Studies on Isoprene Emission from Tropical Trees: Molecular Characterization of Regulatory Mechanism and Optimization of Emission Model

Various plants emit huge amounts of isoprene (C_5H_8), a very reactive volatile hemiterpene into the atmosphere. Isoprene accounts for the largest flux of non-methane hydrocarbon from the biosphere to the atmosphere. Synthesis of isoprene is from the action of the enzyme isoprene synthase (IspS) on its substrate dimethylallyl diphosphate (DMADP). Isoprene is highly reactive and has substantial effects on tropospheric oxidative capacity. Leaf isoprene emission is highly dependent on light intensity and temperature, so it is desired to understand biological mechanisms of how plants regulate their isoprene emission in response to these environmental drivers. This can enable development of mechanistic models that can predict future emissions in a changing climate. This thesis has two major objectives, (i) to explore molecular regulation of isoprene emission capacity in response to temperature and (ii) to optimize the performance of the widely used Guenther 1993 (G-93) isoprene emission model in predicting emissions from tropical tree species.

In the first part, I used the broadleaf tropical tree *Ficus septica* to explore temperature regulation of isoprene emission capacity. *F. septica* was previously demonstrated to cease isoprene biosynthesis when challenged with low temperatures around 12^{o}C, and to re-induce emissions if exposed to warm temperature (30^{o}C) for at least 24 h. I examined gene expression and IspS protein level dynamics during the same temperature treatment regime as in the previous study and concluded that isoprene emission capacity in *F. septica* in response to changing daily temperature can be explained by transcriptional regulation of the IspS gene that apparently controlled the amount of protein in the leaves. Next, I then sought to explore this transcriptional regulation.

In the second study, I conducted a comprehensive analysis of transcriptome data set of *F. septica* under the same temperature regime. I also did metabolite profiling of the substrate supply pathway metabolites and cloned about 1.3kb of the upstream promoter region of *F. septica* IspS. Expression networks and gene ontology analysis of transcriptome gene sets suggested that isoprene biosynthesis has a close relationship with plant hormone signal transduction and circadian rhythm related genes. Their expression profiles together with putative *cis*-acting motifs on IspS promoter suggested that the IspS gene was transcriptionally

regulated by transcription factors of the circadian clock and phytohormone signalling networks, especially those in abscisic acid, jasmonic acid and ethylene signalling.

The last part of this work presents efforts to optimize the G-93 model in predicting emissions from tropical trees. I monitored leaf-scale diurnal isoprene emissions of *F. septica* and *Casuarina equisetifolia* outdoors and compared their temperature and light dependencies to those of temperate poplars. Results show that the G-93 model can fairly simulate emissions during periods of low temperature and low light but performed poorly in predicting emissions during high temperature – high light durations around midday. An iterative optimization method that uses mutual repetitive step-by-step simultaneous optimization of the temperature and light – dependent variables of the G-93 was developed. Parameterization of these two factors by the proposed method improved model performance from explaining 73-77% of emission variations to 81-96%.