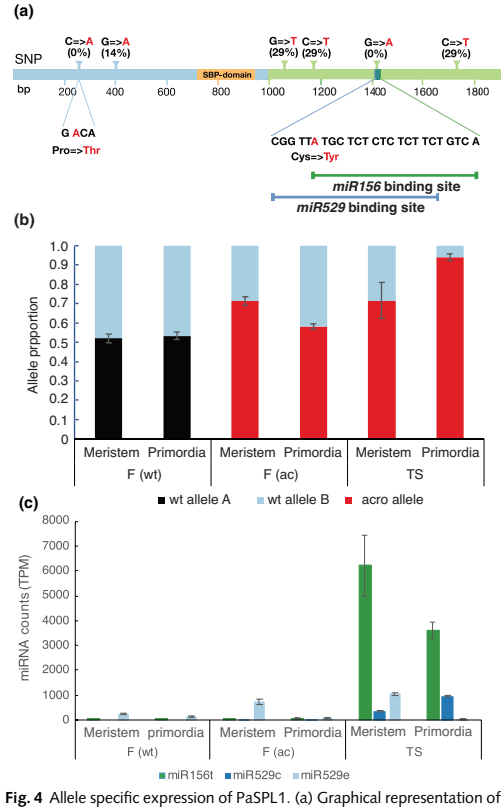

Fig. 4 Allele specific expression of PaSPL1. (a) Graphical representation of the *PaSPL1* transcript. Indicated are the six single nucleotide polymorphism (SNP) positions identified in the *acrocona* allele of *PaSPL1*, the signature SBP-domain and the overlapping binding sites of *miR156* and *miR529*. Red colour indicates the substitution identified in the *acrocona* allele of *PaSPL1*. The percentage shown after each SNP indicates how common a specific SNP is in the *Picea abies* wild-type population. (b) Proportions of transcripts from the wild-type alleles and the *acrocona* allele in the different messenger RNA (mRNA) samples (c) Estimated counts (transcript per million (TPM)) of *miR156t*, *miR529c* and *miR529e*. Each column represents the mean of three biological replicates. Error-bars show standard error. Data points underlying the means are presented in Dataset S2d. F(wt), female wild-type; F(ac), female *acrocona*; TS, *acrocona* transition shoots.

clones derived from the *acrocona* and the wild-type comparator confirmed the presence of this *acro*-SNP at nucleotide 1421.

Expression of *miR156* and *miR529* affected the allelic proportion of expressed *PaSPL1*

To test if the expression of *miR156* or *miR529* affects *PaSPL1* expression levels in an allele-specific manner, we estimated the proportion mRNA expressed from each allele in the transcriptome datasets (Fig. 4b; Dataset S2c). We also assessed *miR156* and *miR529* levels in the same samples (Fig. 4c; Dataset S2d). Wild-type female meristems and primordia expressed low levels of *miR156* and *miR529*, and the two *PaSPL1* alleles were expressed in equal proportions (Fig. 4b,c, left column). In *acrocona* female shoots, the *PaSPL1* allele proportion (*acrocona* vs wild-type) was 70 : 30 in the early meristematic samples and 50 : 50 in the later primordia samples (Fig. 4b, middle column). In the meristem samples, we observed elevated expression of an additional *miR529* variant, *miR529e*, whereas primordia samples expressed low levels of all variants of *miR156* and *miR529* (Fig. 4c, middle column). Similar to the *acrocona* females, the allele proportion in meristematic samples from transition shoots was about 70 : 30. In the later transition shoot samples, the *acro*-allele accounted for almost 95% of all *PaSPL1* transcripts (Fig. 4b, right column). As noted in our differential expression analysis of miRNAs (Fig. S9), *acrocona* transition shoots expressed elevated levels of both *miR156t* and *miR529c*, and in the early meristematic samples, *miR529e* (Fig. 4c, right column). In short, in samples that expressed elevated levels of *miR156* or *miR529*, the allele proportion of *PaSPL1* was affected in favour of the allele which harboured the *acro*-SNP. This pattern was evident in the early meristematic samples and became even more pronounced in the later primordia samples.

MicroR156/529 cleave *PaSPL1* in an allele specific manner

The allele-specific reduction of *PaSPL1* transcripts in *acrocona* transition shoots could be explained by *miR156/529* mediated transcript cleavage. To examine this possibility, we performed 5′ RLM RACE experiments. Putative *PaSPL1* cleavage- and degradation products of the expected size (*c.* 450 bp) could be readily observed in wild-type vegetative samples and, to some extent, in samples from *acrocona* transition shoots (Fig. S11a). Quantification of short degradation products that end at, or downstream of, the *miR156/529* binding-site compared to longer general degradation products that span the entire *miR156/529* binding-site reflects this pattern (Fig. S11b). We identified two putative *PaSPL1* cleavage products from the wild-type vegetative samples by cloning and Sanger sequencing gel-purified DNA-fragments of the expected size. Seventeen out of 24 cloned fragments ended at nt 1427, i.e. within the *miR156/529* binding site, and four fragments ended at nt 1431 (Fig. S11c). By cloning and Sanger sequencing of gel-purified fragments from the *acrocona* transition shoot, we detected two longer degradation products, three additional putative cleavage products that ended at nt 1427, and several shorter *PaSPL1* fragments (Table S6). Furthermore, SNPs present in the cloned DNA fragments showed that the long uncleaved degradation products were expressed from the *acrocona* allele. In contrast, the putative cleavage products that ended at nt 1427, and the shorter fragments, were all from the wild-type allele. This indicates that expression of *miR156* and *miR529* preferentially mediate a cleavage of the wild-type allele of *PaSPL1*, and that this cleavage could explain the differences in the expression levels of the two alleles in *acrocona* transition shoots.

The *acro*-SNP co-segregated with the early cone-setting *acrocona* phenotype

We have previously performed inbred crosses of adult ramets of the *acrocona* mutant (Uddenberg *et al.*, 2013). One-fourth of the resulting siblings displayed an enhanced early cone-setting phenotype and produced cones during the third growth cycle. As these trees have grown older, they now form rounded bushes with no clear apical dominance and regularly produce transition shoots on almost every shoot (Fig. S12). We expect that the causal mutation for the *acrocona* phenotype should be homozygous for the *acro*-allele in the early cone-setting siblings. To test if any of our candidate genes met this criterion, we analysed the segregation pattern of the *acro*-specific SNPs identified in the genes *PaSPL1*, MA_381942g0010, MA_10436587g0010 and MA_21055g010 in a sub-set of trees from the sibling population (Table S7). In this analysis only *PaSPL1* was homozygous for its *acro*-specific SNP in early cone-setting trees.

To provide further support for this segregation pattern, we genotyped the entire segregating inbred sibling population generated in Uddenberg *et al.* (2013) (Table 2; Dataset S2e). In this analysis, 32% of the segregating sibling trees were homozygous for the *acro*-SNP present in *PaSPL1*, 57% were heterozygous, and 11% homozygous wild-type. Among the sibling trees that were homozygous for the *acro*-SNP, 92% displayed either an early cone-setting (21/24) or an intermediate *acrocona* (1/24) phenotype. Only two homozygous trees produced vegetative topshoots, and both of those trees had stunted growth. All heterozygous trees (42/42) produced vegetative shoots only. Similarly, none of the trees that were homozygous wild-type had any cones. Hence, we detected a highly significant ($P < 0.00001$, Fisher's exact test) correlation between trees homozygous for the *acro*-SNP and the early cone-setting phenotype.

We also genotyped wild stands of the *acrocona* mutant which all displayed a semidominant phenotype (Fig. S13). All trees were heterozygous with respect to the *acro*-SNP (Table S8; Dataset S2f), whereas two of the trees were also homozygous for the upstream *acrocona* specific SNP at nucleotide 256 (Dataset S2f). This

Table 2 Genotyping of inbred *Picea abies* var. *acrocona* siblings.

Genotype/phenotype	Wt (G/G)	Het (G/A)	acro (AA)
Apical cone	0	0	21 (88%)
Intermediate	0	0	1 (4%)
Vegetative	8 (100%)	43 (100%)	2 (8%)
Total	8	43	24

suggests that the upstream SNP is not necessary for the enhanced phenotype displayed by homozygous *acrocona* plants.

Discussion

In most of Sweden's planting zones, there is a shortage of domestically produced *P. abies* seeds (Rosvall, 2011). This shortage has two primary causes: irregular cone-setting of *P. abies* and damages to cones and seeds caused by insects and fungi (Almqvist *et al.*, 2010). Conifer breeding and research also face a significant obstacle because of the very long generation times (Flachowsky *et al.*, 2009). Therefore, there is a strong desire to learn more about the molecular mechanism that regulates cone-setting in conifers (Uddenberg *et al.*, 2015). Understanding the genetic mechanism that regulates reproductive phase change in conifers could also increase our understanding of the evolutionary relationship between extant seed plants, i.e. angiosperms and gymnosperms.

In this study, we utilized the unique features of an adult, naturally occurring and presumably heterozygous, *acrocona* mutant. In this *acrocona* mutant, apical shoots on leading branches commonly develop into transition shoots. Like vegetative shoots, the first lateral organs that initiate in *acrocona* transition shoots are needles. Later in the growing season, the *acrocona* transition shoots produce ovuliferous scale-like structures. Hence, we collected samples that allowed us to identify genes expressed in the *acrocona* transition shoots before any morphological signs of the reproductive shift were apparent, and we compared their transcriptome profiles to profiles generated from corresponding wild-type vegetative shoot meristems and female meristems. This selection of shoots allowed us to address the hypothesis that the *acrocona* transition shoots express genes related to reproductive shoot development before the morphological shift. It was also likely that the identified candidate genes would be active in the meristem rather than acting in the down-stream morphological differentiation. Genes upregulated in both transition shoots and female meristems of *acrocona* (relative to wild-type vegetative meristems) would therefore be candidates for genes important for reproductive meristem identity.

Apart from the meristematic samples collected in early August, we also collected primordia samples during the autumn, when lateral organ differentiation occurs. In this growth phase, vascular strands connect to the lateral organs and cellular differentiation occurs within the ovuliferous scales and sterile bracts in female cones, and within the needles in vegetative shoots. Similar cellular differentiation also occurs in the *acrocona* transition shoots.

By combining the results from comparisons of meristem and primordia samples, we identified 14 genes that were upregulated in *acrocona* transition shoots and female cones as compared to wild-type vegetative leading shoots. In line with the hypothesis that these 14 genes represent genes important for reproductive development, we identified *DAL10*, a marker for reproductive shoot identity (Carlsbecker *et al.*, 2003). Among the top candidate genes, we also identified a member of the *SPL* gene family, here named *PaSPL1*. In our phylogenetic analysis of the *SPL* gene family, *PaSPL1* groups together with angiosperm *SPL*-genes that have been shown to regulate flowering (Preston & Hileman, 2013). This is interesting because a certain position in a phylogenetic tree may be indicative not only of shared ancestry, but also of conserved function between closely located genes (Theissen *et al.*, 1996; Tandre *et al.*, 1998). Although sub-functionalization and neo-functionalization frequently occur (Irish & Litt, 2005).

In angiosperms, members of the *SPL*-gene family are key regulators of the age-dependent flowering pathway (Wang *et al.*, 2009; Preston & Hileman, 2013). This pathway also includes *miR156*, which acts as a negative regulator of the *SPL*-genes during juvenile stages and in vegetative meristems. Expression of *miR156*-resistant variants of *AaSPL15* in the perennial herb *A. alpina* is known to induce early flowering and flowering in positions which in wild-type would continue as vegetative shoots (Hyun *et al.*, 2019). We note that this resembles the *acrocona* mutant phenotype. Analysis of the *PaSPL1* sequence revealed that *PaSPL1* harbours a conserved *miR156* binding site located 1421 nucleotides downstream from the start codon. Partly overlapping was also the binding site of *miR529*, a miRNA that has been lost in the core eudicots but that is still present in, e.g. bryophytes and monocots such as *Oryza sativa* (Morea *et al.*, 2016). The occurrence of an overlapping binding site indicates that both miRNAs may negatively regulate *PaSPL1*. In line with this hypothesis, we detected an elevated expression of both *miR156* and *miR529* in wild-type vegetative leading meristem compared with female meristems of both assayed genotypes. This supports the hypothesis that the *SPL/miR156* module of the age-dependent flowering pathway regulates reproductive phase change in conifers – possibly with the additional involvement of *miR529*.

Interestingly, both *miR156* and *miR529* were co-expressed with *PaSPL1* in *acrocona* transition shoot meristems. The SNP present in the overlapping *miR156/529* binding site of the *PaSPL1 acrocona* allele could explain the co-expression: we detected (1) two distinct cleavage products of *PaSPL1* in samples from vegetative shoots cleaved in the putative *miR156/529* target site, (2) specific cleavage of the wild-type allele of *PaSPL1* in heterozygous *acrocona* transition shoots, and (3) a higher expression of the *acrocona* allele (as compared to the wild-type allele) in *acrocona* transition shoots. This indicates that *miR156* and/or *miR529* can mediate *PaSPL1* cleavage, and that this cleave occurs in an allele specific manner, suggesting that the *acro*-SNP renders the *acrocona* allele *miR156/529* tolerant. We note that similar dual cleavage products of *SPL* transcripts have been reported previously in heterologous experiments studying the ectopic expression of *miR156* and *miR529* from *O. sativa* in *A. thaliana* (Morea *et al.*, 2016).

Provided that PaSPL1 regulates female reproductive identity, we would expect a co-segregation of this SNP with the *acrocona* phenotype. Indeed, the *acro*-SNP is absent in a tested set of 35 wild-type genotypes that are part of the Swedish breeding population (Wang *et al.*, 2020). In our previous studies, we have produced an inbred population of the *acrocona* mutant. One quarter segregated with an early cone-setting phenotype among the sibling trees, which we then interpreted as an enhanced homozygous phenotype (Uddenberg *et al.*, 2013). As these plants have grown

older, they only became rounded shrubs, distinct from the heterozygous *acrocona* trees, which displayed a semi-dominant phenotype and grew taller. We have now demonstrated a co-segregation between the early cone-setting phenotype and trees that are homozygous for the *acro*-SNP by genotyping. Importantly, none of the segregating siblings that were homozygous for the wild-type allele displayed any *acrocona* phenotypes.

In conclusion, we propose that cone-setting in the conifer *P. abies* is regulated by conserved elements of the age-dependent flowering pathway. In support of this notion, we provide several independent lines of experimental evidence: (1) Using transcriptome analyses, we demonstrate an anti-correlated expression of *PaSPL1* and *miR156/529* in female and vegetative shoot meristems. (2) Using allele-specific assembly and expression analysis, we identify an *acrocona* specific SNP in the miRNA binding site of *PaSPL1*. We show that the *acrocona* allele of *PaSPL1* is upregulated in transition shoots, along with *miR156* and *miR529* in contrast to the anti-correlated expression in wild-type shoots. (3) Using RLM RACE experiments, we show that *miR156* and *miR529* preferentially mediate cleavage of the wild-type allele of *PaSPL1*. (4) Finally, we demonstrate that among our *acrocona* specific SNPs – in *PaSPL1* and other candidate genes – only the *acro*-SNP in the miRNA binding site of *PaSPL1* co-segregates with the enhanced *acrocona* phenotype. We have, however, not analysed the genomic sequence of *PaSPL1*, and it is possible that other unknown SNPs or perhaps the SNP located at nucleotide 256 in the *PaSPL1* transcript also contribute to the *acrocona* phenotype. Considering these numerous lines of evidence, together with the similarity to the situation in the angiosperm perennial herb *Arabis alpina*, the most parsimonious conclusion is that the early flowering of the *acrocona* mutant is caused by the mutation in the *miR156/miR529* binding site of the *PaSPL1* gene. However, to ultimately prove that the *acro*-SNP alone is responsible for the *acrocona* phenotype we would be required to find, or generate, independent mutations in the *PaSPL1* locus. Our results demonstrate remarkable conservation of this pathway, which is linked to perennity, between species that shared a last common ancestor 300 million years ago. Hence, the age-dependent pathway seems to be crucial to the regulation of reproductive phase change not only in conifers, but also in many other perennial seed plants.

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Author contributions

JFS and OE planned and designed the research. JFS wrote the manuscript with contributions from SA, KJW and NZ. SA, NZ and VN performed the experiments and conducted fieldwork. SA, KJW, WWK and ND analysed the data. JFS, OE, NRS and ON provided supervision, funding and materials. All authors read and edited the final version of the manuscript. SA, KJW and NZ contributed equally to this work. JFS and OE are the joint corresponding authors on the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article or its Supporting Information. The sequencing data is available at the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena/browser/home) under the accession no. PRJEB45942. All custom-made code is available at either https://github.com/karljohanw/clustrast or https://github.com/winni2k/abeona.

References

Akhter S, Kretzschmar WW, Nordal V, Delhomme N, Street NR, Nilsson O, Emanuelsson O, Sundstrom JF. 2018. Integrative analysis of three RNA sequencing methods identifies mutually exclusive exons of MADS-box isoforms during early bud development in *Picea abies*. *Frontiers in Plant Science* **9**: 1625.

Albani MC, Coupland G. 2010. Comparative analysis of flowering in annual and perennial plants. *Current Topics in Developmental Biology* **91**: 323–348.

Almqvist C, Wennström U, Karlsson B. 2010. *Förädlat skogsodlingsmaterial 2010–2050. Tillgång och behov av förädlat frö samt förslag på åtgärder för att minimera brist och maximera genetisk vinst.* Uppsala, Sweden: Skogforsk.

Azevedo H, Lino-Neto T, Tavares RM. 2003. An improved method for high-quality RNA isolation from needles of adult maritime pine trees. *Plant Molecular Biology Reporter* **21**: 333–338.

Blazquez MA, Weigel D. 2000. Integration of floral inductive signals in Arabidopsis. *Nature* **404**: 889–892.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

Bray NL, Pimentel H, Melsted P, Pachter L. 2016. Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology* **34**: 525–527.

Carlsbecker A, Sundstrom J, Tandre K, Englund M, Kvarnheden A, Johanson U, Engstrom P. 2003. The DAL10 gene from Norway spruce (*Picea abies*) belongs to a potentially gymnosperm-specific subclass of MADS-box genes and is specifically active in seed cones and pollen cones. *Evolution & Development* **5**: 551–561.

Carlsbecker A, Sundstrom JF, Englund M, Uddenberg D, Izquierdo L, Kvarnheden A, Vergara-Silva F, Engstrom P. 2013. Molecular control of

normal and *acrocona* mutant seed cone development in Norway spruce (*Picea abies*) and the evolution of conifer ovule-bearing organs. *New Phytologist* **200**: 261–275.

Chen S, Zhou Y, Chen Y, Gu J. 2018. FASTP: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**: i884–i890.

Criscuolo A, Gribaldo S. 2010. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evolutionary Biology* **10**: 210.

Flachowsky H, Hanke MV, Peil A, Strauss SH, Fladung M. 2009. A review on transgenic approaches to accelerate breeding of woody plants. *Plant Breeding* **128**: 217–226.

Florin R. 1951. Evolution in cordaites and conifers. *Acta Horti Bergiani* **15**: 285–388.

Futamura N, Igasaki T, Saito M, Taira H, Shinohara K. 2019. Comparison of fertile and sterile male gametogenesis in *Cryptomeria japonica* D. Don. *Tree Genetics & Genomes* **15**: 30.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J *et al.* 2004. BIOCONDUCTOR: open software development for computational biology and bioinformatics. *Genome Biology* **5**: R80.

Giacomello S, Salmen F, Terebieniec BK, Vickovic S, Navarro JF, Alexeyenko A, Reimegard J, McKee LS, Mannapperuma C, Bulone V *et al.* 2017. Spatially resolved transcriptome profiling in model plant species. *Nature Plants* **3**: 17061.

Gramzow L, Weilandt L, Theissen G. 2014. MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. *Annals of Botany* **114**: 1407–1429.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Hyun Y, Vincent C, Tilmes V, Bergonzi S, Kiefer C, Richter R, Martinez-Gallegos R, Severing E, Coupland G. 2019. A regulatory circuit conferring varied flowering response to cold in annual and perennial plants. *Science* **363**: 409–412.

Irish VF, Litt A. 2005. Flower development and evolution: gene duplication, diversification and redeployment. *Current Opinion in Genetics & Development* **15**: 454–460.

Karlgren A, Gyllenstrand N, Kallman T, Sundstrom JF, Moore D, Lascoux M, Lagercrantz U. 2011. Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. *Plant Physiology* **156**: 1967–1977.

Kim CS, Lee CH, Shin JS, Chung YS, Hyung NI. 1997. A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acids Research* **25**: 1085–1086.

Klintenas M, Pin PA, Benlloch R, Ingvarsson PK, Nilsson O. 2012. Analysis of conifer *FLOWERING LOCUS T/TERMINAL FLOWER1*-like genes provides evidence for dramatic biochemical evolution in the angiosperm FT lineage. *New Phytologist* **196**: 1260–1273.

Kopylova E, No L. 2012. SORTMERNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics* **28**: 3211–3217.

Kozomara A, Birgaoanu M, Griffiths-Jones S. 2019. miRBase: from microRNA sequences to function. *Nucleic Acids Research* **47**(D1): D155–D162.

Li H. 2018. MINIMAP2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**: 3094–3100.

Lindgren K, Ekberg I, Eriksson G. 1977. External factors influencing female flowering in *Picea abies* (L.) Karst. *Studia Forestalia Suecica* **142**: 1–53.

Liu YY, Yang KZ, Wei XX, Wang XQ. 2016. Revisiting the phosphatidylethanolamine-binding protein (PEBP) gene family reveals cryptic *FLOWERING LOCUS T* gene homologs in gymnosperms and sheds new light on functional evolution. *New Phytologist* **212**: 730–744.

Llave C, Franco-Zorrilla JM, Solano R, Barajas D. 2011. Target validation of plant microRNAs. *Methods in Molecular Biology* **732**: 187–208.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESEQ2. *Genome Biology* **15**: 550.

Morea EGO, da Silva EM, e Silva GFF, Valente GT, Barrera Rojas CH, Vincentz M, FTS N. 2016. Functional and evolutionary analyses of the *miR156* and *miR529* families in land plants. *BMC Plant Biology* **16**: 40.

Mouradov A, Hamdorf B, Teasdale RD, Kim JT, Winter KU, Theissen G. 1999. A *DEF/GLO*-like MADS-box gene from a gymnosperm: *Pinus radiata* contains an ortholog of angiosperm B class floral homeotic genes. *Developmental Genetics* **25**: 245–252.

Muller PY, Janovjak H, Miserez AR, Dobbie Z. 2002. Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques* **32**: 1372–1374 1376, 1378–1379.

Niu S, Yuan H, Sun X, Porth I, Li Y, El-Kassaby YA, Li W. 2016. A transcriptomics investigation into pine reproductive organ development. *New Phytologist* **209**: 1278–1289.

Niu S, Yuan L, Zhang Y, Chen X, Li W. 2014. Isolation and expression profiles of gibberellin metabolism genes in developing male and female cones of *Pinus tabuliformis*. *Functional & Integrative Genomics* **14**: 697–705.

O'Maoileidigh DS, Graciet E, Wellmer F. 2014. Gene networks controlling *Arabidopsis thaliana* flower development. *New Phytologist* **201**: 16–30.

Pimentel H, Bray NL, Puente S, Melsted P, Pachter L. 2017. Differential analysis of RNA-seq incorporating quantification uncertainty. *Nature Methods* **14**: 687–690.

Preston JC, Hileman LC. 2013. Functional evolution in the plant *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL)* gene family. *Frontiers in Plant Science* **4**: 80.

R_Core_Team. 2013. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rosvall O. 2011. *Review of the Swedish tree breeding programme*. Uppsala, Sweden: Skogforsk.

Rutledge R, Regan S, Nicolas O, Fobert P, Cote C, Bosnich W, Kauffeldt C, Sunohara G, Seguin A, Stewart D. 1998. Characterization of an *AGAMOUS* homologue from the conifer black spruce (*Picea mariana*) that produces floral homeotic conversions when expressed in Arabidopsis. *The Plant Journal* **15**: 625–634.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* **74**: 5463–5467.

Sauquet H, von Balthazar M, Magallon S, Doyle JA, Endress PK, Bailes EJ, de Morais EB, Bull-Herenu K, Carrive L, Chartier M *et al.* 2017. The ancestral flower of angiosperms and its early diversification. *Nature Communications* **8**: 10.

Schurch NJ, Schofield P, Gierlinski M, Cole C, Sherstnev A, Singh V, Wrobel N, Gharbi K, Simpson GG, Owen-Hughes T *et al.* 2016. How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *RNA* **22**: 839–851.

Smith SA, Beaulieu JM, Donoghue MJ. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences, USA* **107**: 5897–5902.

Soneson C, Love MI, Robinson MD. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research* **4**: 1521.

Spanudakis E, Jackson S. 2014. The role of microRNAs in the control of flowering time. *Journal of Experimental Botany* **65**: 365–380.

Sundell D, Mannapperuma C, Netotea S, Delhomme N, Lin Y-C, Aa S. 2015. The plant genome integrative explorer resource: PlantGenIE.org. *New Phytologist* **208**: 1149–1156.

Sundstrom J, Carlsbecker A, Svensson ME, Svenson M, Johanson U, Theissen G, Engstrom P. 1999. MADS-box genes active in developing pollen cones of Norway spruce (*Picea abies*) are homologous to the B-class floral homeotic genes in angiosperms. *Developmental Genetics* **25**: 253–266.

Tandre K, Svenson M, Svensson ME, Engstrom P. 1998. Conservation of gene structure and activity in the regulation of reproductive organ development of conifers and angiosperms. *The Plant Journal* **15**: 615–623.

Theissen G, Kim JT, Saedler H. 1996. Classification and phylogeny of the MADS-box multigene family suggest defined roles of MADS-box gene subfamilies in the morphological evolution of eukaryotes. *Journal of Molecular Evolution* **43**: 484–516.

Turner I, Garimella KV, Iqbal Z, McVean G. 2018. Integrating long-range connectivity information into de Bruijn graphs. *Bioinformatics* **34**: 2556–2565.

Uddenberg D, Akhter S, Ramachandran P, Sundstrom JF, Carlsbecker A. 2015. Sequenced genomes and rapidly emerging technologies pave the way for conifer evolutionary developmental biology. *Frontiers in Plant Science* **6**: 970.

Uddenberg D, Reimegard J, Clapham D, Almqvist C, von Arnold S, Emanuelsson O, Sundstrom JF. 2013. Early cone setting in *Picea abies* acrocona is associated with increased transcriptional activity of a MADS box transcription factor. *Plant Physiology* **161**: 813–823.

Wang JW, Czech B, Weigel D. 2009. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* **138**: 738–749.

Wang X, Bernhardsson C, Ingvarsson PK. 2020. Demography and natural selection have shaped genetic variation in the widely distributed conifer Norway spruce (*Picea abies*). *Genome Biology and Evolution* **12**: 3803–3817.

Westrin KJ, Kretzschmar WW, Emanuelsson O. 2022. CLUSTRAST: a short read de novo transcript isoform assembler guided by clustered contigs. *bioRxiv*. doi: 10.1101/2022.01.02.473666.

Winter KU, Becker A, Munster T, Kim JT, Saedler H, Theissen G. 1999. MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. *Proceedings of the National Academy of Sciences, USA* **96**: 7342–7347.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Differentially expressed genes.

Dataset S2 Background data.

Dataset S3 MicroRNA statistics.

Dataset S4 Consensus *acrocona* transcripts.

Fig. S1 Genotyping of the acro-single nucleotide polymorphism in *PaSPL1*.

Fig. S2 Hierarchical clustering of differentially expressed genes in early meristematic samples.

Fig. S3 Hierarchical clustering of differentially expressed genes in late primordia samples.

Fig. S4 Expression of candidate genes in control samples.

Fig. S5 Cloning and sequencing of the *PaSPL1* coding sequence.

Fig. S6 SQUAMOSA BINDING PROTEIN-LIKE (SPL) gene family phylogeny.

Fig. S7 Hierarchical clustering of precursor microRNA expressed in meristem.

Fig. S8 Hierarchical clustering of mature microRNAs expressed in meristems.

Fig. S9 Expression of *miR156t*, *miR529c*, and *miR172i*.

Fig. S10 Hierarchical clustering of mature microRNAs expressed in bud primordia.

Fig. S11 *PaSPL1* transcript cleavage estimated by 5′ RNA Ligase-Mediated 5′ Rapid Amplification of cDNA Ends (5′ RLM RACE).

Fig. S12 Phenotypes of inbred *acrocona* siblings after 13 growth-cycles.

Fig. S13 Locations and phenotypes of adult stands of *acrocona* trees.

Table S1 Samples subjected to RNA-sequencing.

Table S2 Primers used in the study.

Table S3 Samples used in 5′ RNA Ligase-Mediated 5′ Rapid Amplification of cDNA Ends (5′ RLM RACE) experiments.

Table S4 The *PaSPL*-gene family.

Table S5 Acrocona specific single nucleotide polymorphisms.

Table S6 Frequency of RNA Ligase-Mediated 5′ Rapid Amplification of cDNA Ends (5′ RLM RACE) products.

Table S7 Genotyping of genes with *acrocona* specific single nucleotide polymorphisms.

Table S8 Genotyping of adult stands of *acrocona* and wild-type *Picea abies*.

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Picea abies is an economically important tree, and does not produce enough seed for research, breeding, and industry. To understand the development of seed cones, we took a variety of approaches. We provided evidence of a mutation that leads to the acrocona mutant phenotype. We performed Spatial Transcriptomics experiments to create a spatiotemporal atlas of gene expression of shoot primordia development. We studied the variation in cone-setting ability and effects of Gibberellic Acid injections in a seed orchard.

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