Tree Physiology 18, 53–58

Accumulation of flavonoids and related compounds in birch induced by UV-B irradiance

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Received February 3, 1997

Summary A growth chamber experiment was conducted to examine the effects of UV-B exposure (4.9 kJ m^{-2} day^{-1} of biologically effective UV-B, 280–320 nm) on shoot growth and secondary metabolite production in *Betula pendula* (Roth) and *B. resinifera* (Britt.) seedlings originating from environments in Finland, Germany and Alaska differing in solar UV-B radiation and climate. Neither shoot growth nor the composition of secondary metabolites was affected by UV-B irradiance, but the treatment induced significant changes in the amounts of individual secondary metabolites in leaves. Leaves of seedlings exposed to UV-B radiation contained higher concentrations of several flavonoids, condensed tannins and some hydroxycinnamic acids than leaves of control seedlings that received no UV-B radiation. At the population level, there was considerable variation in secondary metabolite responses to UV-B radiation: among populations, the induced response was most prominent in Alaskan populations, which were adapted to the lowest ambient UV-B radiation environment. I conclude that solar UV-B radiation plays an important role in the formation of secondary chemical characteristics in birch trees.

Keywords: Betula pendula, Betula resinifera, birch populations, phenolic acids, tannins, ultraviolet-B radiation.

Introduction

The secondary metabolites of birch trees (*Betula* spp.) are mainly phenolic and terpenoic compounds (e.g., Julkunen-Tiitto et al. 1996). The ecological activities of secondary metabolites are diverse and highly variable, and depend on prevailing physiochemical conditions (Appel 1993, Dixon and Paiva 1995). It has been suggested that some phenolics and terpenoids constitute defensive metabolites against herbivory and pathogens (e.g., Reichardt et al. 1984, Palo 1987, Sunnerheim-Sjöberg 1991, Tahvanainen et al. 1991). Secondary compounds also play a role in protective reactions to the oxidative action of pollutants (e.g., Eckey-Kaltenbach et al. 1994, Kangasjärvi et al. 1994).

Solar radiation, and UV-radiation in particular, not only stimulate but also induce the *de novo* synthesis of many secondary compounds (e.g., McClure 1975, Wellmann 1983, Zaprometov 1988), and UV-B exposure causes temporary changes in phenolic concentrations in plant epidermal cells (Li et al. 1993, Liu et al. 1995, Ormrod et al. 1995). Because many phenolics absorb in the UV-region of the spectrum (e.g., Markham and Mabry 1975) and are therefore capable of protecting plant cells from the harmful effects of UV-radiation, it has been suggested that the activation of biosynthetic pathways producing secondary metabolites represents a general plant response to increased UV-B radiation (Flint et al. 1985, Zaprometov 1988, Tevini et al. 1991, Li et al. 1993, Middleton and Teramura 1993, Lois 1994, Lois and Buchanan 1994, Reuber et al. 1996).

Sensitivity to UV-B differs among species, populations and varieties, and is also influenced by environmental conditions (e.g., Sullivan and Teramura 1988, Tevini and Teramura 1989, Bornman and Teramura 1993). Sensitivity to UV-B may be related to epidermal screening activity, because the epidermis can attenuate the major proportion of incoming UV-B radiation (Caldwell et al. 1983, Robberecht and Caldwell 1983, Day 1993, Day et al. 1994). It has been proposed that differences in UV-B radiation along latitudinal or elevational gradients have resulted in plant species from diverse UV-B habitats developing contrasting sensitivities to UV-B radiation (Barnes et al. 1987, Sullivan et al. 1992, Ziska et al. 1992). If so, the considerable qualitative and quantitative variations among birch species and populations in the production of secondary compounds, as a result of differences in plant age and environmental factors (Lavola and Julkunen-Tiitto 1994, Lavola et al. 1994, Julkunen-Tiitto et al. 1996), may result in differences in their UV-resistance.

However, no specific studies of changes in individual flavonoids or other UV-absorbing secondary compounds caused by UV-B irradiance have been conducted in deciduous tree species. Consequently, the main objective of this study was to determine the effects of UV-B exposure on the formation of phenolic compounds in populations of European silver birch (*Betula pendula* Roth) and its conspecific species in North America, Alaskan paper birch (*B. resinifera* Britt.). I hypothesized that in these populations UV-B induces changes in the amounts of UV-absorbing secondary compounds that reflect differences in UV-sensitivity among the populations.

Material and methods

Birch seedlings from three geographical origins were used: seeds of silver birch, *Betula pendula*, were collected from two sites in Finland (Karjalohja: 60.3° N, 23.7° E (F1) and Helsinki: 60.2° N, 25.1° E (F2); 100–200 m) and Germany (Soltau: 52.9° N, 9.8° E; 100–200 m (G1) and Harzburg: 51.8° N, 10.5° E; 200–500 m (G2)), and seeds of Alaskan paper birch, *B. resinifera* were collected from three sites near Fairbanks, Alaska (65.3° N, 140.6° E; 200–500 m (A1, A2, A3), provided by Prof. John Bryant, University of Alaska, Fairbanks, AK). On July 1, 1996, a cloudless day with an aerosol value of zero, the ambient biologically effective UV-B radiation ($UV\text{-}B_{BE}$; Caldwell's generalized plant damage spectrum, action = 1 at 300 nm), determined for each population according to calculations by Björn and Teramura (1993), was 5.6, 4.3 and 3.4 kJ m^{-2} for the German, Finnish and Alaskan populations, respectively.

Seeds were germinated in a greenhouse. One month after germination, the seedlings were transferred to growth chambers and grown in a 1:1 (v/v) mix of perlite and vermiculite and fertilized with nutrients twice a week (100 ml), according to Ingestad (1962). One week after the transfer, irradiation with UV-B was begun. The plants were irradiated for 3 h per day, receiving on average 4.9 kJ m^{-2} of biologically effective UV-B radiation ($UV\text{-}B_{BE}$) supplied by fluorescent lamps (Philips, Ultraviolet-B, TL 40 W/12). Irradiance was measured with a Macam SR9910 spectroradiometer (Macam, Livingstone, Scotland). Cellulose acetate filters were used to remove radiation below 280 nm. The plants in the control chamber received no UV-light. Artificial light was supplied by metal halide discharge lamps (Philips, HPI/T 400 W). Irradiance was about 150 μmol m^{-2} s^{-1} at the beginning of the experiment and later it was increased to about 220 μmol m^{-2} s^{-1}. The chambers provided a 16-h photoperiod, day/night temperatures of 22/18 °C and a humidity of 65–80%.

The seedlings were grown for 76 days and then harvested and air-dried to obtain shoot biomass. An air-drying method was used because it does not change the quality or quantity of secondary metabolites in leaf samples of deciduous species (Julkunen-Tiitto and Tahvanainen 1989, Keinänen and Julkunen-Tiitto 1996). One half of the second youngest, fully expanded leaf from the top of each air-dried seedling was prepared for analysis as described by Lavola et al. (1994) and Meier et al. (1988) (*B. pendula*, $n = 10$; *B. resinifera*, $n = 5$). Secondary metabolites were identified by high performance liquid chromatography (HPLC). Elution solvents were A (aqueous 2.1% tetrahydrofuran + 0.25% *o*-phosphoric acid) and B (100% methanol). The following gradient was used: at 0 min 2% of B in A, at 4 min 12% of B in A, at 30 min 36% of B in A, and at 45 min 56% of B in A. The eluent was simultaneously monitored at 280 nm for phenolic acids and DPPG and at 360 nm for flavonoids. Secondary metabolites were quantified against commercial standards: salicin (Roth, Karlsruhe, Germany) for 3,4'-dihydroxypropiophenone-3-β-D-glucopyranoside (DPPG), chlorogenic acid (Aldrich, Steinheim, Germany) for all phenolic acids, myricetin-3-*O*-rhamnoside for all myricetin-derivatives, and quercetin-3-*O*-rhamnoside for all quercetin-derivatives (all flavonoid standards were purchased from Roth, Germany). Identification of components was based on their retention times and spectral data, as described by Keinänen and Julkunen-Tiitto (1997). Condensed tannins (proanthocyanidins) were determined by the vanillin:HCl-test (Julkunen-Tiitto 1985).

Tests of ANOVA were made for the statistical evaluation of data between species and populations within species.

Results

A 4.9 kJ m^{-2} day^{-1} dose of biologically effective UV-B radiation did not alter shoot biomass production compared with that of seedlings that received no UV-B ($F = 0.565$, $P = 0.455$ for *B. pendula* and $F = 0.072$, $P = 0.79$ for *B. resinifera*), and did not cause any visible symptoms of leaf injury. Shoot biomass production differed significantly between species ($F = 34.8$, $P = 0.0001$) and between birch populations ($F = 5.671$, $P = 0.0015$ for *B. pendula* and $F = 5.430$, $P = 0.0113$ for *B. resinifera*). Shoot biomass production of *B. pendula* populations was considerably higher (3–5 g_{DW}) than that of *B. resinifera* populations (1.5–3 g_{DW}).

Eight flavonoids, three phenolic acids and one phenolic glucoside were found in both birch species. The cinnamic acid derivatives were tentatively identified as *trans* forms of 3- and 5-*p*-coumaroylquinic acids (Ossipov et al. 1996, Keinänen and Julkunen-Tiitto 1997). In all seedlings, UV-B irradiation increased the concentrations of most flavonoids (Figures 1 and 2), hydroxycinnamic acids (Figure 3) and condensed tannins (Figure 4); however, the response to UV-B was both species-specific and population-specific. The increase was flavonoid biased in the seedlings in *B. pendula* populations (Figures 1 and 2), whereas in seedlings in *B. resinifera* populations the increase was phenolic acid biased (Figure 3). Exposure of seedlings to UV-B irradiation increased the amounts of tannins more in *B. resinifera* populations (over 100% increase in each population) than in *B. pendula* populations (about 50% increase) (Figure 4). It was not determined if the observed accumulation of different phenolics was a consequence of increased synthesis or decreased turnover rates.

Among the populations, there were differences in both the constitutive and the UV-B induced concentrations of secondary metabolites (see Figures 1–4). There were also some UV-B induced differences in the responses of populations from different geographical locations. Exposure of seedlings of the Finnish, German and Alaskan populations to UV-B increased the amounts of quercetin-3-rhamnoside by 15, 36 and 86% (Figure 1), chlorogenic acid by 0, 22 and 92% (significant geographic location × UV-treatment interaction, $F = 9.526$, $P = 0.0002$) (Figure 3), cinnamic acid II by 12, 23 and 64% (Figure 3) and condensed tannins by 38, 55 and 132% (significant geographic location × UV-treatment interaction, $F = 4.722$, $P = 0.0109$), respectively (Figure 4). In contrast, the concentration of myricetin-3-galactoside increased by 52, 116 and 188% in seedlings of the Alaskan, German and Finnish populations, respectively (significant geographic location × UV-treatment interaction, $F = 5.038$, $P = 0.0082$) (Figure 1).

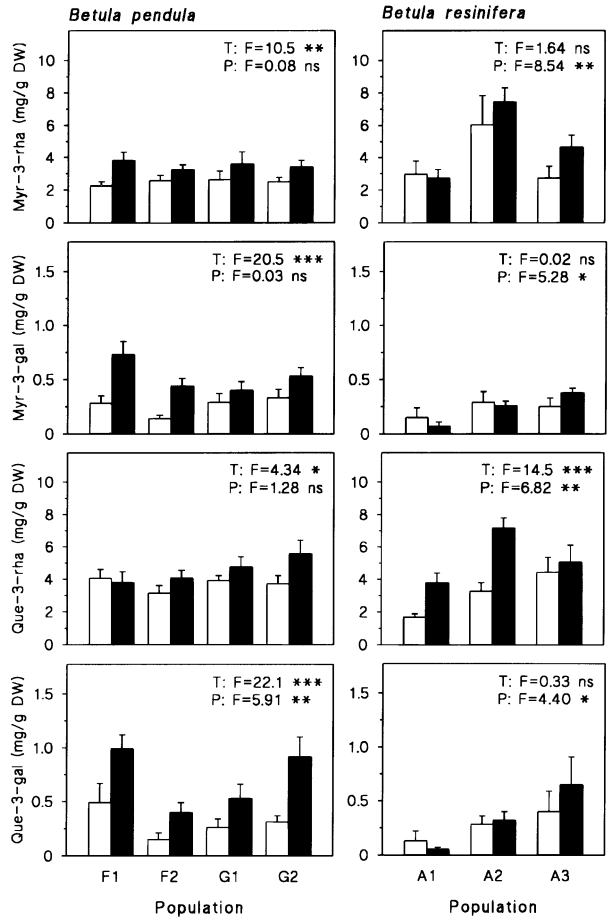

Figure 1. Changes in the main flavonoid concentrations (mean ± SE) in the leaves of *Betula pendula* and *B. resinifera* in control seedlings (no UV-B) (□) and in seedlings exposed to UV-B irradiation (■). The result of ANOVA (*F*- and *P*-values) is shown for each flavonoid component (UV-treatment = T, population = P, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Myr = myricetin, que = quercetin, rha = rhamnoside, gal = galactoside.

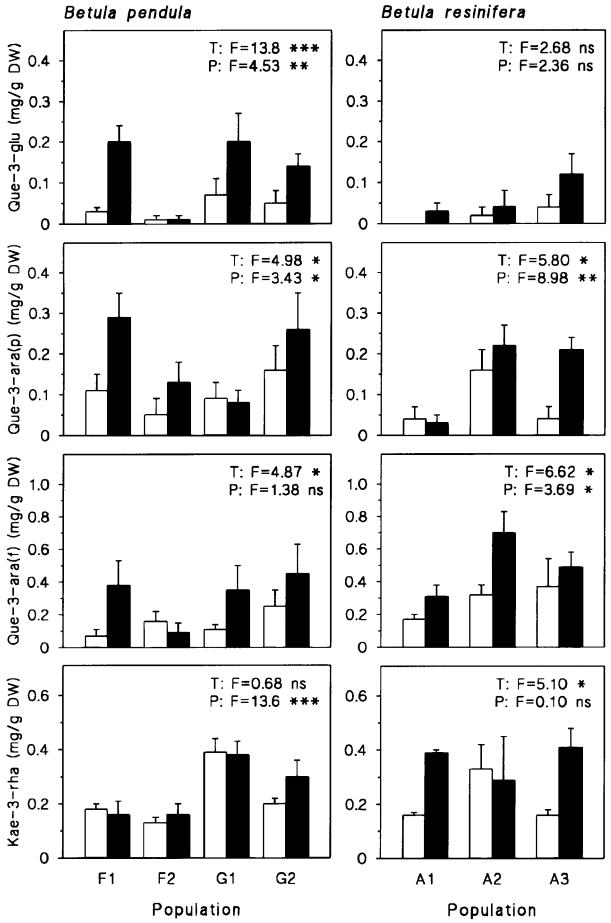

Figure 2. Changes in the minor flavonoid concentrations (mean ± SE) in the leaves of *Betula pendula* and *B. resinifera* in control seedlings (no UV-B) (□) and in seedlings exposed to UV-B irradiation (■). The result of ANOVA (*F*- and *P*-values) is shown for each flavonoid component (UV-treatment = T, population = P, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Que = quercetin, kae = kaempferol, glu = glucuronide, ara(p) = arabinopyranoside, ara(f) = arabinofuranoside, rha = rhamnoside.

In addition, there was considerable variation within a population, especially in the amounts of minor flavonoids (Figure 2), which partly masked the effect of UV-B.

Discussion

Compared to ambient UV-B doses estimated by model calculations (Björn and Teramura 1993), the treatment UV-B exposure of 4.9 kJ m^{-2} day^{-1} was higher than the ambient dose for Alaskan birch populations, similar to the ambient dose for Finnish populations and lower than the ambient dose for German populations. The treament UV-B dose did not affect shoot growth of the seedlings but caused a marked accumulation of leaf phenolics. In contrast, in previous studies, a low ambient UV-B environment enhanced the sensitivity of seedlings to UV-B irradiation, and the intensified responses were reflected both in growth and secondary metabolites (e.g., McClure 1975, Zaprometov 1988, Bornman and Teramura 1993).

The concentrations of seven of the eight individual flavonoids detected in *B. pendula* and four flavonoids found in *B. resinifera* were significantly higher in UV-B-treated seedlings than in control seedlings. However, the qualitative flavonoid composition of UV-B irradiated seedlings did not differ from that of seedlings that had not received UV-B. Flavonoid accumulation was accompanied by a UV-B-induced increase in phenolic acids and condensed tannins. Similar accumulations of UV-absorbing compounds in leaves, described as total flavonoid content, have previously been reported in response to supplementary UV-B (e.g., Robberecht and Caldwell 1983, Flint et al. 1985, Barnes et al. 1987, Li et al. 1993, Ormrod et al. 1995, van de Staaij et al. 1995). Increases in cinnamic acids and chlorogenic acid in response to UV-B irradiation have also been found in some herbaceous species (Lott 1960,

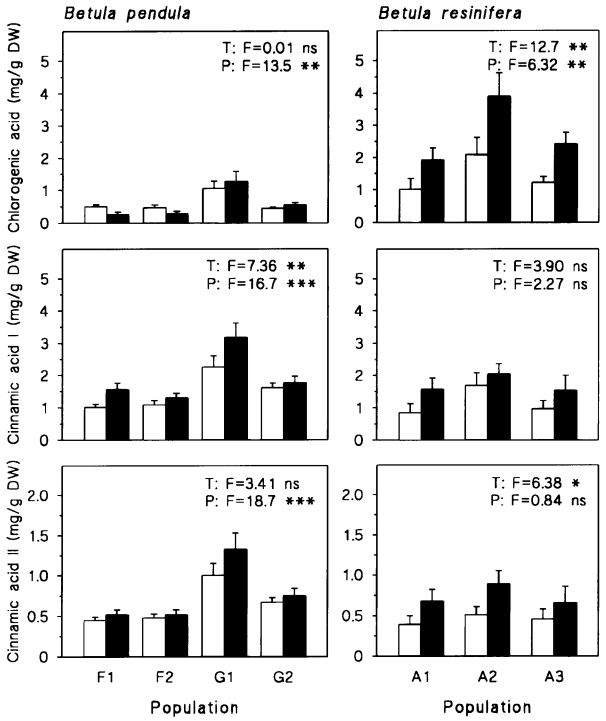

Figure 3. Changes in the phenolic acid concentrations (mean ± SE) in the leaves of *Betula pendula* and *B. resinifera* in control seedlings (no UV-B) (□) and in seedlings exposed to UV-B irradiation (■). The result of ANOVA (*F*- and *P*-values) is shown for each phenolic acid component (UV-treatment = T, population = P, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

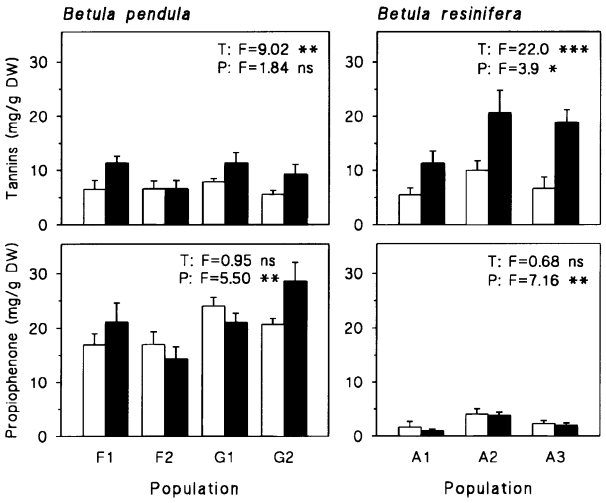

Figure 4. Changes in the condensed tannin and 3,4'-dihydroxypropiophenone-3-β-D-glucopyranoside (DPPG) concentrations (mean ± SE) in the leaves of *Betula pendula* and *B. resinifera* in control seedlings (no UV-B) (□) and in seedlings exposed to UV-B irradiation (■). The result of ANOVA (*F*- and *P*-values) is shown for both phenolic components (UV-treatment = T, population = P, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

Koeppe et al. 1969, Li et al. 1993, Liu et al. 1995, Ormrod et al. 1995, Sheahan 1996). However, in a study of a conifer and a deciduous tree species, Sullivan et al. (1996) found no change in the concentrations of UV-absorbing compounds in response to UV-B radiation at doses similar to and higher than that used in the present study. It has been suggested that these qualitative differences in the UV-B absorbing compounds of different species underlie the differences among species in UV-screening capability and the disparity in UV-B responses.

In the present study, the UV-B treatment had no effect on shoot growth and did not result in any leaf injuries perhaps indicating that the UV-B-induced formation of phenolics had some protective function. In particular, the increase in concentrations of specific flavonoids (myricetin and quercetin derivatives), cinnamic acid derivatives, and chlorogenic acid may play a protective role against photo-oxidation damage by UV-B radiation (Larson 1988, Li et al. 1993, Lois 1994, Liu et al. 1995, Reuber et al. 1996, Sheahan 1996). In a leaf, flavonoids absorb UV-radiation at 200–380 nm and cinnamic acid derivatives mainly absorb at 270 to 350 nm, attenuating UV-B even more effectively than flavonoids (e.g., Markham and Mabry 1975, Sheahan 1996, Lavola et al. 1997). Because several flavonoids and phenolic acids are antioxidants, they may scavenge the free oxygen radicals generated by UV-light (e.g., Husain et al. 1987, Larson 1988, Foyer et al. 1994). It has been suggested that a specific flavonoid is responsible for UV-protection (e.g., Lois and Buchanan 1994, Ballaré et al. 1995, Liu et al. 1995, Reuber et al. 1996, Lavola et al. 1997).

The concentration of condensed tannins increased in response to UV-B irradiation. However, under nutrient-limited growth conditions the tannin concentration of birch seedlings is not affected by UV-B irradiance, although the amounts of some flavonoids increase (Lavola et al. 1997), perhaps implying a minor role of tannins in UV-protection. Tannin accumulation in seedlings may merely reflect an age-dependent reaction (e.g., Chalker-Scott and Krahmer 1989, Julkunen-Tiitto 1989), because plants subjected to UV stress, with no nutrient limitation, appear to grow "physiologically older" than plants not exposed to UV-B. Thus, birch seedlings exposed to UV-B have thicker leaves (more cuticle) than control seedlings. Leaf thickening has been shown to be a normal structural response of terrestrial plants to UV-B radiation (e.g., Bornman and Teramura 1993, Sullivan et al. 1996).

The birch populations differed in the production of shoot biomass and secondary metabolites. Also, the responses of the two birch species to UV-B exposure differed. The European silver birch (Finnish and German populations) accumulated more flavonoids than the Alaskan paper birch, but the accumulation of phenolic acids and tannins showed the reverse trend. There was also some correlation between the UV-B-induced responses and the native UV-environment of the populations; that is, some phenolics (quercetin-3-rhamnoside, chlorogenic acid, tannins, cinnamic acid II) increased in the order: Finnish < German < Alaskan populations. Thus, among the populations studied, the greatest response of Alaskan populations to UV-B may imply their weaker adaptation to higher than ambient UV-B radiation. This finding partly supports the observations that plants originating from locations with a naturally

high UV-B irradiance (high altitude, low latitude) exhibit less sensitivity to enhanced UV-B irradiance than plants originating from locations with a low UV-B irradiance (low altitude, high latitude) (Robberecht and Caldwell 1983, Barnes et al. 1987, Sullivan and Teramura 1988, Sullivan et al. 1992, Ziska et al. 1992).

Based on present and earlier results (Lavola et al. 1997), I conclude that birch seedlings have considerable amounts of constitutive UV-B-absorbing compounds that are further UV-B-inducible, because the change in light quality toward the UV-region strongly activated flavonoid metabolism. Therefore, UV-B radiation modifies the expression of both the constitutive and the inducible secondary metabolites in birch leaves and thus the whole chemical resistance mechanism in birch (cf. Tuomi et al. 1988). Each of the birch populations counteracted the harmful action of UV-B by improving its origin-specific production of UV-B absorbing secondary compounds. However, modification of UV-sensitivity (in terms of UV-absorbing compounds) may depend more on phenotypic plasticity of the secondary metabolism induced by the prevailing growth conditions than on the UV-B-adaptation of the populations.

Acknowledgments

I thank Sinikka Sorsa for technical assistance, Markku Keinänen, Johanna Hakulinen and Marja-Riitta Juntheikki for their help in processing the work, Kenneth Meaney for revising the manuscript and Riitta Julkunen-Tiitto for guidance in many aspects of the work. This research was supported by the Academy of Finland.

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