UMEÅ UNIVERSITET

# The significance of ethylene and ETHYLENE RESPONSE FACTORS in wood formation of hybrid aspen

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Akademisk avhandling

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## Abstract
The woody tissues serve to stabilise plants, store nutrients and translocate water and minerals. The formation of wood, or 'secondary xylem', follows a well-defined developmental gradient which is initiated by cell division activity in the vascular cambium. The 'xylem cambial derivatives' then expand before deposition of the secondary cell wall (SCW), which is where most of the biomass of wood originates. After this, some cells of the xylem typically undergo programmed cell death (PCD). Cellulose and lignin are chemical components of the SCW that provide structural support and water impermeability, respectively. The chemical composition of the SCWs is also important economically since it affects the efficiency of wood processing during pulping and enzymatic hydrolysis. Two dominant xylem cell types of angiosperm tree species like *Populus* are the fibers and the vessel elements. Fibers are important for the mechanical strength of the wood and provide the majority of the wood biomass. Vessel elements join endwise to form hollow tubes, or vessels, for the purposes of water and solute transport in the stem.

Formation of wood is a complex process, subject to multiple levels of regulation. Plant hormones are important for wood formation, and ethylene signalling has been shown to stimulate cambial activity, affect the ratio between fibers and vessel elements, as well as the expansion of the cambial derivatives. Ethylene is also involved in the 'tension wood' response of stems that are displaced from their original vertical position. Formation of 'tension wood' generates a force that lifts the stem back to the upright growing position. What remains unknown is the molecular link between ethylene signalling and wood formation. The work in this thesis focuses on providing this link using the model tree species hybrid aspen (*Populus tremula x tremuloides)*.

Using a state-of-the-art transcriptomic database that spans all phases of xylem differentiation in hybrid aspen wood, from cell division through xylem cell expansion to xylem maturation (SCW deposition and PCD), the expression of the ethylene pathway related genes was investigated during normal wood formation. The analyses reveal ethylene perception and transcriptional reprogramming is possible across all zones of wood formation. Previously uncharacterised components were identified that may be important contributors to wood formation. Furthermore, although ethylene is known to affect the ratio between the abundance of the vessel elements and the fibers, genetic evidence is lacking. Using the tension wood response and transgenic trees modified in ethylene signalling, it was shown that ethylene is a negative regulator of vessel formation and important for a functional tension wood response. Furthermore, characterisation of two transcription factors (TFs), belonging to the ethylene response factor (ERF) gene family, suggests that aspects of xylem cell division, expansion and subsequent SCW formation, including lignification, can be affected by ERF85 and ERF139 in an ethylene-dependent manner. Phase transitions during wood formation need to be controlled spatiotemporally, and transcriptional regulation by these ERFs seems to be part of such control to establish correct transitions between cell expansion, secondary cell wall formation and lignification. The work presented here also identifies promising additions to the toolkit available for forest tree biotechnology and molecular breeding programmes.

## Keywords
ethylene, wood formation, hybrid aspen, *Populus*, ETHYLENE RESPONSE FACTOR, ERF85, ERF139, cambium, lignin, xylem expansion, secondary cell wall, tension wood, cambial derivative cell fate, vessel element, time-lapse photography

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